International Journal of Pharma Research

Vol.4 • Issue 2 ISSN 0975-3532 July - December 2013

Indexed in Index copernicus and google scholar

I J P R

The Research Publication from
PSG COLLEGE OF PHARMACY
Coimbatore 641 004, Tamil Nadu, INDIA

www.psgpharma.ac.in

No Processing Charge & Publication Fee

INTERNATIONAL JOURNAL OF PHARMA RESEARCH (IJPR)
THE OFFICIAL PUBLICATION OF PSG COLLEGE OF PHARMACY

International Journal of Pharma Research (IJPR) is the official publication of PSG College of Pharmacy, Coimbatore, Tamilnadu, India. It mainly covers the articles from different areas of pharma research from Academia, Industry and Community & Hospital Pharmacy.

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An Overview of Current Developments In Oral Disintegrating Tablets (ODT's)

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Received Date : 16.09.2013 Accepted Date : 13.11.2013

ABSTRACT

The current work focuses on need of product extension by oral dispersible tablets (ODT) of an existing marketed product in its terminal period of patent. While designing ODTs, it is mandatory to consider the physiochemical and pharmaceutical aspects of the drug as well as the biopharmaceutical aspects. Methods of preparation include freeze drying, cotton candy, molding, spray drying, compaction and mass extrusion. With a historical background on ODTs, a detained explanation is given not only on the preparation methods, but also on various evaluation parameters. In the evaluation process, although many parameters are of that of the tablets, the standard pass range varies so as to meet the requirement of a fast disintegrating properties of the formulation.

INTRODUCTION

In the present pharmaceutical scenario, research is focusing towards patient-friendly as well as patient complied dosage forms. Thus, a vast spectrum of formulation technologies has been observed which accounts safety and efficacy of the formulations. As it takes huge investment, both in terms of cost and time, a lot of risk factors are hidden in coming out with a new molecular entity including active pharmaceutical ingredient (API). Thus towards the termination period of patency (10 to 15 years) of a product, competitors are investing research on product extensions rather than investing on a new active drug molecule offering great scope for bioavailability and bioequivalence studies (BA/BE). Oral disintegrating tablet (ODT) is one of the best examples of dosage forms supporting the above discussion since the need depends on augmenting the bioavailability and patient compliance. Various synonyms of ODT's include Oro Dispersible tablets, Quick Disintegrating tablets, Fast Dissolving tablets, Rapid Disintegrating tablets and Rapimelts.[1] The US pharmacopoeia approved all the above named terms as Oral Disintegrating Tablets (ODT's). According to US pharmacopoeia " ODT's are the one which disperses rapidly within 3 min in the buccal cavity before swallowing" thus these are those dosage forms that are intended to dissolve in the buccal saliva and facilitate for swallowing without water. These came into existence when the first ODT's were formulated to increase the ease of administering vitamins in pediatric use. In the recent contemporary ODT technologies, micro particles incorporated with the API are punched into tablet form by which bitter tasted drugs can be prepared in a palatable form and also improves the stability of the drug. This ODT was not disintegrated through dissolution but by effervescence. Three companies Catalant Pharma Solutions (formerly named as Scherer DDS) in the UK, Cima labs in the US and Takeda pharmaceutical company in Japan had taken the first step for the development of ODT's.

Table - 1 : USFDA approved ODT'S products:

S. No	USFDA approved products	Year	Type of formulation
1	Claritin (Loratidine)	Dec 1996	Zydis formulation
2	Klonopin (Clonazepam)	Dec 1997	Zydis formulation
3	Maxalt (Rizatriptan)	Dec 1998	Zydis formulation

Table - 2 : ODT'S Manufacturing methods / technologies along with patented companies.

S. No	Method/Technologies	Patented companies
1	Ora salv	Cima labs
2	Dura salv	Cima labs
3	Wow tab	Yamanouchi Inc
4	Flash dose	Fuisz Tech Inc
5	Adva tab	Fuisz Tech Inc
6	Cotton candy process	Eurand Tech

OraSolv™ from Cima Labs used direct compression technique was capable of using less compression force. DuraSolv™ a second generation ODT from Cima Labs gave a better mechanical strength. Wow tabTM patented by Yamanouchi Inc. used compression molding. Flash dose and AdvaTabTM by Fuisz tech Inc. and Eurand Tech. employed cotton candy technique and microencapsulation method respectively. While the former was effective in taste masking the later is known for its rapid less than 30 sec disintegration. OraQuick™ technology by K. V. Pharmaceuticals does not utilize any solvents and provides faster production. QuickDis™ from Lavipharm Laboratories Inc. comes out with bucal fast dissolving films. NanoCrystal™ from Elan's proprietary focuses on reducing the particle size less than 2 microns. MeltEase™ Technology developed by Nutrition Formulators, allows tablet dissolution in less than five seconds for a 400 mg tablet. Other patented technologies includes Frosta technology, Multi flash technology etc. Many geriatrics and pediatrics feel difficulty in swallowing (Dysphagia) the conventional tablets which enhances the scope of using ODT's.[2]

Pediatrics, geriatrics, psychiatry and bed-ridden with Dysphagia are the most benefitted among the patient community. ODT's are great help for travelers who need not consider water for swallowing since ODT's are designed to disintegrate in the buccal cavity. During Upper Respiratory Infections (URI's) chocking of a conventional medicaments can be avoided by considering ODT's. Other merits extends to stability, bitter taste masking, enhanced bioavailability, rapid absorption and acute treatment. ODT's contain hygroscopic substances and there is a chance of microbial contamination if not properly stored. So care should be taken while storage of these dosages forms. In order to safeguard the stability and safety of the product special packing is required which should me mostly considered.[3]

PHYSICO CHEMICAL ASPECTS

Molecular weight: Should be low to facilitate easy penetration of drug through the mucosal linings. Solubility is also an important aspect to be taken care while formulating an ODT.

Crystal morphology: Water-soluble drugs has various formulation challenges as they form eutectic mixtures, resulting in freezing-point depression and the formation of a glassy solid which may collapse upon drying because of loss of supporting structure during the sublimation process. Such collapse sometimes can be avoided by using various matrix-forming excipients like mannitol which can induce crystallinity and therefore impart rigidity to the amorphous composite.[4]

Compressibility – the particles should be easily compressible and should withstand the compression force and also should be rigid after compression and this should not cause any problem during shipping and transport. This emphasizes the need for disintegrating the tablets which must pass the friability test.[5]

Particle size: The easiest size of tablet to swallow is 7 to 8 mm diameter while the easiest size to handle was larger than 8mm therefore the tablet size that is both easy to take and easy to handle is difficult to achieve.[6]

Hygroscopicity: Since the ODT's contain ingredients that have water molecules, it is desirable to consider the stability and integrity in terms of physical nature and specialized packing of product is recommended.[7]

BIOPHARMACEUTICAL ASPECTS

Since the size of an ODT should be small, dose of the active ingredient should be limited. The application of technologies used for ODTs is limited by the amount of drug that can be incorporated into each unit dose. For lyophilized dosage forms, the drug dose must be lower than 400 mg for insoluble drugs and less than 60 mg for soluble drugs[7]. This parameter is particularly challenging when formulating a fast-dissolving oral films or wafers.Usually ODT's are used for immediate action to achieve the acute pharmacological response. Suitable half-life of the drug of ODT's shall be 3 to 5 h to avoid instability problems and better absorption. Drugs which require frequent dosing may be considered for ODT's since consuming water along with the medicament may be avoided. Since the drug is exposed to salivary pH (7.4), stability of the drug as well as the excipients used

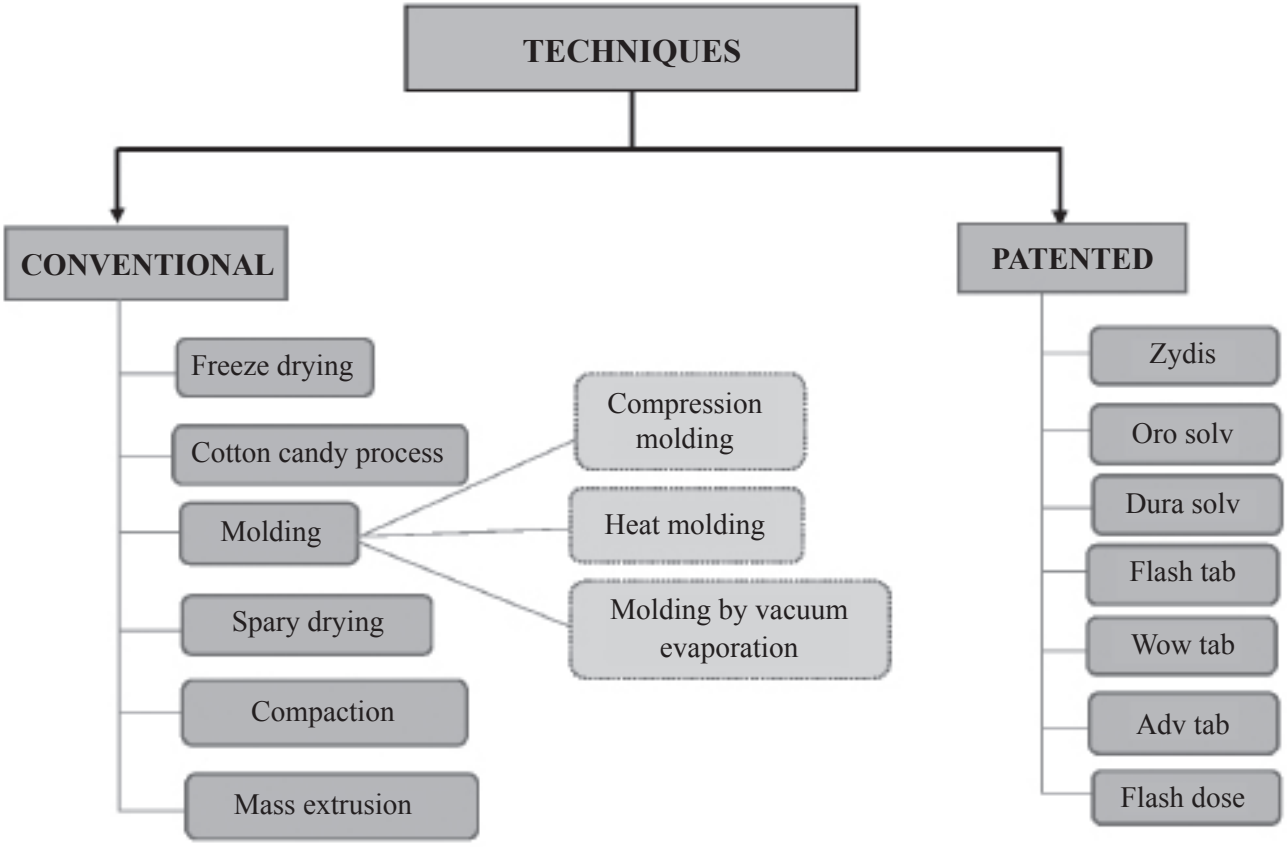
in ODT formulation is very important. For localized action within the buccal cavity (mouth ulcer, gingivitis) the drug must be permeable.[8]

FORMULATION ASPECTS

Swallow ability should be such that it should leave negligible or no residue in the mouth after oral administration. Bioavailability should be more (>10%). Palatability in the oral cavity – as most drugs are unpalatable when the ODT dissolves in the oral cavity releases the active medicament and produces discomfort for the patient to swallow. So flavoring and sweetening agents are added to facilitate easy administering of the ODT.[9]

Mechanical strength of tablets is very important as the tablets should dissolve in the oral cavity, low compaction force is required so as to make the tablet friable and can be easily disintegrated when comes in contact with saliva. The drug should be very stable and it should not undergo any kind of degradation before and after entering the stomach.[10] Fast disintegration is either achi eved by the process/ technique or by the role of specified excipients.

Figure1. Various approaches of product development techniques in ODTs



Freeze drying (lyophilization technique):

This is so called because the water is sublimated from the product after freezing and is carried out in four phases.[11]

1. Pretreatment : Here the product is concentrated first, then formulation reversion which is nothing but the addition of components to increase the stability is done. The material is brought below its eutectic zone which finally results in an increase in the porosity and relative surface area thereby saliva can penetrate through pores and aids in disintegration. This process can result in adverse thermal effects which in turn results in instability of the drug. So this is carried out

at a non-elevated temperature. But the drawback of this technique is that it is time consuming, expensive and packaging will be difficult and also the concentrations of drugs may vary from one tablet to another during this process.

2. Freezing : In this phase the material is cooled below its triple point (Triple point is the lowest temperature at which solid and liquid phases coexist). This ensures sublimation. Then the product is frozen slowly at -50 to -80^0 C.

3. Primary drying : In this phase heat is produced by conduction and is supplied to the material to facilitate sublimation of water. About 95% water is sublimated. Partial vacuum controls the pressure and speeds up the sublimation. Then a condenser (at – 50^0 C) facilitates re solidification of water vapor.

4. Secondary drying : This is done to remove unfrozen water molecules. Here the temperature is raised than that of the primary phase to break the links between water molecules and frozen material. The pressure is lowered to facilitate desorption.

By the end of the freeze drying process the final water residue will be very low (1 to 4%).

Ibrahim & EL.Setouhy *et.al.* (2010), prepared oral disintegrating tablets of Valsartan by freeze drying process.

1. Cotton candy process (candy floss method) :[12]

Here saccharides and polysaccharides are processed into amorphous flossy by flash melting and centrifugal force which acts simultaneously and thus an ODT matrix is formed. This matrix is crystallized partially to provide a compound which will show good flow property and compressibility. Then the active ingredients and suitable excipients are added in required quantities, blended and finally compressed. As high temperature is maintained, this method may not suitable for thermo labile substances. Fuisz technology, Ltd employed flash dose technology in manufacturing ODT's for which cotton candy is the base.

2. Molding :[12]

This employs the use of water soluble ingredients so as to facilitate complete and rapid dissolution of the tablet. Here power blend is moistened with hydro-alcoholic solvent and then molded into a tablet. The pressure should be lower than that of conventional tablet compression. Cima labs Inc, Ethypharm, Yamanouchi Pharma, Eurand, Élan Corp these companies are employed DuraSolv™, OraSolv™, FlashTab™, WowTab™, Ziplets™, FastMelt™ technologies respectively in the manufacturing of ODT's , for which molding is the base. Following are different techniques of molding.

a. Compression molding: powdered blend is moistened with alcoholic solvent and compressed to form a wet mass which is then air dried and finally the solvent is removed.

b. Heat molding: drug is dissolved into a molten matrix and directly molded to ODT's. Tis method involves preparing a suspension containing the drug, agar, and sugar (e.g., mannitol or lactose), then pouring the suspension into the blister packaging well, solidifying the agar solution at room temperature to form a jelly, and finally drying approximately at 30^0 C under vacuum.

c. Molding by vacuum evaporation without lyophilization: at standard pressure solvent is made to evaporate from the drug. This vacuum drying will density the matrix thereby improves the mechanical strength of the tablets. Anongnart mesnukul et.al., prepared Indomethacin oral disintegrating tablets by molding method.

3. Spray Drying:[12]

This technique will produce powders with high porosity and fine characteristics which can dissolve rapidly within 20 seconds upon placing in aqueous medium. In this method, an aqueous composition containing supporting matrix and other additives are spray dried to form highly porous and fine powder (drop size 20 - 180µm depending on nozzle and pressure 50 – 300 bars) to which the active ingredients are added and then the tablet is compressed. Allen et al., had employed this method in which he used hydrolyzed and unhydrolyzed gelatins as supporting agent for matrix, mannitol as a binder, Sodium starch glycolate as disintegrant. Alternatively, gelatin can be used as a supporting agent and as a matrix, mannitol as a bulking agent and sodium starch glycolate or cross carmellose or crosspovidone are used as superdisintegrants. To this acid (citric acid) or alkali (sodium bicarbonate) is added to enhance further dissolution. The prepared suspension was spray dried to get porous powder which is compressed into tablets which gives disintegration within 20 sec.

4. Compaction :[12]

It is the most employed technique. This requires low cost of manufacturing, and easy to manufacture. All the ingredients are weighed, sieved, mixed in required proportions and finally compressed into a tablet by applying some compaction force. Using disintegrates, in single or combination results in disintegration and dissolution of the compressed tablets (1.0-2.0 kp hardness). Yash Paul *et.al*., prepared Zidovudine ODT's by compaction method. Jayadev patil *et.al.,* prepared ODT's containing Loratidine.

5. Mass extrusion :[12]

This method is mainly employed to formulate taste masked granules. Here the blend is softened by using a solvent mixture (water-soluble polyethylene glycol and methanol) and this softened mass is extruded out as a cylindrical shaped extrudates which can be further cut into even segments using a heated blade to form tablets. Vinod et al., had reported on mass extrusion which is very simple and practical method since thermal conditions need not be considered[13]. Although the author has not used in the production of ODT's, this technique will be of great use and wise enough to consider in the scale up of ODT's. Alpana et al., formulated Rizatriptan benzoate ODT's by the mass extrusion method[14].

6. Sublimation

The purpose of sublimation is to generate micropores to increase the effective surface area. This enhances the contact points between the solid surface and the medium. In separate studies conducted by Heinemann et al., and Knitsch et al. explored high volatile substances like camphor, naphthalene, urea, and urethane, ammonium bicarbonate, ammonium carbonate, benzoic acid to generate the micropores[15-17]. Solvents such as cyclohexane and benzene were also suggested for the generation of porosity in the matrix, but gained less significance because of toxicity. Yet in another study, Koizumi et al. prepared tablets by Mannitol (matrix), and camphor (sublimating agent) and achieved rapid salivary disintegration in 10-20 s[18].

Excipients basis for ODTs

Earlier we have mentioned about various excipients used in the ODT formulations. But still to augment the disintegration/ dissolution rates several other adjuvants are also incorporated. For example, incorporation of highly hydrophilic excipients and effervescent agents further accelerate the process of disintegration. It is surprising to know that below the critical disintegrant concentration (CDC), tablet disintegration time is inversely proportional to disintegrate concentration whereas, above CDC, disintegration time remains approximately constant or even increases.[19]

Usually sugar based bulking agents as excipients like mannitol, maltilol, maltose, fructose, isomalt, dextrose, xylitol, sorbitol, starch hydrolysate, polydextrose and, lactilol which display high aqueous solubility and sweetness, and hence impart taste masking property and a pleasing mouth feel are considered. Care must be taken not to increase the bulk of ODTs which is obviously not reasonable.

API	Indication	Excipients (role)	Methods employed	Chief investigator (year)
Sildenafil citrate	Anti-hypertensive, Erectile dysfunction	Lactose monohydrate (diluent) Mg.stearate (lubricant) Sodium Starch Glycollate(SSG) (disintegrant) Mango flavor (flavoring agent) Colloidal silicon dioxide (glidant) Povidone K30 (binder)	Direct compression	*Rajeev Hiremath et al. 2013*
Rizatriptan benzoate	Anti-migraine	Eudragit (taste masking) Cross carmellose sodium (CCS) (disintegrant) Cross povidone (CP) (disintegrant) Sodium stearyl fumerate Sodium saccharin (sweetening agent) Peppermint oil (flavoring agent)	Mass extrusion	*Alpana.P kulkarni et al. 2012*
Lornoxicam	NSAID	PVP K30 (binder) MCC 102 (filler) HPMC K4 (hydrophilic polymer) HPMC K15 (hydrophilic polymer) Mg. stearate (lubricant) Talc (glidant)	Wet granulation	*Nabin karna et al. 2012*
Rosuvostatin Metoprolol succinate (Bilayered)	Hypercholesterolemia Hypertension	HPMC K100M (hydrophilic polymer) Sodium alginate (disintegrant) Xanthan gum (binder)	Wet granulation	*Nilesh R.Khule et al. 2012*
Loratidine	Antihistamine	Mannitol (diluent) Starch (binder) CCS (disintegrant) Citric acid (effervescent) Sodium bicarbonate (effervescent) Aspartame (sweetening agent) Mint flavor (flavoring agent)	Direct compression	Jayadev patil et al. 2011

API	Indication	Excipients (role)	Methods employed	Chief investigator (year)
Zidovudine	Anti-retroviral	Surelease(E7 19010) (polymer and taste masking) Ac-di-sol	Direct compression	*Yash paul et al.2011*
Tramadalol hydrochlo-ride		Eudragit E100 (binder) CP (disintegrant) CCSd (disintegrant) SSG (disintegrant)	Mass extrusion	*Mansing et al. 2011*
Metoprolol succinate	Anti-hypertensive	Guar gum (binder) Carbapol (hydrophobic polymer) CCSd (disintegrant) CP (disintegrant) Mg.stearate (lubricant) Talc (glidant)	Wet granulation	*Sathyaraj et al. 2011*
Lisinopril	Anti-hypertensive	CP (disintegrant) CCSd (disintegrant) MCC (filler) Mg.stearate (lubricant) Talc (glidant)	Kneading technique and direct compression	*Suresh et al. 2011*
Nifedipine	Angina pectoris	EC (hydrophobic polymer) HPMC (hydrophilic polymer) Acetone (solvent) Methanol (solvent) KH2PO4	Wet granulation	Katayoun et al. 2011
Candesartan Cilexitil Captopril	Nephritic syndrome	EC (hydrophobic polymer) MCC (filler) Acetone (solvent) Liquid paraffin	Emulsion Solvent evaporation	Vinay et al. 2011
Primaquine	Antimalarial	HPMC (hydrophilic polymer) PVP K90 (binder) EC (hydrophobic polymer) Sodium CMC Sod. Alginate (stabilizing agent) Mg.stearate (lubricant) Talc (glidant)	Wet granulation	Swathi et al. 2011

API	Indication	Excipients (role)	Methods employed	Chief investigator (year)
Amlodipine besylate	Chronic stable angina, Hypertension, Vasospastic angina	CP (disintegrant) CCSd (disintegrant) SSG (disintegrant) Aspartame (sweetening agent) Mg.stearate (lubricant) Lactose (diluent) Talc (glidant)	Direct compression	*Mohan et al. 2010*
Valsartan	Hypertension, Congestive heart failure.	Mannitol (diluent) Spray dried lactose (diluent) Sucrose (sweetening and diluent) Pregelatinized starch (binder) Sodium alginate(disintegrant) Sorbitol (diluent) Xanthan gum (hydrophobic polymer) Pectin (emulsifying and thickening agent) Acetonitrile	Freeze drying	*Ibrahim et al.2010*
Phenira-mine maleate	Anti-allergic, Urticaria, Angioedema.	Pregelatinized starch (binder) SSG (disintegrant) CCSd (disintegrant) CP (disintegrant) MCC (filler) Mannitol (diluent) Aspartame (sweetening agent)	Effervescent technique	*Swamy et al. 2009*
Ondansetron HCl	Anti-emetic	CP (disintegrant) CCSd (disintegrant) Chitosan powder (disintegrant, binder) Glycine (wetting agent) Spray dried lactose (diluent) Potato starch (binder and thickening agent)	Direct compression	*Honey et al. 2009*
Nateglimide	Anti-diabetic	HPMC 2910 (hydrophilic polymer) HPMC 2208 (hydrophilic poly-mer) MCC (filler) Mg.stearate (lubricant) Maerogol 6000	High shear mixing and direct com-pression	Chisato et al. 2009

API	Indication	Excipients (role)	Methods employed	Chief investigator (year)
Indomethacin	NSAID Analgesic Antipyretic	PEG 4000 (solvent) PEG 400 (solvent) Sodium hydrogen orthophosphate (emulsifying agent) HCl (buffers) KH2PO4 Xanthan gum (hydrophobic polymer) Lactose (diluent)	Melting and mold technique	Anongnart et al. 2009
Cinnarazine	Anti-emetic Motion sickness	MCC (filler) Chitosan (binder) Glacial acetic acid (Citric acid (buffering agent, acidifier) Aspartame (sweetening agent) Menthol (flavoring agent) Colloidal SiO2 (adsorbent) Mg.stearate (lubricant)	Wet granulation	Mitesh et al. 2009
Famotidine	Anti-ulcer	SSG (disintegrant) CCSd (disintegrant) Camphor (carminative) Sodium saccharin (sweetening agent) Mannito l(diluent) PVP (binder) Talc (glidant) Mg.stearate (lubricant)	Wet granulation	Furtado et al. 2008

API	Indication	Excipients (role)	Methods employed	Chief investigator (year)
Pantoprazole	Anti-ulcer	CP (disintegrant) CCSd (disintegrant) SSG (disintegrant) L-HPC Pregelatinized starch (binder) Sodium bicarbonate (alkalizing agent) Potassium bicarbonate (alkalizing fumerate) Agar (binder) Guar gum (binder) Camphor (carminative) Menthol (flavoring agent) Thymol (anti-oxidant, cooling agent) Aspartame (sweetening agent) Talc (glidant) Sodium stearyl fumerate (lubricant)	Direct compression Sublimation	Chaudhari et al.2008
Indomethacin	NSAID	Camphor (carminative) Ammonium bicarbonate Mannitol(diluent) Colloidal SiO2 (adsorbent) Spray dried lactose(filler) Mg.stearate(lubricant) Sodium saccharin(sweetening agent) PVP (binder)	Non aqueous wet granulation	Singh et al. 2008
Roxithromycin	Bronchitis Diphtheria Sinusitis Pneumonia Trench fever	Agar (binder) Spray dried lactose (diluent) Mannitol (sweetening and solvent) Guar gum (binder) Potassium bromide (anti convulsant)	Direct compression	*Vijay et al. 2008*

Evaluation Parameters on Powders and Multiparticulates (Pre Formulation)

Characterization and evaluations are necessary to perform at the preformulation level which rule outs any pharmaceutical compatibility problems and ensures all excipients can be considered for the selected formulation and suitable for tablet compression. Usually one needs to perform each experiment 6 times to get statistically significant results.

1. Pharmaceutical incompatibility studies

It is essential to find out the interactions between drug/s-excipient/s, and if so which has to be substituted with a compatible ingredient. Earlier TLC used to perform by spotting a solution of drug alone (reference) and with physical mixture (PM) in geometric ratio stored for 48 h (test) and observed under UV light as well as by a spray reagent and the R_f values of the test must be comparable with that of the reference. But to have a reliable confirmatory data one need to go for FTIR (KBr pellet method) or DSC (thermograms) studies. The samples prepared usually are drug alone and PM. If interaction is noticed by the change/ absence of the significant peaks of the drug, or emergence of a new peak, then drug with each excipient must be sampled. It is highly appreciable to conduct the interaction study on the final formulation.

2. Bulk density (gm/cm³) : It is the ratio of untapped mass of powder to the total volume of powder, gives the amount of substance occupying a certain volume. It includes void spaces and particles[20]. Bulk density is very important in the size of containers needed for handling, shipping, and storage of raw material and blend. It is also important in size blending equipment and is determined by

$$\rho b = M / Vo$$

where M = Weight of the sample, V = Apparent volume of powder.

3. Tapped density(gm/cm³) : is the ratio of tapped mass of powder to the total volume of powder. It includes only particles but not void spaces, is determined by

$$\rho tap = M / Vf,$$

where M = Weight of the sample, Vf = Tapped volume of powder.

4. Carr's index (I) : is a measure of the propensity of a powder to be compressed. It is determined from the bulk and tapped densities. In theory, the less compressible a material the more flowable it is. As such, it is measures of the relative importance of interparticulate interactions. In a free flowing powder, such interactions are generally less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater inter particle interactions and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in the Carr's index which is calculated using the following formula.

3. Spray Drying : 12

This technique will produce powders with high porosity and fine characteristics which can dissolve rapidly within 20 seconds upon placing in aqueous medium. In this method, an aqueous composition containing supporting matrix and other additives are spray dried to form highly porous and fine powder (drop size 20 - 180μm depending on nozzle and pressure 50 – 300 bars) to which the active ingredients are added and then the tablet is compressed. Allen et al., had employed this method in which he used hydrolyzed and unhydrolyzed gelatins as supporting agent for matrix, mannitol as a binder, Sodium starch glycolate as disintegrant. Alternatively, gelatin can be used as a supporting agent and as a matrix, mannitol as a bulking agent and sodium starch glycolate or cross carmellose or crosspovidone are used as superdisintegrants. To this acid (citric acid) or alkali (sodium bicarbonate) is added to enhance further dissolution. The prepared suspension was spray dried to get porous powder which is compressed into tablets which gives disintegration within 20 sec.

$$\text{Carr's index(I)} = \frac{tapped\ density - bulk\ density}{tapped\ deisity}$$

5. Hausner's ratio(H) : is the ratio of tapped density to the bulk density.

6. Angle of repose (θ) : is the maximum angle possible between the height of the pile of powder to the horizontal plane. Either fixed funnel method or orifix method is commonly employed and calculated using the following formula

$$\text{Tan } \theta = h/r$$

where θ = Angle of repose, h = Height of the cone, r = Radius of the cone base.

Table 4 : comprehensive standard values of various derived properties

Flow property	Carr's index(I)	Hausner's ratio(H)	Angle of repose (θ)
Excellent	≤ 10	1.00-1.11	25-30
Good	11-15	1.12-1.18	31-35
Fair	16-20	1.19-1.25	36-40
Passable	21-25	1.26-1.34	41-45
Poor	26-31	1.35-1.45	46-55
Very poor	32-37	1.46-1.59	56-65
Very very poor	>38	> 1.60	>66

7. Drug content (mg or %) : Micro matrices of drug equivalent to the drug was weighed and dissolved in a minimum amount of a suitable solvent. This solution is filtered and the filtrate is taken in a 100ml volumetric flask and made up the volume with 7.4 phosphate buffer solution. This solution was analyzed for drug content by measuring absorbance of the drug using UV spectrophotometer. Drug content is very important as this data can be deputed for other parameters like drug loading, entrapment efficiency for microencapsulation and in drug release.

8. Percentage yield (%) : The yield of formulating micro matrices was calculated using the weight of the final product after drying with respect to the initial total weight of the drug and polymer used for preparation of micro matrices and percentage yield was calculated as per the formula.

$$\text{Percentage yield (\%)} = Pm/\ Tm \times 100$$

where, Pm and Tm are practical and theoretical masses of the micro matrices, respectively.

9. Moisture content (%) : Moisture was determined by loss on drying. Micromatrices were dried at ambient temperature by keeping 1000 mg of microspheres in desiccators until a constant weight was achieved. The % moisture content was calculated using the formula.

$$\text{Moisture content (\%)} = \frac{initial\ weight - final\ weight}{initial\ weight} \times 100$$

10. In vitro drug release: The drug release was studied using USP type II apparatus at 37 ± 0.5°C and at 50 rpm (mimicking oral mechanical moments) using 900ml of phosphate buffer pH 7.4 as dissolution medium. 1ml of the sample solution was withdrawn at predetermined time intervals, filtered, diluted suitably and analyzed spectrophotometrically. Equal amount of the fresh dissolution medium should be replaced immediately after withdrawal of the test sample.

EVALUATION PARAMETERS FOR ODT'S (POST FORMULATION)

Since ODT is a solid unit dosage form (tablet), all the evaluations of a conventional oral tablet must be investigated; only the standards will vary.[21]

1. Hardness (kg/cm²) : Ten tablets were taken and each tablet is placed in constant with the lower arm (plunger) of Monsanto hardness tester and the reading is set to zero and noted. Then the arm (plunger) is forcibly pressed against the spring by tightening the threaded bolt till the tablet gets into pieces. The force for the tablet to break is indicated on the gauge by the pointer.

$$T = 2F/\pi dt$$

where F is the crushing load and d is the diameter,t is the thickness of the tablet.

2. Friability (%) : Twenty ODTs should be used utilizing a USP-type Roche friabilator. Pre-weighed tablets where placed in a plastic chambered friabilator

attached to a motor revolving at a speed of 25 rpm for 4 min (USP limit – 0.5 to 1 %). The tablets were then reweighed ad the percentage weight loss (friability) was calculated :

$$\text{Friability} = \frac{\textit{initial weight of tablet - final weight of tabelt}}{\textit{initial weight}} \text{ X } 100$$

3. Weight variation (%) : Twenty tablets were randomly selected from each formulation and weighed using a digital balance:

$$\text{Weight variation} = \frac{\textit{average weight - individual weight}}{\textit{average weight}} \text{ X } 100$$

Table 5 : Standards of weight variation for ODTs

IP/BP	USP	limits
80 mg or less	130 mg or less	10%
More than 80 mg or less than 250 mg	130 mg to 324 mg	7.5%
250 mg or more	More than 324 mg	5%

4. Thickness (mm): Tablets are taken and they are placed between both arms of vernier calipers and readings on the scale were observed and noted. Finally, the average values are calculated. The thickness variation was calculated by taking ten tablets and thickness is measured with a screw gauge micrometer.

5. Drug content (mg or %) : One tablets is taken and is powdered and powder equivalent to 100 mg of the drug was weighed and taken and 5 ml of methanol was added, diluted with 6.8 phosphate buffer solution and the volume is made up to 100 ml followed by sonication for 15 minutes and then filtered and suitable dilutions are made and checked for drug concentration by any suitable analytical methods (HPLC, UV spectroscopy).[22]

Table 6 : Standards of drug content for ODT's :

S.no	Standards	Limits
1	IP	<10mg or 10%
2	BP	<2mg or 2%
3	USP	<25mg or 25%

Table - 7 : Standards of drug content for ODT's:

S.No	Number of Tablets	IP/BP/USP	
		Range of %deviation of Average values (%)	None outside (%)
1	10	NMT 1 tablet 85-115	≥75 or ≤125
2	20	NMT 2 tablets 85-115	≥75 or ≤125
3	30	NMT 3tablets 85-115	≥75 or ≤125

6. Moisture uptake study: This is done to assess the physical and chemical stability of the formulation. Moisture is absorbed on the surface of solid drugs and increases the rate of decomposition, causing agglomeration and leaching of the active. Here ten tablets are taken, weighed and placed in desiccator which contain calcium chloride at 37^0 C. After 24 h complete loss of moisture occurs which followed by re-weigh and expose to 75% RH. This has been done at room temperature for 2 weeks and tablets are again reweighed and the percentage increase in the weights can be determined by finding out the difference between weights before and after exposure to moisture.

7. Wetting time : This experiment mimics the action of saliva in contact with the tablet. Five circular tissue papers were placed in a petri dish of 10cm diameter. Ten milliliters of water containing 0.5% nigrosine, a water soluble dye was added to the petri dish. The dye solution was to identify complete wetting of the tablet surface. A tablet was carefully placed on the surface of the tissue paper in the petridish at 25^0 C. The time require for water to reach the upper surface of the tablets and to completely wet them was noted as the wetting time. Wetting time was recorded using a stopwatch.[23]

8. Water absorption ratio : Ability of an ODT to disperse greatly depends on the capacity of water sorption. Water absorption ratio, R can be determined by following equation: $R = \frac{Wb - Wa}{Wa} \times 10,$

where Wb and Wa are the weights before and after sorption respectively.[24]

9. In vitro Disintegration test : This is to measure the time required for the tablets to disintegrate into particles under a given set of conditions. The tablet is placed in the basket which contains a mesh with an aperture of diameter 2mm (# 10). This basket is suspended in the disintegration media and moved up and down at the rate of 28 to 32 cycles per minute with 50 to 60 mm distance from bottom and top. Media of 0.1N HCl or simulated gastric fluid (pH 4.5 buffer) or simulated intestinal fluid (pH 6.8 buffer) of 900 mL at 37 ± 2^0 C is selected, although the drug release is expected in the stomach. The time at which the tablet starts disintegrating is noted and the time taken for the complete disintegration of tablets is noted down. If 1 or 2 tablets fail, repeat for 12 tablets.[25] Oro dispersible tablets must disintegrate within 1 minute.

It is interesting to note that texture analyzer is also used to perform disintegration but gained minor significance by Abdelbary *et al*.[26]. They have used texture analyser TAXT2i, RHEO, France for determination of in vitro disintegration test by calibrating with 5 Kg load. Briefly this is composed of an elastic spring that maintains the suspension of the movable cylindrical platform (with a perforated gird) from the base of the recipient. The tablet after keeping on the gird is completely dry and is not in contact with the disintegration medium. The probe descends until a trigger force is detected where it gets in contact with the tablet placed on the grid and pushes the whole system downwards, where the elastic spring contracts. Hence, the tablet touches the medium and starts disintegrating. At this point, the TA apparatus is set to maintain a predetermined nominal force (50 g) for a given period of time (60 s). The TA measures the penetration distance as the tablet is compressed while submerged in the medium and. time–distance profiles generated by the texture analysis software are obtained, thus enabling the calculation of the starting and ending disintegration times.

10. Oral disintegration test : Oral disintegration time was assessed by randomly administering the tablets in healthy volunteers. The volunteers were asked to rinse their mouth thoroughly before the test. Tablets were placed on the tongue and a chronometer was started immediately. They were asked to produce a tumbling motion without biting the tablet. The time at which the last noticeable granule has disintegrated was noted and the chronometer was stopped. The volunteers were asked to wash their mouths and again the same experiment was repeated. The average of triplicate measurements represented an individual oral disintegration time. For each rapid disintegrating tablet (RDT) examined, the mean oral disintegration time was calculated as well as the standard deviation and the coefficient of variance should be employed.[27]

11. In-vitro Dissolution : The drug release can be studied using USP type II apparatus at 37 ± 0.5^0C , 50 rpm (to mimic GI motility) using 900 ml of phosphate buffer pH 7.4 as dissolution medium. Aliquot quantity of the sample solution was withdrawn at predetermined time intervals and equal amount of the fresh dissolution medium was replaced. The withdrawn samples were filtered, diluted suitably and analyzed so that percentage drug dissolved can be calculated.[28]

12. Stability study : Reports show accelerated stability study was carried out at 40 ± 2^0C in a humidity chamber having 75% RH for 1 month to 6 months (ICH guidelines) or some times accelerated stability studies based on *Arrhenius method* by subjecting to elevated temperatures. Some investigators have also taken Micro Particles, Minimatrices, and Microspheres into account which will be an integral part of a tablet.

CONCLUSION

Our review points out that ODT's are the best and most preferable type of dosage forms which offers high patient compliance, better safety and efficacy of the drug when compared with other conventional dosage forms which comes only as tablets and not as any other formulations. Also these afford more suitability for pediatrics and geriatrics who has difficulty in swallowing the tablets and also for people who do not carry water with them. It will be more advantageous for the researchers to formulate the marketed products in the form of ODT's rather than going for new formulations. After all one can expect the outcome of more novel technologies for ODT's in the future days.

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A validated HPTLC method for the estimation of flavonoid in the roots of *Aegle marmelos*

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Received Date : 31.07.2013 Accepted Date : 27.09.2013

ABSTRACT

Aegle marmelos (L) Correa., commonly known as bael, is widely used herb in Ayurveda. In the present study, densitometric method has been developed for the validation of rutin content present in the roots of *Aegle marmelos*. Compounds were separated from the ethyl acetate and methanolic extracts of the plant, by analyzing on silica gel $60F_{254}$ plate using ethyl acetate -acetic acid- formic acid -water, 100:11:11:26 (v/v) as mobile phase. Detection by measurement of absorption by 254 nm. The Rf value was found to be 0.35. The Rutin content of the extracts were calculated statistically, comparing with Rutin standard. The developed methods were found to be precise and accurate. The linear range of the method was 400 -1000 ng per band. The amount of Rutin content in ethyl acetate and methanol extracts were ranging from 0.32 - 0.54 and 0.12-0.28 mg per gm respectively. This technique will be used for routine standardization of the Rutin content of *Aegle marmelos* extracts.

INTRODUCTION

The roots of *Aegle marmelos* (L) Correa., commonly known as Bael belonging to the family Rutaceae used in Ayurvedic system of medicine since antiquity. This plant grows wild in the sub-Himalayan tract, central and southern India[1].

Various reports indicate, the methanolic extracts from the root of *Aegle marmelos* inhibited the beating rate by approximately 50% of cultured mouse myocardial cells[2]. Recent studies have revealed potential health benefits of Aegle such as antibacterial[3], anti ulcer[4], anti allergic[5] and as an effective antidiarrhoeal agent[6]. Alcoholic extracts of the roots and fruits showed hypoglycemic activity[7] and the stem bark able to inhibit the in vitro proliferation of human tumor cell lines [8].

In *Aegle marmelos*, the bioactive compounds are reported to be coumarins[1, 5], alkaloids[1, 8], and sterols[3,4]. Recently, the concept of marker-based standardization of herbal drugs is gaining momentum. Identification of major and unique compounds in herbs as markers and development of analytical methodologies for monitoring them are the key steps involved in marker-based standardization. Being the major active principles largely responsible for bio-potency of many crude drugs, flavonoids are recognized as one of the marker compound. There are some reports on the presence of total phenolics and flavonoids in roots were estimated through colorimetric technique[9], but attempt to validate the Rutin content of *Aegle marmelos* are not available. Hence, in this study, a simple, rapid and sensitive HPTLC method has been described to quantify Rutin content in the roots of *Aegle marmelos*.

2. Experimental

2.1. Reagents, standard solutions, and materials

Solvents used were n-hexane, chloroform, ethyl acetate and methanol which were analytical grade and obtained from Qualigens. Flavonoid standard Rutin was purchased from Himedia.

Stock solution (2mg mL^{-1}) of the standard was prepared daily in methanol immediately before use.

2.2 Plant material

The roots of *Aegle marmelos* were collected in the month of April 2010 and further processed. The drug was authentified by Botanical Survey of India BSI/ SRC/5/23/669. Voucher specimens (No: 06) were deposited at PSG college of Pharmacy, Coimbatore, Tamilnadu, India.

2.3 Extraction procedure

Sample of coarsely powdered roots of *Aegle marmelos* were extracted with successive solvents with increasing polarity which were n-hexane, chloroform, ethyl acetate and methanol by cold maceration technique. The successive extracts obtained were distilled off from the solvents and water soluble extracts (ethyl acetate and methanol) were lyophilized.

2.3.1 Sample preparation

The lyophilized extracts were weighed and re-extracted with methanol for sampling in HPTLC.

2.4 Thinlayer chromatography

TLC was performed on Silica gel 60F254 TLC plates (E Merck, Germany) with ethyl acetate -acetic acid-formic acid -water, 100:11:11:26 (v/v) as mobile phase[10] sample and standard at different aliquots of 1,2,3,4,5 µl were applied to the plates as 5 mm wide from the bottom, by means of pressurized nitrogen gas (150 kg/ cm^2) through CAMAG Linomat V fitted with a 100 µl syringe.

Ascending development, with the mobile phase consisting of solvent was performed in a twin –trough glass chamber (10x10cm) obtained from CAMAG, with previously saturated mobile phase for 30 minutes at room temperature (25 ± 2° C) and relatively humidity (60 ± 5%).Subsequent to the development, the TLC plates were dried in air flow.

Plates were then scanned at 254 nm (deuterium lamp) with the CAMAG TLC scanner 3 (slit dimension: 3mm x 0.45mm, scanning speed: 20mm s^{-1}). The Peak areas & peak heights were recorded. The calibration curve for Rutin was obtained by plotting the peak heights Vs concentrations and peak areas versus applied concentrations.

2.5 Analytical method validation

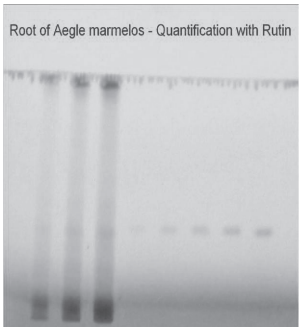
The method was validated for linearity and range, accuracy and precision, Specificity, limit of detection (LOD) and limit of quantification, in accordance with ICH guidelines[11].

Linearity was assessed by applying different volumes (1-4µL per band) of the standard stock solution (between 200 and 1000ng L^{-1}, k=3) three times at the lowest and highest concentration.

The precision of the method was determined by analyzing standard solutions in triplicate at three concentrations on the same day and on different days for inter day and intra day precision respectively. Precision was expressed as SD and RSD of the series measurements.

2.5.1 Reproducibility

To perform the reproducibility of the assay, three independently prepared samples was analyzed every 3 hr within 24 hr at room temperature.



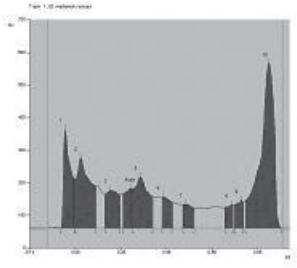


Fig - 1

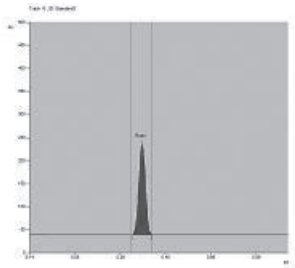


Fig - 2

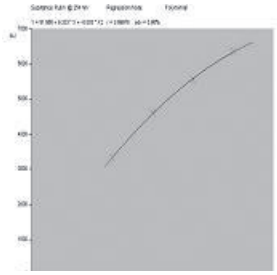


Fig - 3

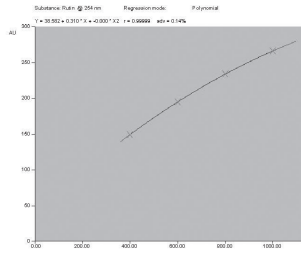


Fig - 4

3. Results and discussion

In order to quantify Rutin in *Aegle marmelos* extracts, the TLC procedure was optimized. The mobile phase consisting of ethyl acetate-acetic acid-formic acid-water (100:11:11:26,v/v/v/v) gave a sharp, thin, and well defined peak at Rf = 0.35 (Picture :1, Figure:2)

The developed HPTLC method showed a good correlation coefficient (r^2 =0.9995 ±0.0001) in the concentration range 200-1000 ng /spot with respect to peak area. Measurement of peak height and peak area(Figure:3,Figure :4)at four different concentration levels showed low values of SD for intra and inter day precision of the whole method was found and tabulated in Table :1.

Table : 1 - Intra and inter day precision of HPTLC method (n=3)

Amount (ng/spot)	Mean area	Intra day precision		Mean area	Inter day precision	
		SD	%RSD		SD	%RSD
400	3311.90	1.42	0.14	3308.28	1.63	0.16
600	4605.01	1.84	0.18	4618.06	2.12	0.21
800	5545.79	2.04	0.20	5548.12	2.51	0.25

The TLC results showed the presence of free Rutin in the ethyl acetate and methanolic extracts obtained from roots of *Aegle marmelos* in comparison with authentic sample (Fig.1). There have been no previous reports of Rutin in *Aegle marmelos* extracts using the same TLC method.

According to chromatography in a thin layer of silica gel, spots in the ethyl acetate and methanol extracts of *Aegle marmelos* have similar colour to the standard of Rutin, and the retention factors(Rf) are 0.35, which are also the same as the standards. The quantity of the Rutin present in the ethyl acetate extract and methanolic extract were compared and found that ethyl acetate extract contains higher quantity of Rutin ie 0.32-0.54 mg per gm than that of methanolic extract which have 0.12-0.28 mg per gm tabulated in Table 2.

S.No	Method property	Value
1.	Linearity Range (ng/band)	400-1000
2.	Correlation coefficient (r)	0.99978
3.	Limit of detection(ng/spot)	80
4.	Amount of Rutin present in the EtoAc extract (mg/gm) Methanolic extract (result of multiple analysis)	0.32 - 0.54 0.12 - 0.28

4. Conclusion

Compared to the other techniques, HPTLC is more convenient for high sample throughput and thus adopted for quality control. The performance data of the validation showed that the HPTLC method is highly suited for quantification of Rutin. Furthermore, the proposed method can be extended to study further qualitative and quantitative analysis under nonspecific markers as per WHO guidelines[12].

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Prevalence of Cancer In Malwa Area of Punjab

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Received Date : 08.08.2013 Accepted Date : 21.11.2013

ABSTRACT

The objectives of our study was to identify individuals showing the warning sign /symptom of cancer, to locate &find the number of already diagnosed existing cancer cases and to determine the number of death due to cancer in the last five years. Door to door survey was conducted among the four districts of Malwa area including Faridkot, Ferozepur, Moga and Ludhiana. Based on the survey Moga was observed at higher risk of cancer cases. Moga has higher incidences of suspected, confirmed and death cases of cancer as compared to other districts. Major cause of cancer being the indiscriminate use of pestcides, consumption of tobacco, alcohol and unsafe drinking water. Hence provisions should be made to avoid use of pesticides and provide safe drinking water to the population.

Key words : Malwa, Cancer, Deaths

INTRODUCTION

Punjab is one of India's most prosperous states and this prosperity has been largely due to its success in the every culture especially in green revolution. But unfortunately this cause of prosperity has changed the face of Punjab. Malwa area of Punjab is known as Cancer Bowl of country (SHSRC)[1]. The incidence of cancer is much more in Punjab as compared to other states of India. To aware the people's of Punjab about the cancer and its severity a train has been started from "Bathinda to Bikaner" and has been designated as "Cancer Train".

Cancer has become one of the ten leading cause of death in developed nation. Cancer in uncontrolled multiplication and spread of abnormal form of body's own cells[2]. It is estimated that of ten million new cases of cancer diagnosed every year, over half are from the developing world[3] . National Cancer Registration Programme indicate that the leading sites of cancer are oral cavities and lungs in males and cervix and breast in females which accounts for over 50% of all cancerous deaths in India (NCCP)[3].

METHOD

Survey Technique; Door to door survey was done in the four districts of Malwa region. Cross sectional study was conducted by the pre-designed and pre-evaluation questions. Standard questionnaire as established by govt. of Punjab was used and it was associated with interview. Questionnaire was printed in two languages Punjabi and English. Using the village profile Performa the information was recorded about village population, cropping pattern, water sources. Information was gathered if there was any member in the household who had signs and symptoms of cancer.

Sampling was random and no inclusion-exclusion criteria was maintained as the survey was conducted an almost whole population.

Study Tools; A detailed district wise environmental profile of the area was prepared. Information was gathered related to the source of water, tobacco use, use of pesticide in last 2 years. It also included inquiries on symptoms of cancer, diabetes, heart diseases and asthma among family members of household's deaths in households during last 2 years. For any suspected case of cancer in the household, a detailed history of symptoms, signs, investigation, and treatment was taken, which was reviewed by two physicians to diagnose cancer cases.

RESULTS:

Prevalence of suspected cancer cases is much higher in Moga as compared to other districts. There are 0.2% deaths, 0.9% suspected cases and 0.3% confirmed cancer cases in Moga .and 0.01% deaths ,0.07% suspected cases,0.05%confirmed cases in ludhiana and 0.04% deaths,0.189% suspected cases,0.03% confirmed cases in faridkot.and 0.03% deaths and 0.02%Suspected cases and 0.025% confirmed cases in ferozepur as shown in table 1.

Uterus (16 out of 142) and breast (37 out of 142) are the main sites of cancer in case of females while mouth and esophagus cancer is mostly seen in case of males (Table2). In Faridkot most f the cancer patients are in the age group of 30-44 (Table 3).

Unhygienic drinking water, handling with pesticides use of tobacco and consuming alcohol as shown in table 4 can be the leading causes of cancer.

TABLE NO. 1: Distribution of cancer morbidity and mortality in four districts of Malwa area

Parameters/District	Faridkot	Ferozepur	Moga	Ludhiana
(a) Dist.surveyed : 4	10570	12211	647	17849
(b) Household	91181	175729	3254	81280
(c) population				
Gender distribution	51045	94586	1718	43582
(a) Male	40136	81143	1536	37698
(b) Female				
c) Age distribution	30257	55838	1360	39605
<30	28403	45078	724	24588
30-44	17670	50270	464	10508
45-59	14851	24546	406	6579
>60				
(d) Deaths reported in last 10 years	4	65	6	15
(e) suspected cancer cases	173	45	31	56
(f) histological confirmed cases	28	41	12	41

TABLE NO. 2: Most common sites of cancer

	Districts							
	Faridkot (N=28)		Ferozepur (N=45)		Moga (N=12)		Ludhiana (N=56)	
Cancer type	Male	Females	Males	Females	Males	Females	Males	Females
Uterus	0	5	0	4	0	1	0	6
Colon/Rectum	1	1	2	1	0	0	3	3
Mouth/Tongue	1	0	0	0	1	1	4	1
Kidney	3	0	2	1	0	1	0	3
Oesophagus	3	2	6	0	0	2	4	1
Blood	1	1	2	2	0	1	0	1

Breast	0	2	0	10	0	0	0	14
Skin	0	2	3	3	0	0	0	1
Liver/Gall bladder	2	3	3	2	1	1	3	2
Brain	0	0	1	1	0	0	3	2
Any other	1	0	0	2	1	2	3	2

TABLE NO. 3: Age and sex distribution of suspected cases in study

Age	CASES (N=173) Faridkot		CASES (N=45) Ferozepur		CASES (N=31) Moga		CASES (N=56) Ludhiana	
	M	F	M	F	M	F	M	F
<30	11	23	3	6	2	2	2	6
30-44	17	50	4	0	0	11	4	11
45-59	3	27	8	12	2	6	9	12
>60	14	28	6	6	3	5	8	4
TOTAL	45	128	21	24	7	24	23	33

Discussion:

Most of the suspected cases of cancer were found in Moga(0.9%) followed by Faridkot(0.189), Ludhiana (0.07) and Ferozepur (0.02). This shows that Moga is at higher risk of cancer as the number of deaths and confirmed cases are also higher in Moga as compared to the other districts.

It was observed that most of the risk factors like participation in cultivation, usage of pesticides, spraying and storage of pesticides, consumption of alcohol, tobacco and unhygienic water are the main causes of cancer. Cancer of female reproductive system (uterus and breast) is more common in females. Previous

TABLE NO.4: Pattern of usage of drinking water and other risk factor among cases and control

VARIABLES	CASES (N=173) Faridkot	Control (N=240) Faridkot	CASES (N=45) Ferozepur	Control (N=65) Ferozepur	CASES (N=31) Moga	Control (N=40) Moga	CASES (N=56) Ludhiana	Control (N=70) Ludhiana
(A) Source of drinking water-								
a) Tube well	40	0	3	1	13	0	3	0
b) Tap water	28	3	21	2	10	2	30	5
c) Hand pump	100	0	21	2	8	1	23	1
(B) Tobacco Uses-								
a) Smokers	0	1	2	0	0	0	2	1
b) Chewing tobacco	4	0	0	0	2	0	1	0
(C) Drinking habits								
a) Alcohol drinking	9	2	5	1	2	1	6	2
(D) use of pesticide								
a) urea	071	21	026	12	020	09	016	05
b) Any other	101	20	004	02	000	01	025	03
c) None	001	00	015	03	010	02	015	02

surveys[4] conducted in Punjab also showed that females are at higher risk of reproductive cancer. In case of males mouth and esophagi are the leading sites of cancer[5]. The cause can be the consumption of tobacco and alcohol. Tobacco has been reported as one of the leading cause of lung and esophageal cancer while consumption of tobacco and alcohol shows synergistic effects[6,7]. Lung cancer has been the leading site of cancer in Delhi, Mumbai, and Bhopal while second and third leading sites in Bangalore and Chennai respectively as per the Atlas of cancer in India.

Alcohol alone is one of the major reasons of liver cancer[8]. Heavy drinking can lead to cirrhosis, a condition where the liver is repeatedly damaged and scar tissue builds up. A review of evidence in 2012 concluded that having 1 drink a day (around 1.5 units) could increase the risk of breast cancer by 5%[9] and the risk increases in case of woman drinkers. Several studies have found that each additional 10g of alcohol drunk a day increases the risk by about 7-10%[10, 11, 12.]

Usage and storage of pesticides are also observed as one of the reasons of cancer. Punjab being the state of green revolution, farming is the one most important mode of earning, so the higher incidences of pest control. Although usage of urea has been reported by many cultivators but there is use of other pesticides like heptachlor, Ethion and formaldehyde. Moreover, containers of pesticides were used for the storage of food items. There are reports of wide spread contamination of diet, liquid milk and butter with pesticide residues from Punjab[13, 14, 15.] A study conducted in cotton growing area of Bathinda to see the chronic effects of pesticides among rural children on the ability to perform developmental task has shown that in more than 80% children exposed to pesticides performed significantely worse than the less exposed children of Anandpur Sahib of Ropar[16] .

Other reason for the cause of cancer was found to be drinking of unhygienic and unsafe water. Water of Malwa area has been reported with the presence of heavy metals like arsenic (0.015ppm), selenium (0.09ppm) and mercury (0.004ppm) which are above the permissible limits[3]. A study carried out in Orsk city of Russia found that the high carcinogenic risk is associated with arsenic in water[17]. other studies also support the increased risk of cancer associated with presence of arsenic in drinking water[18,19.]

In conclusion the cancer cases and deaths are higher in Moga due to the cocktail of risk factors which were more common use of tobacco and alcohol, consumption of high levels of heavy metals in water and excessive pesticide use. It is difficult to know the single cause of cancer in the area, therefore, a multipronged strategy to provide safe water supply, discouraging the indiscriminate use of pesticides, tobacco and alcohol is recommended.

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RESEARCH ARTICLE

Study of prescription pattern of antihypertensive medications in Type - 2 Diabetic patients

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Received Date : 26.08.2013 Accepted Date : 29.10.2013

ABSTRACT

Hypertension is highly prevalent in diabetic patients and its control is very important to prevent the complications. Various classes of drugs are available in the market to treat Hypertension. A cross sectional study was conducted prospectively to observe prescription pattern of antihypertensive medications in diabetic patients in a tertiary care hospital in south India. A total of 735 Diabetes Mellitus patients were admitted from Jan to Oct 2008. 49% of the total diabetic patients had hypertension. Majority of patients were in the age group of 60-69 years and male predominance was observed. Monotherapy was commonly prescribed to about 52.2% of the patients. Among monotherapy, calcium channel blockers (40.4%) were the mostly prescribed drug class. Out of the calcium channel blockers, amlodipine (93.4%) was the most prescribed drug. In dual drug therapy, combination of calcium channel blockers and angiotensin converting enzyme inhibitors were the most prescribed drugs in about 19.2% patients. Antihypertensive therapy for patients with complications and those with other co morbidities along with hypertension and diabetes, calcium channel blockers were the mostly prescribed class of drugs except for patients with Ischemic heart disease. Calcium channel blockers are the most commonly prescribed drug in diabetic hypertensives.

Key words : *Hypertension, diabetes, prescription pattern, calcium channel blockers*

INTRODUCTION

Diabetes mellitus (DM) comprises a genetically and clinically heterogeneous group of chronic metabolic disorders that are characterized by hyperglycemia.[1] Hypertension in diabetes is a widespread, substantial, and treatable cardiovascular risk factor. The prevalence of hypertension in the diabetic population is 1.5–3 times higher than that of nondiabetic age-matched groups. Hypertension ultimately affects approximately 20–60% of patients with type 2 DM, depending on age, ethnicity, and obesity.[2]

The Joint National Committee (JNC) VII guidelines for treating hypertension are consistent with recommendations from the American Diabetes Association (ADA) and the World Health Organization (WHO). Recommended therapy for Hypertension with Diabetes should begin with an Angiotensin-converting enzyme (ACE) inhibitor or an AngiotensionII receptor blockers (ARBs), titrated upward as needed. If Blood Pressure remains above the target level (130/80 mm Hg), either a thiazide diuretic or Calcium Channel Blockers (CCBs) should be added to the treatment regimen.[3]

Kasturba Hospital is a tertiary care university hospital in the west coast of India. Approximately 2000 diabetic patients are treated as inpatients every year. Nearly 50% of these patients present with hypertension along with diabetes, and various classes of drugs are used for treating hypertension. Treatment targets from guidelines cannot always be achieved in everyday clinical practice. Hence it is important to monitor trends in hypertension control in defined population (Type 2 Diabetes), as limited data exists on the patterns of anti-hypertensive use in this population and if they are consistent with current guidelines. Thus there is a need exist for robust data from the specified population through a

systematic review of drug usage. Aim of the present study is to analyse the prescription pattern on the use of anti-hypertensive drugs in type 2 diabetic patients.

Methodology :

A cross-sectional observational study was carried out prospectively among diabetic patients admitted in the medicine wards of tertiary care hospital in coastal Karnataka. The study group consisted of all the Type 2 Diabetes Mellitus patients admitted in various medicine wards during January 2008-October 2008. After the clearance from institutional ethics committee and those who met the following criteria were included in the study.1) Patients admitted in the Medicine wards who are diagnosed to have type 2 diabetes with hypertension as per International Statistical Classification of Disease and related health problems (ICD) diagnostic criteria irrespective of age and sex. 2) Diabetic hypertensive that had other co morbidities were also included in the study. Diabetic patients who were admitted in the wards other than medical wards and patients who were discharged against medical advice were excluded. Medication chart of each patient was reviewed to obtain prescription pattern of hypertension in diabetic patients, and assessment of prescription pattern of hypertension in diabetic patients along with their co morbidities and complications. On the basis of therapy, patients were categorized to those on monotherapy, dual therapy and multi drug regimen. Drug combinations of dual and multi drug therapy were further evaluated to determine the types and combination of different classes of antihypertensive used.

Results :

Out of the total 735 Diabetic patients, 360(49%) patients had hypertension. Among the 360 patients, 219(61%) were males and 141(39%) were females. About 200(55.5%) patients out of 360 patients were classified as elderly hypertensive (age ≥ 60 years). Average age of diabetic patients with hypertension was found to be 61.1 ± 10 years. Only 310(86%) of these 360 patients gave previous history of hypertension and 128(41.3%) had hypertension for less than 5 years. Others were unaware of hypertension.

At admission mean systolic BP was found to be 134.4±19 mmHg and Mean diastolic BP was 83.1±10.4 mmHg. All demographic characteristics are represented in the Table 1.

Table.1 : Demographic Characteristics of Diabetes with hypertensive patients

Gender wise distribution (N=360) :							
N=360	Male			Female			
	219(61%)			141(39%)			
Age group-wise distribution(N=360) ;							
Age groups in Years	20-29	30-39	40-49	50-59	60-69	70-79	80-89
No (%)	0	2(0.6)	42(11.6)	116(32.2)	121(33.6)	64(17.8)	15(4.2)
Duration of hypertension(N=310) :							
History of HTN in years	Newly diagnosed			1-5	6-10	≥10	
No (%)	57(18.39)			128(41.3)	69(22.25)	56(18.06)	

Management of hypertension in diabetic patients :

Of the 360 diabetic patients with hypertension, 337(94%) patients were administered pharmacological treatment and 23(6%) patients were given non-pharmacological treatment with lifestyle modification, salt restrictions etc. Out of 337 patients on medications, 188(55.78%) were on Monotherapy, 104(30.86%) were on dual therapy, 37(10.97%) were on triple drug regimen and 8(2.37%) patients were on four drugs.

Monotherapy :

In patients on monotherapy 188(55.78%), calcium channel blockers (CCB) were the most prescribed drug in 76(40.4%) patients, followed by Angiotensin Receptor blockers (ARB) in 45(23.9%) patients, Angiotensin converting enzyme inhibitors (ACEI) in 37(19.6%) and Beta Blocker (BB) in 26(13.8%) . 1(0.5%) patient each was on alpha 2 agonist clonidine and diuretic indopamide. Two (1%) patients were on alpha1 blocker control release prazosin. Amlodipine was the most commonly prescribed CCBs among 71 (93.4%) patients. Details are shown Table 2

Table2: Individual Drug Use Pattern in Monotherapy (n=188) with Antihypertensive

THERAPY	NO:OF PATIENTS(percentage) n=188
CCB	**76(40.4)**
Amlodipine	71(93.4)
Diltiazem	3(4)
Nifedipine	2(2.6)
ARB	**45(23.9)**
Losartan	36(80)
Telmisartan	9(20)
ACEI	**37(19.6)**
Enalapril	21(56.75)
Ramipril	14(37.8)
Lisinopril	2(5.4)
BB	**26(13.8)**
Atenolol	12(46.15)
Metoprolol	11(42.3)
Nebivolol	2(7.7)
Carvidilol	1(3.8)
Alpha 1 blocker(prazocin)	**2(1)**
Alpha 2 agonist(clonidine)	**1(0.5)**
Diuretic(Indopamide)	**1(0.5)**

Dual Therapy :

Out of 104 patients who were on dual therapy, the most commonly prescribed combination was ACEI and CCB in 20(19.2%) patients, of them 10(50%) patients were on combination of ramipril and amlodipine. 16 (15.3%) patients were on a combination of BB and CCB, of them 8(50%) were on atenolol and amlodipine. ARB + CCB were given for 13(12.5%) patients followed by BB and ACEI combination in 11 (10.55%) patients. Other combinations in dual therapy are explained in detail in the table 3.

Table.3 : Pattern of drug use combinations in Dual Therapy

Combination Therapy	No: of patients(percentage)n=104
ACEI+CCB	20(19.2)
CCB+BB	16(15.4)
ARB+CCB	13(12.5)
BB+ACEI	11(10.6)
BB+D	7(6.7)
ARB+D	6(5.7)
ARB+BB	5(4.8)
CCB+AA	5(4.8)
ACEI+D	4(3.8)
CCB+D	4(3.8)
AB+D	2(1.9)
AB+ACEI	2(1.9)
AB+BB	1(0.96)
AA+D	2(1.9)
AA+ARB	1(0.96)
AA+CCB	1(0.96)
AA+ACEI	1(0.96)
ARB+ACEI	1(0.96)
D+D	1(0.96)
CCB+CCB	1(0.96)

(ACEI-Angitensin converting enzyme inhibitors, CCB-Calcium Channel Blockers, BB - Beta blocker, ARB - angitension II receptor Blocker, D-Diuretic, AA - Alpha 2 agonist, AB - Alpha 1 blocker

Triple drug combinations :

Out of 337 patients, only 37 patients are on three drug combination regimen. Details are shown in table 4

Table 4: Patients on Three Drug Regimen

Combination Therapy	No: of patients(percentage) N=37
ACEI+BB+D	5(13.5)
ACEI+2D	3(8.1)
BB+CCB+D	3(8.1)
D+CCB+AA	2(5.4)
ACEI+CCB+BB	2(5.4)
ARB+CCB+D	2(5.4)
ARB+BB+D	2(5.4)
ARB+CCB+BB	2(5.4)
AA+AB+CCB	2(5.4)
2D+AA	1(2.7)
2D+BB	1(2.7)
2D+ACEI	1(2.7)
2CCB+D	1(2.7)
3D	1(2.7)
ACEI+CCB+D	1(2.7)
AA+BB+D	1(2.7)
AA+CCB+ACEI	1(2.7)
CCB+ARB+AA	1(2.7)
CCB+BB+AA	1(2.7)
CCB+BB+ACEI	1(2.7)
ARB+2BB	1(2.7)
BB+2CCB	1(2.7)
BB+CCB+ARB	1(2.7)

(ACEI-Angitensin converting enzyme inhibitors, CCB - Calcium Channel Blockers, BB - Beta blocker, D - Diuretic, AA - Alpha 2 agonist, AB - Alpha 1 blocker)

Four drug combinations :

Among 337 patients only, 8 patients are on four drug regimen details are shown in table 5.

Table 5 : Patients on Four Drug Regimen

Combination therapy	No: of patients N=8
CCB+AB+BB+D	1(12.5%)
ARB+ACEI+CCB+AA	1(12.5%)
BB+AB+CCB+D	1(12.5%)
ACEI+D+BB+AA	1(12.5%)
BB+ACEI+D+CCB	1(12.5%)
BB+ARB+D+AA	1(12.5%)
BB+CCB+AB+D	1(12.5%)
BB+CCB+ACEI+D	1(12.5%)

(ACEI-Angiotensin converting enzyme inhibitors, CCB - Calcium Channel Blockers, BB - Beta blocker, D - Diuretic, AA - Alpha 2 agonist, AB - Alpha 1 blocker)

Pattern of Prescription of Antihypertensive with other Existing Co morbidities and Complications :

Out of the total 735 Diabetic patients, 360 patients had hypertension and diabetes. Among this 360 patients, 163(45.3%) patients were had other comorbidities and diabetic complications which required specific choice of class of antihypertensive. 21(12.9%) patients had Bronchial asthma(BA), 8(4.9%) patients had chronic obstructive pulmonary disorder(COPD), 22 (13.5%) patients had Nephropathy, 14(8.6%) patients had Anemia ,5(3%) Patients had Rheumatoid arthritis (RA), 70 (43%) patients had Ischemic heart disease (IHD) and 23(14.1%) patients had Hyperlipidemia (HLD).

On analysis of the overall use of antihypertensive drugs in patients with other co morbidities along with diabetes and hypertension, calcium channel blocker was found to be the mostly prescribed class of drugs, specifically amlodipine, as monotherapy and in combination. Whereas in Ischemic heart disease (70 patients), Beta blocker was the commonly used drug class in about 30 (42.85%) patients, mainly metoprolol in 19(63.3%) patients followed by Angiotensin converting enzyme inhibitors in 25(35.71%) patients with ramipril being the mostly prescribed drug among this class in 16(64%) patients. The use of CCBs in these groups of patients is shown in Table 6.

Table 6: Use of CCBs as Antihypertensive in Diabetes along with various Co morbidities and Complications

Co morbidity and complications	No: of patients N=163	Percentage of patients on CCB
BA	21	85.71%
COPD	8	62.5%
Nephropathy	22	86.36%
Anemia	14	48.38%
RA	5	20%
IHD	70	32.85%
HLD	23	34.78%

Overall Drug Utilization

In the overall drug utilization pattern, calcium channel blockers were prescribed in majority (48.3%) of patients among 337 patients of diabetes with hypertension who received pharmacological treatment. Mainly amlodipine was prescribed in 147(43.6%) patient, followed by ARBs (Losartan -19.6%), BB (metaprolol-11%, atenolol-11%), ACEI(enalapril-10%) and Diuretics(frusemide-5.6%).

Discussion :

The purpose of the study was to evaluate the prescription pattern of antihypertensive in Type 2 diabetic patients with hypertension admitted in the medicine wards of a tertiary care hospital in South Karnataka. One of the major observations of the study was 49% of the Type 2 diabetic patients were found to have hypertension. This finding is consistent with the data revealed by one of the study conducted at the same study site which showing about 40% of diabetics to be hypertensive and other studies4,5 which showed that in India about 50% of diabetics have hypertension.

With regard to the demography of the Type 2 diabetic patients with hypertension, majority of the patients (83.6%) were falling under the age group of 50-79 years which shows the predominance of elderly population. Average age of patients with both Diabetes and Hypertension was found to be 61.1+10 years of age. This observation was consistent with the result of the study done by David6 wherein the Prevalence of hypertension in diabetes was mostly seen within a range of 60+12 years.

Gender wise distribution showed a male predominance (61%) than females, which is supported by one study conducted by Johnson & Singh (2005)[7] In the current study the number of men admitted are more compared to women in the hospital during the study period, this might have also led to such finding. Whereas in majority of the studies conducted female predominance was shown in diabetic patients with hypertension.[8,9]

In the present study, 41.3% of the hypertensive patient had a history of hypertension for 1-5 years. In the study conducted by Waleed et al (2009)[9] it was observed that the duration of history of hypertension was 7.2+7.5 years.

The patients who were diagnosed to have hypertension with diabetes were grouped in to different stages as per JNC VII guidelines, It was observed that majority of the patients were in the pre-hypertensive stage (37%). This observation is consistent with the observation of a study conducted by Sipahi et al (2006)5 in which majority of patients (57.3%) were in Pre-Hypertensive stage. This might be because most of the patients have a controlled blood pressure with drugs, along with lifestyle modifications. Mean systolic BP was found to be 134.4+19mmHg and the mean diastolic BP was 83.1+10.4mmHg .These finding were consistent with the study carried out by Azimi-Nezhad et al (2008)[10] wherein the mean systolic BP was found to be 135.53 ± 21.64mmHg, and the mean diastolic BP was found to be 87.08 ± 15.36mmHg at the time of admission. In the present study, it was observed that JNC VII guidelines are strictly followed in treatment of patients as the practicing physicians follow their based on their experience with patients.

Therapy was studied in detail focusing on the prescription pattern of different class of antihypertensive and the individual agents among these classes. In the present study, patients were mainly put on monotherapy. This observation was in accordance with the observation of the study carried out by Jhaj et al (2001)[11] wherein majority (48.8%)of patients were put on monotherapy followed 41.8% of the patients being given two or more drugs. In our study, the mostly prescribed drug class was calcium channel blockers in 40.4% patients which are supported by a study conducted by Odili et al (2008)[12] in which 31% of patients were on CCBs. It was then followed by Angiotensin receptor blocker in 36(80%) patients, Angiotensin converting enzyme inhibitors in 37(19.6%) and beta blockers in 26(13.8%) patients. CCBs are beneficial in treating hypertension in patients with diabetes as stated by Chobanian et al (2003).[13]

In our study, among the calcium channel blockers, amlodipine was prescribed the maximum in about 93.4% of the patients. Use of amlodipine was supported by a study conducted by Adigun et al (2003)8 in which Amlodipine 2.5–10 mg/day were used in 51% of the cases. As per the standard literature, the available evidences does not suggest that CCBs are harmful in diabetic patients, but it does suggest that they are not as protective (for renal complication) as ACEIs. 23.9% patients in the present study were on ARBs. Even though ARBs can be considered as first-line agents,

they are commonly used as second-line alternatives, especially for patients who develop an ACE inhibitor-induced cough. In this study ARBs are prescribed more commonly than ACEIs. Among the ARBs losartan was commonly prescribed. These agents were found to delay the development of diabetic nephropathy and slow the progression of renal disease, thereby reducing renal complications as supported by a study wherein the effect of losartan on renal and cardiovascular outcomes in Type-2 DM patients was determined (Deferrari et al., 2002).[14]

With regard to Dual therapy, 20(19.2%) patients on dual therapy was put on ACEI+CCB combination, 16 patients were on BB+CCB, 13 patients were on ARB+CCB, 11 patients were on BB+ACEI. The commonly used drugs in these classes are enalapril, amlodipine, losartan, metoprolol and atenolol. Dual therapy was prescribed less compared to monotherapy in this study, might be because majority of the patients were in Pre-hypertensive stage with a controlled BP <140/90mmHg at the time of admission. Combination of drugs is usually preferred when aggressive control on BP is needed as per standard guidelines.

This propensity to use ACEIs and CCBs is in keeping with the trend of increasing use of these drugs; accompanied by a decreasing utilization of β-blockers and diuretics, even in developed countries (Jhaj et al., 2001).[11] Beta-blockers are by and large not recommended in DM, because of their possible adverse effects on glucose metabolism. But in patients with underlying cardiac problems it is used with caution as it is preferable in those patients. Diuretics are preferred as second agents if a combination therapy is indicated, as low dose diuretics are much less likely to produce the metabolic abnormalities like increasing fasting glucose and serum cholesterol values (Saseen et al., 2005).[3]

This combination of ACEI and CCB is supported by the ACCOMPLISH (Avoiding Cardiovascular Events in Combination Therapy in Patients Living with Systolic Hypertension) study. This study was designed to compare the generally recommended combination of an ACEI with a diuretic and the combination of ACEI and CCB. In ACCOMPLISH there was a small, but statistically significant, difference in blood pressure of 0.9 mmHg systolic and 1.1 mmHg diastolic, which favored the ACEI plus CCB combination. Safety and efficacy of amlodipine add-on therapy was shown by ADHT (Amlodipine Diabetic Hypertension Efficacy Response Evaluation Trial) (Kloner et al., 2008).[15]

One of the major observations in the study was that, the control of BP was found to be similar with no much significant difference between the patients who were on Monotherapy (133.5+13.6/83.8+8.5mmHg) as well as on Dual Therapy (132.5+13.3/83.4+9.3mmHg). This shows that irrespective of the number of drugs in the treatment regimen, the target BP was attained since the regimen was planned based on the individual patient need.

With regard to the antihypertensive therapy in patients with complications and other co morbidities along with hypertension and diabetes, CCBs were the mostly prescribed class of drugs. Among calcium channel Blockers amlodipine was found to be the most prescribed individual drug.

The efficacy of calcium channel blockers in reducing blood pressure and the safety when used along with Angiotensin converting enzyme inhibitors or Angiotensin receptor blockers to slow down the progression of diabetic nephropathy is supported by the study conducted by Nathan et al (2005)[16] in which they also showed that CCBs prevent CVD events as effectively as the other antihypertensive drug classes.

Treatment of Bronchial asthma with calcium channel blocker in our study was supported by other studies conducted by Kivity et al (1992)[17], Svedmyr et al (1984)[18] and Jhaj et al (2001).[11] No patient with bronchial asthma and COPD was prescribed a β-blocker as it is contra indicated in this condition (Schwartz et al, 1980).[19]

Patients with IHD were treated with beta-blockers as per the standard guideline. 42.85% patients were given beta-blocker specifically metoprolol in about 63.3% patients but very few patients were on CCBs which is also a drug that is indicated in this condition given by standard literature.

Hyperlipidemia is one of the cardiovascular risk factor, and controlling it is important to the overall care of hypertensive patient. Alpha blockers have been shown to have favorable effects by reducing LDL cholesterol and increasing HDL cholesterol. However data from ALLHAT study revealed that alpha blocker do not

reduce the cardiovascular risk as much as thiazide diuretics though thiazides diuretics and beta blockers may adversely affect serum cholesterol values (Saseen, 2005)[3].Whereas ACEI and Calcium Channel Inhibitors have no effect on serum lipids .Hence they are preferred to be used in patients with hypertension along with hyperlipidemia. In the present study, 34.78% of the patients were on CCBs and all the patients with hyperlipidemia along with hypertension were given amlodipine.

In the present study, it was found that alteration in the prescription of anti hypertensive was not necessary for co morbidities like anemia and rheumatoid arthritis along with DM and hypertension. As per the standard guideline, there is no contraindication of any class of antihypertensive or specific drugs to be used in case of co morbidities like anemia and rheumatoid arthritis along with DM and hypertension. In our study, 48.38% of anemic patients were given CCB and 20% of the patients with rheumatoid arthritis were given CCB. Thus the study revealed that in the overall drug utilization pattern, Calcium channel blockers stood first being the mostly prescribed drug class, followed by ARBs, ACEIs, Diuretics and BBs.

Conclusion

The present study was able to identify the anti-hypertensive drug use in diabetic patients. It is observed that calcium channel blockers are commonly prescribed drug either as Monotherapy or as multiple therapy in patients of diabetic with hypertension without any problem. Monotherapy was observed more for treating hypertension in diabetic patients compared to the combination therapy. Overall JNC VII guideline was not strictly adhered to as the clinicians preferred to design dosage regimen based on their experience and local conditions.

Acknowledgement : Authors would like to acknowledge the all the units of medicine at Kasturba hospital, Manipal for their support.

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RESEARCH ARTICLE

Anti-inflammatory effect of Curcuma longa extract- An *In vitro* and *In vivo* study

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Received Date : 26.09.2013 Accepted Date : 03.12.2013

ABSTRACT

Curcumin is one of the major constituents of Curcuma longa has lots of medicinal values in traditional medicine. This dried rhizome powder extract had been studied extensively in animal models and proved with anti-inflammatory activity and its effectiveness in reducing inflammation in osteoarthritis, rheumatoid arthritis and as an antiproliferative agent in few malignancies.To determine the effect of C. longa extract in histamine induced Bronchospasm in animal model. To compare anti-inflammatory activity and percentage inhibition of fibroblast cell growth of C. longa extract with methyl prednisolone. The dried rhizomes of Curcuma longa were powdered to prepare successive extract as per the standard technique using chloroform and ethanol as solvents adapting Soxhlation procedure. In fibroblast cell growth study 100, 300, 600, 900 and 1200µg of the extract was used. To assess the bronchodilatation 50, 100, 200mg/kg dose of curcumin extract was used. Guinea pigs exposed with three different doses of extract were studied at weekly interval after daily oral administration to prevent the histamine aerosol induced bronchospasm using histamine chamber to observe the occurrence of PCD (Preconvulsive Dyspnea). In connective tissue fibroblast cell culture study after 24h of the drugs (prednisolone or extract) treatment, the cell viability was measured by 10µl of MTT. The intensity of formazan blue formation was measured at 570nm. The IC50 was calculated by using Grapad PRISM software. C. longa extract produces definite connective tissue fibroblast cell growth inhibition when compared to prednisolone and protect animals against histamine induced bronchospasm at the doses level between 100-200mg/day.C. longa extract has a role in chronic inflammation based on the inhibitory role on connective tissue cell fibroblast proliferation when compared with the known established anti-inflammatory agent methyl prednisolone.

Key words : *Curcumin Anti-inflammatory Fibroblast cell*

INTRODUCTION

Curcumin is an active principle obtained from the plant curcuma longa which is not a new therapeutic tool but proved long time back with lots of medicinal values in traditional medicine.[1] The dried rhizome powder extract had been studied extensively in animal models and proved with anti-inflammatory activity.[2] Many human studies had proved its anti-inflammatory action and its effectiveness in reducing inflammation in osteoarthritis and its benefits in rheumatoid arthritis.[3] Because this extract widely used for anti-inflammatory action in arthritis and help in healing a wound we had planned to compare the effectiveness with known anti-inflammatory agent steroid. Many animal and *in vitro* studies express the effectiveness of curcumin shown to promote apoptosis, inhibit telomerase activity in tumor development few malignancies.[4] Many Research reported that yellow pigment curcumin has the ability to modulate the COX-2, inducible nitric oxide synthase (iNOS) to bring out the anti-inflammatory changes.[5] These references strongly reflect the anti- inflammatory potency but no comparison between the strongest anti-inflammatory agent corticosteroids. Hence this study had planned to rule out the potency of C. longa extract in fibroblast cell culture model using methyl prednisolone as a standard anti-inflammatory agent.

Material & Methods

Drug Prepar ation

Locally purchased fresh rhizomes of Curcuma longa, after botanical confirmation allowed to dry at cool place for 2-3months, were powdered to prepare successive extract as per the standard technique using chloroform

and ethanol as solvents adapting Soxhlation procedure. The extract was dried and kept under desiccators for future use. Ethanol has shown good solubility for prednisolone and for curcumin successive extract. 2mg/ml stock was prepared for both the drugs using ethanol. The doses such as 100, 300, 600, 900 and 1200µg/ml were prepared for both the drugs by using growth medium and used *in vitro* cell culture study. To assess the protective effect in histamine induced bronchoconstriction study curcumin extract 50, 100, 200mg/kg dose was used. The drugs were administered as fine suspension prepared in fish oil for better absorption[4] and administered orally in adult guinea pig except for animals in reproductive phase with the body weight of 450-600g.

Fibroblast Cell culture study- L6 cell line (rat myoblast cell line) was obtained from National Centre for Cell Science (NCCS), Pune. It was maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% serum (FBS), amphotericin (3µg/ml), gentamycin (400µg/ml), streptomycin (250µg/ml) and penicillin (250 units/ml) in a carbon dioxide incubator at 5% CO_2. L6 cells show a steady growth rate with a doubling time of 12 to 24 hours.

About 700 cells/well were seeded in 96 well plate using culture medium, for 24hours and allowed to grow using CO_2 incubator, then the viability was tested using trypan blue dye with help of haemocytometer. After 24hrs, based on the doubling time again 95% viability was confirmed with trypan blue dye and with help of haemocytometer. Then new medium with prednisolone and curcumin in the concentration of 100, 300, 600, 900 and 1200µg/ml were added at respective wells and kept incubation for 24hrs.

After 24hrs of the drug treatment the medium was changed again for all groups and 10µl of MTT (5mg/ml stock solution) was added and the plates were incubated for an additional 4h. The medium was discarded and the formazan blue, which was formed in the cells, was dissolved with 50µl of DMSO. The optical density was measured at 570nm. The percentage toxicity was calculated by using following formula.

% Toxicity = 1 - treated cells/ untreated cells x 100

IC_{50} for drugs were calculated using Graphpad PRISM software tool.

Histamine induced Bronchospasm study

Animals- Adult guinea pig both sex with body weight of 450-600 gram was used in the study. The guinea pigs were housed in metal cages. The animals are feed with regular feed and water ad libitum. The animals are maintained at ambient temperature 25±2^{0}C and 55% relative humidity. The Institutional Animal Ethics Committee of PSG & IMSR approved the study and the study was conducted strictly following the guidelines of ICMR, New Delhi.

Control Group : Three Animals were first observed for normal respiratory rate and frequency and depth of respiration as control. The same animals were exposed to histamine induced bronchospasm individually using 2% histamine aerosol under constant pressure with a help of Standard histamine chamber for 2min on day 0 without any pretreatment as control animal. The end point of preconvulsive dyspnea (PCD) will be determined from the time of aerosol exposure to the onset of dyspnea that was leading to the appearance of convulsions.[6] As soon as PCD had commenced, the animals were removed from the chamber and exposed to fresh air. This PCD will be taken as day 0 value and as control.

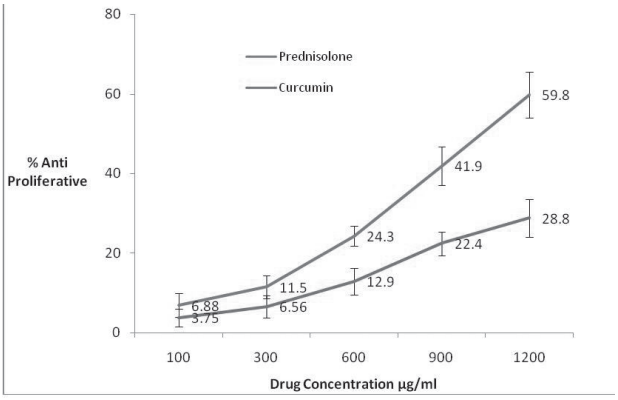
Curcumin group : 6 animals were pretreated orally daily with Curcuma longa successive extract. The extract was administered after 12 hours of starvation, at the volume of 1ml/kg of body weight. All animals were treated initially with 50mg/kg/day as single dose during the first week and were allowed to expose histamine induced bronchospasms similar to that of control group for 2min (120sec) within 24h and the same measured after the first week. During second week all animals were treated with 100mg/kg/day as single dose and exposed to histamine after a week. The same protocol was repeated in third week and all animals were treated with 200mg/kg/day of curcumin as single dose. Animals exhibited with severe bronchospasm in any of the group were immediately exposed to steroid + salbutamol nebulizer solution & normal air using nebulizer to revert the histamine induced lung changes. All animals were observed for a period of 1week in their own environment with their normal water and food after completion of the study for any pathological changes.

Results

Fibroblast Cell culture study

The antiproliferative study of curcumin in fibroblast cell growth has been represented in figure 1. The effect of curcumin was compared to prednisolone as standard drug. In comparison to prednisolone the anti proliferative effect of curcumin was comparative less. The IC50 value of curcumin will be greater than 1200µg/ml. However curcumin exhibited dose dependent growth inhibition of fibroblast cells.

Effect of curcumin in PCD

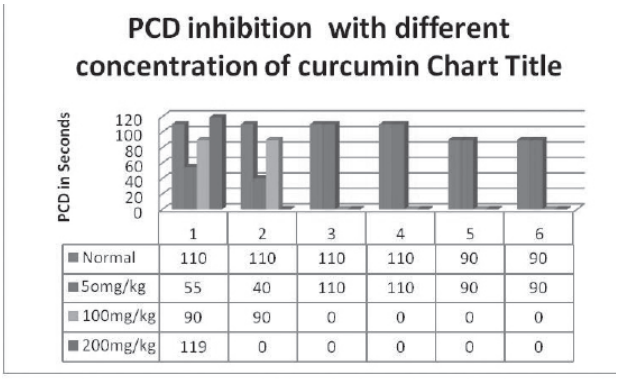


GROUP	PERCENT INHIBITION	S.E.M.	STATISTICAL SIGNIFICANCE
C100	3.75	2.17	* P < 0.05
P100	6.88	2.94	
C300	6.56	2.77	*** P< 0.001
P300	11.5	2.82	
C600	12.9	3.29	*** P< 0.001
P600	24.3	2.51	
C900	22.4	3.01	*** P< 0.001
P900	41.9	4.78	
C1200	28.8	4.79	*** P< 0.001
P1200	59.8	5.78	

HS -*** , S *

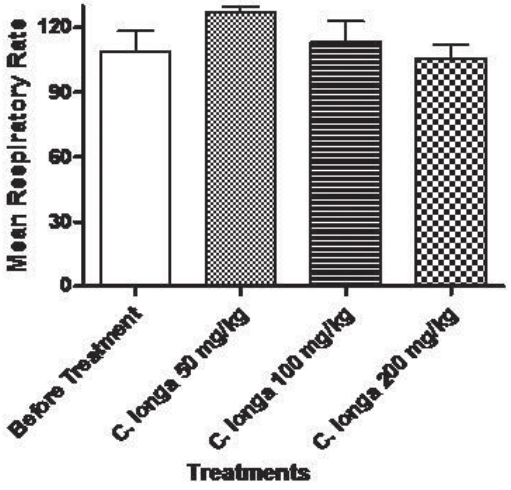
The effect of curcumin extract on the onset of PCD in guinea pigs after 2% histamine aerosol administration is given as percentage protection in figure 2. The results indicate all the control animals exhibited PCD and the onset of action in seconds is in the range of 90 to 118. Similar results were observed with 50mg/kg treated curcumin extract. In the second week sixty seven percent of animals treated with curcumin extract (100mg/kg) have got protected from histamine aerosol induced PCD. The onset of action in these animals was found to be (90-120 seconds). Similarly in the third week animals received 200 mg/kg of curcumin extract exhibited 87% protection and only one animal out of six exhibited PCD symptoms.

Effect of curcumin extract in respiratory rate



Respiratory rate in comparison to control group, curcumin treated animals did not differ significantly and exhibited similar response after 24h and one week of curcumin treatment (Figure 3)

Discussion



In this study doses were selected by dose escalation method. Both C. *longa* and methylprednisolone were not effective in the lowest concentration. Only in the dose range between 100-1500 µg/ml, both the molecules have shown reduction in the cell count. In comparison to methylprednisolone as standard, C. *longa* extract showed 50% anti-inflammatory activity, by reducing fibroblast inhibition in the standard environment provided. In skin injury, the inflammatory cells also produce growth

factors necessary for the production of extra cellular matrix by fibroblasts and produce blood vessels in the healing wound. Initially there is activation of the extrinsic, and later the intrinsic pathway, forming fibrin and its subsequent polymerization into a gel. The main function of the fibroblasts is to maintain the structural integrity of connective tissues by continuously secreting precursors of the extracellular matrix.

The fibroblasts synthesize collagen, elastin, and the proteoglycans of the ground substance of connective tissue8. Many of the fibroblasts bound to the collagen fibers of connective tissue remain sessile for long periods, but in the event of an injury that destroys tissue, they are capable of migrating to the site of injury at a rate of about 1μ/min to participate in the repair of damage. Dividing fibroblasts are rarely seen in normal tissue, but they proliferate in response to tissue damage and become active in the synthesis of the fibrous and amorphous component of connective tissue needed for repair of the injury[7].

Methyl prednisolone is a known anti-inflammatory agent, with inhibitory effects on arachidonic acid synthesis and secondary inflammatory substances like cyclo-oxygenase-2, lipooxygenase, cytokines, TNF-α, IL-1, IL-2, IL-6, IL-8 & IL-12. In cell culture study, both C. longa extract and methyl prednisolone inhibited myoblast cell growth. Curcumin might also act by the same mechanism[9]. However, Curcumin had a different potency, ie at 1200μg/ml and achieved 50-60% effect of methyl prednisolone. Hence, curcumin could be useful in the treatment of clinical conditions associated with chronic inflammatory action. Curcumin suppresses the bronchial smooth muscle cell proliferation and airway wall thickness and partly through the platelet derived growth factor[10]. Whereas, in clinical testing curcumin at 1000 mg twice daily supplementation did not significantly affect post bronchodilator FEV1, ACT scores in atabel permanent asthma patient. Even though *in vitro* evidence showed that Curcumin has anti-inflammatory properties and can inhibit allergic cytokine responses from lymphocytes *in vitro* evidence showed that Curcumin has anti-inflammatory properties and can inhibit allergic cytokine responses from lymphocytes *in vitro*[11]. Whereas, in clinical testing Curcumin at 1000 mg twice daily supplementation did not significantly affect post bronchodilator FEV1, ACT scores in persistent atopic asthma patients.[12]

Oral administration of C. *longa* extract significantly inhibited porcine pancreatic elastase -induced pulmonary inflammation and emphysema in association with the induction of antioxidant gene expression and inhibition of chemokine gene expression. Furthermore, it was found that oral administration of curcumin significantly inhibited CS-induced pulmonary inflammation and emphysema as well[12]. In allergic and acute inflammatory situations, cells such as eosinophils, mast cells and neutrophils play a role in the fibroblast/inflammatory cell interaction. Eosinophils are prominent cells in allergic asthma released from the bone marrow as CD34 precursors and recruited to the airways by prostaglandin D2, cysteinyl leukotrienes, cytokines such as IL-5[13]. Eosinophils stimulate fibroblast migration and proliferation whereas mast cells interact with smooth muscle cells, and besides the production of mediators such as leukotriene LTD4, prostaglandin PGD2 and histamine and also contribute to fibrogenesis more in smooth muscle as part of the remodeling response[14]. Both mast cells and eosinophils are important sources of the zinc-dependent matrix metalloproteinases and their degradation of matrix proteins and proteglycans are important in remodeling of the airways[15] and Curcumin improves the steroid sensitivity of oxidant treated monocytes, which are commonly absent in chronic obstructive pulmonary disease[16]

In this study, guinea pig showed protection with 100-200mg/kg single oral dose administration of curcumin against PCD when animals were exposed to 1% histamine aerosol. Only in lower dose 50mg/kg animals were not protected and showed increase in respiratory rate proved that it prevent inflammatory cells activity at the bronchial smooth muscle cellular level which share some mechanism related to bronchial hyperactivity[9]. This experimental study exhibits the role of Curcumin in respiratory disease like bronchial hyperactivity and some important role in the prevention of COPD.

Conclusion : Curcumin has definite value in the chronic inflammation based on its inhibitory role on connective tissue cell fibroblast function when compared with known established potent anti-inflammatory agent methyl prednisolone. This could be further explained with cell growth in presence of cell medium with cellular growth material or medium to remove fibrinonectin to cross check the cellular activity.

Acknowledgement

We thank our PSG Management, Principals of PSG Medical College and PSG college of Pharmacy to provide immense support in the form of providing infra structure and encouragement to achieve this study. We appreciate and thank all the PSGIMSR animal house persons for their extended Support to complete the study successfully.

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Pharmacoeconomic Evaluation of add on Therapy of Vildagliptin Against Pioglitazone In Type 2 Diabetic Patients: A Prospective Observational Study

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Received Date : 13.09.2013 Accepted Date : 18.11.2013

ABSTRACT

Pharmacoeconomic evaluation of addition of vildagliptin against pioglitazone by using HbA1C reduction as the primary outcome. Materials and Methods: One year study was designed to compare vildagliptin against pioglitazone as add on therapy in type 2 diabetes patients, inadequately controlled by other established combination therapies. Patients added with vildagliptin (n=62) or pioglitazone (n=48) were selected. The primary efficacy endpoint was change in HbA1c from baseline to follow up value. Secondary endpoints included FBS and PPBS. Patient's medication adherence was assessed using Morisky Medication Adherence Scale (MMAS-8) and treatment satisfaction was assessed by Diabetes Treatment Satisfaction Questionnaire (DTSQ). Direct medical costs were also analyzed. Results: Both the drugs exhibited significant reduction in HbA1c. FBS was found to be more reduced in patients using pioglitazone while PPBS was more effectively reduced by vildagliptin. Majority of patients exhibited excellent adherence in case of both drug groups. Satisfaction was found to be comparatively more with pioglitazone group. Statistical significance was not established in any of these outcomes. ($P > 0.05$) Individual drug cost was significantly less with pioglitazone. Conclusion: Vildagliptin is found non inferior to pioglitazone in glycemic control, as add on therapy, with good tolerability profile. But cost analysis clearly indicates that vildagliptin is much expensive and contributes to the financial burden in developing country like India where majority of patients lack health insurance and diabetes become a common disease even in very low income people as well. All these factors emphasize importance of pioglitazone with unique action on adipose tissues.

Key words : Gliptins, Pioglitazone, Add on therapy, HbA1c, Direct medical cost.

INTRODUCTION

Diabetes is a chronic metabolic disease with alarming increase in prevalence. According to recent estimates, approximately 285 million people worldwide (6.6%) in the 20–79 year age group have diabetes and by 2030, 438 million people (7.8%) of the adult population is expected to have diabetes[1]. According to Indian Council of Medical Research – India Diabetes (ICMR-INDIAB) Study, prevalence in India is progressing rapidly across the nation, reaching a total of 62.4 million persons with diabetes.[2] Because of the rising prevalence, the management of this chronic condition will become a serious clinical and financial burden to patients as well as all health-care systems[3] Although lifestyle modification is the most cost-effective intervention for the prevention of diabetes, this alone may not be sufficient to control progression of disease for many of the patients[4]. Oral anti diabetic drugs (OADs) are the first line of drug treatment for type 2 diabetes, in which metformin is the first drug of choice worldwide. However, the progressive nature of this disease usually requires a combination of two or more oral agents in the long term, often before initiating insulin therapy[5].

Sulfonylurea and/or pioglitazone are used in case of

patients who are not responding to metformin. But patients using sulfonylureas are at an increased risk of hypoglycaemia and weight gain while pioglitazone is associated with fluid retention and weight gain. Research and development efforts to improve glycemic control with minimum side effects, lead to a new option i.e., the Dipeptidyl peptidase (DPP)-4 inhibitors. Vildagliptin is the second dipeptidyl peptidase 4 inhibitor which is presently approved in 70 countries including India and has been launched in 37 countries. They enhance the effects of incretins GLP-1 and GIP (the main insulinotropic peptides of intestinal origin released into circulation after meals), by inhibiting enzyme dipeptidyl peptidase 4 and increase glucose-mediated insulin secretion and suppress glucagon secretion[6, 7]. A number of extensive clinical studies have demonstrated that Vildagliptin significantly reduce HbA1c when used as monotherapy and in combination with other traditional agents. In the current scenario, it is prescribed mostly as an add on agent to metformin ± sulfonylurea. But the drug is quite expensive.

There have been only few studies that have compared DPP-4 inhibitors as add on to metformin ± sulfonylurea against pioglitazone which is the alternate option. Besides, studies considering cost analysis are again rare. The present prospective study was designed as a pharmacoeconomic comparison of effectiveness of addition of vildagliptin against pioglitazone in type 2 diabetic patients.

MATERIALS AND METHODS

The study was carried out in Department of Endocrinology of Amrita Institute of Medical Sciences (AIMS), Kochi. A non-experimental (Observational), prospective follow up study of one year was designed and data collection was carried out for a period of 10 months (September 2012-June 2013). Patients who were diagnosed to have type 2 diabetes under endocrinology department and satisfied the study criteria were selected for the study. Patients of either sex aged ≥ 18 years with type 2 diabetes mellitus, prescribed with either vildagliptin or pioglitazone, having HbA1c 6-12.5 % and willing to participate in the study were included. Those with active liver disease (Alanine transaminase/ Aspartate transaminase more than 3 times normal), severe renal insufficiency (Serum Creatinine more than 2mg/dl) were excluded. The study was carried out after getting approval from the Research and Ethics Committee. Informed consent was obtained from the patient and/or patient's care givers before interviewing them.

The sample size was calculated with reference to published literatures, approximate no of prescriptions of study drugs per month and feasibility of completing the study with in time. The patients added with vildagliptin (n=62) or pioglitazone (n=48) were selected. The details of the patients, pertaining laboratory data and drug therapy details were collected from the medical records as well as direct interview of the patients or his/her care givers using a pre-designed data collection form. The data collection form provided the information regarding the demography of the patient which includes age, sex, duration of diabetes, family history, social history etc, patient's co morbid conditions and any observed side effects. Other hypoglycemic agents and concurrent medications for comorbid conditions were continued.

Baseline HbA1c of the patients were noted before the addition of either vildagliptin or pioglitazone. The patients were followed up after 3-6 months. The primary efficacy endpoint was change in HbA1c from baseline to follow up value. Secondary endpoints included FBS and PPBS. Any elevation in LFT, RFT and any side effects occurred in either of the group during the study was also recorded. During the follow up period, patient's medication adherence was assessed using Morisky Medication Adherence Scale (MMAS-8) and treatment satisfaction was assessed by Diabetes Treatment Satisfaction Questionnaire (DTSQ). Direct medical cost analysis too was carried out and the considered costs includes individual tablet cost of vildagliptin and pioglitazone, cost of laboratory blood tests like HbA1c, FBS, PPBS and LFT and appointment fee.

STATISTICAL ANALYSIS

Statistical analysis was done using computer software SPSS 21.0 version. Changes in outcomes from baseline to endpoint were compared between vildagliptin group and pioglitazone group. The significance of the study results were assessed using Independent samplet test, Paired samplet test and ANOVA.P value of <0.05 was considered significant. Statistical tests were all one tailed and the results were represented as average reduction from baseline to endpoint.

RESULTS

Demographic and Baseline Characters :

A total of 160 patients were screened from September 2012 to June 2013. Among them, 48 subjects were excluded as they failed to fulfill inclusion and exclusion criteria. Out of the 112 patients, 2 patients were lost to follow up. The baseline demographic characters and other parameters are represented in Table 1.

Table 1 : Summary of Baseline characters

Total no of patients	110
Patients using Vildagliptin (%)	61.81
Patients using Pioglitazone (%)	38.19
Male patients (%)	57.27
Female patients (%)	42.73
Mean Age ± SD of the patients	53.41 ± 9.68
Patients with family history of diabetes (%)	61.54
Mean ± SD of duration of Diabetes (Years)	10.92 ± 6.44
Mean ± SD BMI of patients using vildagliptin (kg/m2)	26.07 ± 3.39
Mean ± SD BMI of patients using pioglitazone (kg/m2)	24.80 ± 4.71
Mean ± SD ALT (SGPT) (IU/L)	54.61 ± 19.59
Mean ± SD AST (SGOT) (IU/L)	29.72 ± 2.29
Mean ± SD of Serum Creatinine (mg/dl)	1.02 ± 0.17

Efficacy End Points :

Primary Efficacy Endpiont:
(Glycosylated Haemoglobin)

All the patients who are added with Vildagliptin and Pioglitazone had similar baseline A1C. Both drugs showed significant reduction in HbA1c from baseline to endpoint (3-6 months) when added to the other hypoglycemic agents. Vildagliptin group showed an average HbA1c reduction of 0.79 (n=62, baseline HbA1c: 6-12.5) and pioglitazone group showed an average HbA1c reduction of 0.61 (n=48, baseline HbA1c: 6-12.5).

Reduction was again calculated from 3 different baselines which are represented in Table 2. Both vildagliptin and Pioglitazone also showed significant reductions and comparatively higher HbA1c reduction in patients with a baseline HbA1c greater than 10%. (1.44% & 1.11% respectively)

Table 2 : HbA1c Reduction of Vildagliptin & Pioglitazone from Different Baselines

Baseline A1c	Drug	No of patients	Average reduction
≤ 7	Vildagliptin	9	0.1
	Pioglitazone	15	0.33
7.1 -9.9	Vildagliptin	37	0.68
	Pioglitazone	24	0.53
≥ 10	Vildagliptin	16	1.44
	Pioglitazone	9	1.11

Both the groups were also categorized and compared as add on to specific drug baselines.

HbA1c reduction of Vildagliptin Vs Pioglitazone as 2nd line agent:

As add on to metformin the average reduction of HbA1c by vildagliptin (n=9), and pioglitazone (n=4) was 0.97 & 0.45 (baseline: 6.5-11) respectively and as add on to sulfonyl urea, reduction was 0.43 & 0.48 (n=6, baseline 6.5-11 for both) respectively. Vildagliptin showed more reduction than pioglitazone when used as second line agent.

HbA1c reduction of Vildagliptin Vs Pioglitazone as 3nd line agent:

The average reduction of HbA1c by vildagliptin (n=21, baseline: 7-11.5) and pioglitazone (n=17, baseline: 7-11.5) when added to patients inadequately controlled by metformin and sulfonylurea was 1.06 & 0.84 respectively. Both drugs showed high A1c reduction when used as third line agent, with vildagliptin showed comparatively more reduction.

HbA1c reduction of Vildagliptin Vs Pioglitazone As Add On To Metformin + Sulfonyl Urea+ Acarbose

The average reduction of HbA1c by vildagliptin and pioglitazone (n=4, baseline: 6.5-11.5) was 0.52 & 0.17 respectively. Vildagliptin showed better reduction when added with other 3 drugs.

HbA1c reduction of Vildagliptin Vs Pioglitazone As Add On To Metformin + Sulfonyl Urea+ Insulin

The average reduction of HbA1c by vildagliptin (n=8, baseline: 8-12) and pioglitazone (n=3, baseline: 8-12) was 1.01 & 0.93 respectively. Both drugs show similar significant A1c reduction when co prescribed with insulin.

Secondary Efficacy Endpiont: Fasting Blood Sugar and Post Prandial Blood Sugar

Table 3 depicts reduction of FBS and PPBS by vildagliptin and pioglitazone, when added to patients inadequately controlled by other hypoglycemic agents. FBS was found to be more reduced in patients using pioglitazone while PPBS was more effectively reduced by vildagliptin.

Table 3 : FBS and PPBS reduction by vildagliptin and pioglitazone

Drug	FBS (Baseline: 120-220)		PPBS (Baseline: 200-300)	
	No.of patients	Average Reduction	No.of patients	Average Reduction
VILDAGLIPTIN	30	4.84	16	15.54
PIOGLITAZONE	19	11.23	7	12.74

Other clinical parameters and adverse reactions observed :

Both treatments were well tolerated. Adverse reactions observed were mild and self limiting. Weight gain due to pioglitazone was most commonly found, followed by edema due to pioglitazone. Compared to pioglitazone adverse reactions were less reported in patients using gliptins. Significant changes in liver enzyme levels, Creatinine levels or severe episodes of hypoglycemia were not observed in either of the drug groups.

Medication Adherence and Treatment Satisfaction of patients :

Medication adherence and satisfaction of patients regarding the add on treatment were analyzed using MMAS-8 scale and DTSQ respectively. In the case of both drug groups, majority of patients exhibited excellent adherence. On comparing vildagliptin and pioglitazone, not much difference was found in adherence behavior by the patients while patients having poor compliance were observed more in vildagliptin group. Regarding Satisfaction, most of the patients showed excellent satisfaction about the treatment. Pioglitazone using patients were found comparatively more satisfied. None of the patients showed low score/ poor satisfaction in both groups. However while comparing the mean value of individual item in DTSQ questionnaire, satisfaction score of all items were found to be more in patients taking pioglitazone. Score of 2 items of DTSQ regarding the perceived frequency of hyperglycemic and hypoglycemic symptoms were comparable among both groups.

The scores MMAS-8 and DTSQ are shown in table 4 & and individual mean score comparison is shown in table 5.

Adherence score	**Excellent (8)**	**Medium (6-8)**	**Poor (<6)**
	% of patients	**% of patients**	**% of patients**
Vildagliptin	52.55	37.23	10.22
Pioglitazone	49.55	45.04	5.41
DTSQ score	**Excellent (24-36)**	**Medium (12-23)**	**Poor (0-11)**
	% of patient	**% of patients**	**% of patients**
Vildagliptin	74.34	25.66	-
Pioglitazone	86.07	13.92	-

Table 5: Diabetic Treatment Satisfaction Individual Item Mean Score

Drug	Flexibility	Continuing therapy	Convenience	Current treatment	Understanding about diabetes	Recommend to others
Vildagliptin	4.77	4.34	3.73	3.70	4.34	4.59
Pioglitazone	4.96	4.63	3.87	3.91	4.91	5.58

Direct Medical Cost of Patients :

Table 6 depicts the direct medical cost of the patients. Monthly cost of brands most commonly prescribed is shown below. Mandatory laboratory tests to be done are similar for vildagliptin and pioglitazone. But the individual tablet cost varies a lot. Comparing vildagliptin and pioglitazone, vildagliptin containing brands are much expensive.

Table 6 : Direct Medical Cost of Patients:

DRUG	DOSE	FREQUENCY	COST (MONTHLY)
VILDAGLIPTIN (ZOMELIS/GALVUS/JALRA)	50 MG	BD	Rs. 1140
VILDAGLIPTIN+METFORMIN (ZOMELIS MET)	50/500 MG	BD	Rs. 1200
VILDAGLIPTIN+METFORMIN (ZOMELIS MET)	50/1000 MG	OD	Rs. 660
PIOGLITAZONE (PIOZONE)	15 MG	OD	Rs. 210
PIOGLITAZONE +METFORMIN (PIONORM M)	15/500 MG	OD	Rs.126
COST OF LABORATORY TEST AND APPOINTMENT			
HbA1c		Rs. 300	
FBS Or PPBS		Rs. 50	
LFT		Rs. 230	
APPOINTMENT		Rs. 150	

Discussion :

As add on to metformin the average reduction of HbA1c by vildagliptin (n=9), and pioglitazone (n=4) was 0.97 & 0.45 respectively and as add on to sulfonyl urea, reduction was 0.43 & 0.48 (both n=6) respectively. Baseline HbA1c was 6.5-11%. G. Bolli et al[8] compared the efficacy and tolerability of vildagliptin against pioglitazone as add-on to metformin monotherapy. The study enrolled type 2 diabetic patients on metformin dose of ≥ 1500mg/day and had A1C of 7.5–11.0%. the average reduction of HbA1c by vildagliptin (n=295) and pioglitazone (n=281) was 0.9 & 1.0 respectively. The study concluded that the efficacy of vildagliptin is non-inferior to that of pioglitazone as add on to metformin. A study by Garber A J et al[9] analyzed the effects of vildagliptin in patients inadequately controlled with a glimepiride (4mg OD). They found an A1c reduction of 0.6±0.1% in patients receiving vildagliptin 50 mg daily and 0.7 ± 0.1% in those receiving 100 mg daily which is comparable to present study. Another study by B. Charbonnel et al[10] analyzed efficacy and tolerability of add-on pioglitazone therapy to failing metformin monotherapy (850–2,550 mg/day). The mean reduction from baseline in HbA1c was 0.89% for pioglitazone. Less reduction in the present study compared to the above studies may be due to short no of patients. The increased/near maximal dose of metformin used by patients in above study may also contributes to high reduction.

Both vildagliptin and pioglitazone are mostly used as add on to metformin and sulfonyl urea. Bosi et al[11], analyzed vildagliptin effect Vs placebo (n=182, baseline A1c: 7.5-11%) and the between group difference was 1.1 ± 0.1%. Aljabri K et al[12] analyzed addition of pioglitazone against bedtime insulin to maximal doses of sulfonylurea and metformin. HbA1C levels lowered by pioglitazone were –1.9% ± 1.5%. In the present study also the effectiveness is compared between vildagliptin and pioglitazone. The average reduction of HbA1c by vildagliptin and pioglitazone (n=21 & 17 respectively, baseline: 7-11.5) was 1.06 & 0.84 respectively. Compared to the above studies, a very high difference in A1c reduction was not found with present study.

The secondary outcomes of present study were fasting blood sugar and post prandial blood sugar. Average reduction in both the parameters for 2-3 months are calculated and compared between vildagliptin and pioglitazone. For fasting blood sugar vildagliptin showed an average reduction 4.84mg/dl while pioglitazone reduced 11.23mg/dl. In the case of postprandial blood sugar, average reduction shown by vildagliptin and pioglitazone were 15.54 & 12.74 respectively. G. Bolli et al[8] in a study compared the efficacy and tolerability of vildagliptin against pioglitazone and found that Pioglitazone decreased FPG to a greater extent than vildagliptin. A study of vildagliptin was conducted in patients with impaired Glucose tolerance by Julio Rosenstock et al[13] and found that Prandial glucose excursions were reduced ($p < 0.001$). They found that the known effects of vildagliptin on incretin levels and islet function in type 2 diabetes were reproduced in subjects with IGT, with a 32% reduction in postprandial glucose excursions. Similar to these studies, FBS was found to be more reduced in patients using pioglitazone while PPBS was more effectively reduced by vildagliptin in the present study.

A French Population-Based Study was done by Michel Tiv et al[14] about the medication adherence in type 2 diabetes. They found that 39% of patients reported good medication adherence, 49% medium adherence and 12% poor adherence. In the present study medication adherences of all the study patients were measured. Differing from the above study, most of the patients exhibit excellent adherence (53.89) towards the medication, followed by medium (38.32) and poor adherence (7.79). Michel Tiv et al[14] also pointed out that the factors significantly associated with poor adherence in multivariate analysis were socio-demographic factors: age, 45 years, non-European geographical origin, financial difficulties and being professionally active; disease and therapy-related factors. In present study also financial difficulties and busy profession related schedules were reported as reason for poor adherence by majority of patients.

Sitagliptin was compared to pioglitazone as add on to metformin by Shalini Chawla et al[15] in which Treatment satisfaction was assessed using the Diabetes Treatment Satisfaction Questionnaire. There was no significant difference in the improvement in treatment satisfaction produced in the 2 groups, although this was higher with sitagliptin. Present study analyzed

treatment satisfaction of all the study patients. When vildagliptin was compared to pioglitazone, satisfaction score of all items were found to be more in patients taking pioglitazone. But no significant difference was found in overall satisfaction score of vildagliptin and pioglitazone, similar to the study of Shalini chawla et al.

Vildagliptin was compared to pioglitazone in each sub groups as discussed above. Compared to use as 2nd line agent, both drugs showed high A1C reduction when used as third line agent. Both vildagliptin and pioglitazone showed high reductions when baseline HbA1c >10. When calculating the overall reduction of addition of vildagliptin against pioglitazone in patients inadequately controlled by other hypoglycemic agents, Vildagliptin showed an average HbA1c reduction of 0.79. (n=62, baseline HbA1c: 6-12) while pioglitazone exhibited average reduction of 0.61(n=48, baseline HbA1c: 6-12). This shows that both the drugs have individual effect in reducing HbA1c, but between group difference was negligible. Statistically significant difference in A1c reduction was not established in any of the groups ($p > 0.05$). Although FBS was found to be more reduced in patients using pioglitazone and PPBS was more effectively reduced by vildagliptin in the present study, statistical significance was not established when compared between groups. Therefore considering the effectiveness in glycemic control both drugs are similar, neither is found superior to the other.

While reviewing adverse reactions of present study, Pioglitazone using patients were found to have more reports, especially weight gain (15 patients) and pedal edema (9 patients) while only small no of patients of vildagliptin group reported ADRs, nausea and/ or vomiting (4 patients). But most of them were self limiting and mild.

Another important concern is the reports of risk of bladder cancer associated with pioglitazone. Laurent Azoulay et al[16] conducted a retrospective cohort nested case control study regarding the use of pioglitazone and the risk of bladder cancer and provided evidence that pioglitazone is associated with an increased risk of incident bladder cancer. However the highest rate was observed in patients exposed for more than 24 months (rate ratio 1.99) and in those receiving cumulative dosage greater than 28000mg (rate ratio 2.54). Isabelle N. Colmers et al[17] conducted a systematic review and meta-analysis to evaluate the association and found that the cohort studies of thiazolidinediones and of pioglitazone specifically showed significant associations with bladder cancer (pooled RR 1.15 & 1.22 respectively, 95% CI).Carlo Piccinni et al[18] also done the same by retrieving cases from the U.S. Food and Drug Administration Adverse Event Reporting System (FDA AERS) between 2004 and 2009. They found that Reporting odds ratio was indicative of a definite risk for pioglitazone.(4.30 [95% CI 2.82–6.52])

However when considering gliptins, they are also not completely out of such serious risks. Some of the very recent clinical findings suggest that incretin based medications are associated with the risk of certain rare conditions like pancreatitis and pancreatic cancer. Butler et al[19] studied 7 individuals treated by sitagliptin and 1 by exenatide and found enlarged pancreas (40%) due to proliferation of exocrine pancreas. Another important finding was presence of cell hyperplasia in 3 cases and neuroendocrine tumor in 1case among the 8 individuals with incretin therapy. A study by Michael Elashoff et al[20] assessed the association of exenatide or sitagliptin with pancreatitis, thyroid cancer and also with any other cancer using FDA AERS database. In patients administered exenatide or Sitagliptin pancreatitis has been reported > 6-fold more frequently when compared with other therapies (OR=10.68 and 6.74 respectively; 95% CI). The numbers of pancreatitis events reported with gliptins are 718 for sitagliptin and 43 for linagliptin and the number of pancreatic cancer are 81 for sitagliptin and 1 for linagliptin. The plausible mechanism emerged from studies was that GLP-1 simulates proliferative signaling in human pancreatic ductal epithelium and the pancreatic duct gland was found responsive to these signals. Vildagliptin is not included here as it is not approved in US due to concerns of hepatic toxicity. Since it is coming under the same class, the class effect cannot be completely excluded.

Many developed countries calculated the economic burden of diabetes by applying various pharmacoeconomic techniques and consistent finding across these studies were that the disease poses a significant economic burden. As far as a developing country like India is concerned where majority of the patients are not even covered by health insurances, the impact will be much higher. Although more than 70% of diabetes related cost is attributed to its

complications, burden also comes from charges due to consultation, laboratory tests, medicines, hospital stay etc. There are only a few studies from India on the direct cost of diabetes care. In the present study, the direct medical cost of patients taking vildagliptin and pioglitazone were calculated. Cost of consultations and mandatory laboratory tests are similar for vildagliptin and pioglitazone. Difference is in the individual tablet cost. Vildagliptin is usually given as 50mg, BD for which the monthly cost is Rs.1140 while pioglitazone (15mg, OD) costs only Rs. 210/month. It is clear that the difference is more than 5 times, which demands due consideration.

CONCLUSION :

Considering the primary outcome, both vildagliptin and pioglitazone reduce HbA1c in similar manner, especially from baseline HbA1c > 10. The reduction was analyzed separately from various baselines and as 2nd line agent, 3rd line agent etc and non inferiority of vildagliptin was established in all these cases. The ADRs observed were more with pioglitazone, but majority of the cases were self limiting. Another flaming issue is the reports of association of pioglitazone with bladder cancer. But experts point out only weak association since the increased risk is reported only with a cumulative dose of 28,000 mg. As majority of people are prescribed 15mg/ day, the chance of risk is indeed rare. On the other hand gliptins are also not out of threat as there are emerging issues of pancreatitis and pancreatic cancer with use of gliptins.

On analyzing the medication adherence, patients with poor adherence was found to be less in pioglitazone group. Moreover overall and individual score of treatment satisfaction was found to be more with pioglitazone. In both these cases, although statistical significance is not established, cost may be a prime concern. It is noteworthy that the per tablet cost of pioglitazone is only 1/5th the cost of vildagliptin. Since type 2 diabetes is a chronic disease with alarming increase in prevalence, patients have to take medicines every day. Especially in a developing country like India , where majority of patients are lower income group without any health insurance, the cost reduction of single tablet have grand impact in reducing overall financial burden. Hence while choosing in between the above drugs with similar outcome, cost minimization should also be considered that welcomes the use of pioglitazone.

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RESEARCH ARTICLE

Neutralisation of Cobra Venom Effects by Indian Medicinal Plants

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Received Date : 24.10.2013 Accepted Date : 12.11.2013

ABSTRACT

Ayurvedic system of medicine makes use of hundreds of plants in the treatment of snakebite. Although these are time tested, there has been no scientific validation of their efficacy. To develop herbal remedies in this area, detailed evaluation of folkloric plants in a systematic manner is necessary. In the present research, the aqueous extracts of the areal parts of *Hedyotis corymbosa*, fruit peel of *Punica granatum* and tubers of *Cyperus rotundus* were tested for the neutralization of various snake venom effects. The extracts were prepared by the methods adopted by Vishavaidya's in Kerala and tested against freeze dried cobra venom (*Naja naja*). Parameters like inhibition of lethal activity and neutralization of various enzyme activities were studied to evaluate the anti venom effect. All the three extracts antagonized various venom effects and prevented lethality in albino mice. The results of the study proved the venom neutralizing capacity of the extracts and thus support their traditional use in snake bite therapy.

Key words : *Naja naja, antivenom activity, Hedyotis corymbosa, Punica granatum, Cyperus rotundus*

INTRODUCTION

Snake bite is a major cause of death in many parts of the world especially in India. It is estimated that deaths due to snake bite in the Indian subcontinent is more than 25000 every year[1]. *Naja naja* and *Viper russelli* are the common snakes in India and a large number of deaths occur due to envenomation by these snakes[2]. The most effective and accepted therapy for snake bite is the immediate administration of anti venom. In spite of the high cost for this therapy, there are many demerits including hypersensitivity and untoward serum reactions[3]. In this context, many attempts have been made over the years for the development of snake venom antagonists from plant sources. A number of plants have been used by traditional healers for the treatment of snake bite and many of these are not scientifically evaluated[4]. *Hedyotis corymbosa* (Rubiaceae) is a spreading, annual weed plant found in the fields throughout India, usually found during the rainy season. It is well known for its effectiveness in jaundice and other liver disorders. *Cyperus rotundus* (Cyperaceae) is a perennial plant mentioned in Ayurveda for treating fevers, digestive disorders, wounds, bruises etc. *Punica granatum* (Punicaceae) is a fruit bearing deciduous shrub cultivated throughout India. In ancient medicine, it has been used for treating diarrhea, dysentery, haemorrhoids and to arrest bleeding. The present study investigates the venom antagonizing activity of these three plants used for snake bite therapy by the 'Vishavaidyas' (traditional healers of venom) in Kerala.

MATERIALS AND METHODS

Venom :

The freeze dried cobra venom was obtained from Irula snake catcher's Industrial co-operative society, Chennai, India. It was dissolved in 0.9% saline and centrifuged. The supernatant was used as venom and expressed in terms of dry weight.

Animals :

Male albino mice (18-20g) were used for the experiments. All experiments were approved by the Institutional animal ethics committee. (Approval no: 149/1999/CPSCEA)

Plants :

The plants *Hedyotis corymbosa* (HC), *Punica granatum* (PG), *Cyperus rotundus* (CR) were collected locally from Kottayam, Kerala, India and authentified. The parts used were the areal parts of HC, fruit peel of PG and the tubers of CR.

Preparation of extract :

For the preparation of plant extracts, method followed by the 'Vishavaidya's was adopted. The plants were shade dried, ground and extracted by boiling with water for 4 h. Then it was filtered while hot through a muslin cloth and the filtrate was evaporated to get a dry residue. These dry extracts were then stored in a refrigerator until use.

Inhibition of Lethality[5] :

Various concentrations of cobra venom in 0.2ml physiological saline were injected subcutaneously to male albino mice and the lethal dose was determined. To assess the inhibition of lethality in vitro, various doses of venom were mixed with a fixed dose of plant extract and incubated at 37^0C for 60 min followed by subcutaneous injection in to mice. In vivo study involved the subcutaneous injection of various doses of venom and oral administration of fixed dose of plant extracts. In both studies, three mice were used at each dose level.

Inhibition of myotoxicity of venom[6] :

The myotoxicity of venom is expressed in terms of plasma creatinine phosphokinase (CPK) and is quantitatively estimated in relevant blood samples. To learn the inhibition of myotoxicity, a fixed dose of venom (15 µg) was incubated with various concentrations of plant extracts at 37^0C for 30 min and injected in to mice (right gastroenemius). Blood samples from various groups of mice at the end of 4 h were used for the estimation of CPK activity. The ability of plant extracts were expressed as ED values which is the minimum dose that caused 50% inhibition of enzyme activity.

Inhibition of plasma protease activity[7]:

Various concentrations of plant extracts were incubated with a fixed dose of venom protein (15 µg) at 37^0C for 30 min and injected in to mice (right gastroenemius). Blood samples from various groups of mice at the end of 4 h were used for the estimation of plasma protease activity. Enzyme activity of venom alone served as the control. Inhibitions by various doses of plant extracts were estimated and ED of each was determined.

Inhibition of acetyl cholinesterase activity[8]:

Blood samples for acetyl cholinesterase activity was prepared in a similar manner. The resultant mixture was then injected to the right gastroenemius of male albino mice. Inhibitions by various doses of plant extracts were estimated in comparison with venom control and ED values were determined graphically.

RESULTS

Inhibition of lethality:

10µg venom per mouse was found to be lethal by subcutaneous route. In the in vitro studies, various extracts were incubated with venom and then administered subcutaneously to mice. All the three extracts exhibited significant neutralisation of venom induced lethality. In vivo studies also proved the efficacy of these extracts in antagonizing the venom induced lethality although the fold of neutralization was less compared to in vitro studies. CR was found to be the most effective in both cases (Table 1).

Table 1. Effect of test extracts in inhibiting venom induced lethality

Extract (mg)	Venom neutralized in vitro (µg)	Neutralisation fold*	Venom neutralized in vivo (µg)	Neutralisation fold*
HC (5)	400	40	25	2.5
PG (5)	350	35	20	2.0
CR (5)	550	55	40	4.0

* Calculated with respect to the lethal dose of cobra venom (10 µg)

Inhibition of myotoxic activity:

This was done by estimating the CPK activity in blood samples of mice. A graded dose response was found when increasing doses of plant extracts were tested against a fixed dose of venom (Figure 1) and ED value was determined graphically. CR showed maximum inhibition of CPK activity with an ED value of 22 µg followed by HC.

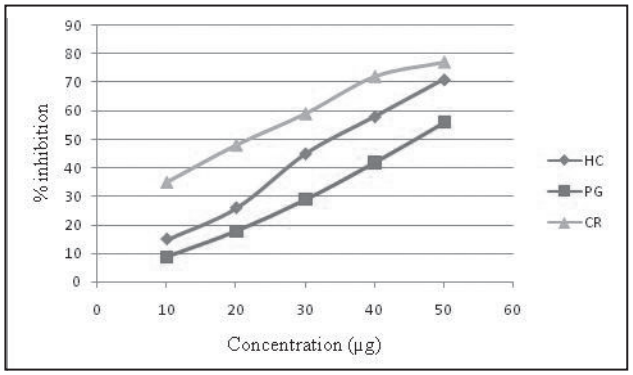

Figure 1. Pattern of plasma CPK inhibition by plant extracts

Inhibition of plasma protease activity:

There was significant reduction in plasma protease activity when the test extracts at various dose levels were incubated with venom. The results are expressed as ED value which is defined as the minimum dose of extract that caused 50% inhibition of enzyme activity. The efficacy of extracts were in the order CR>HC>PG (Figure 2).

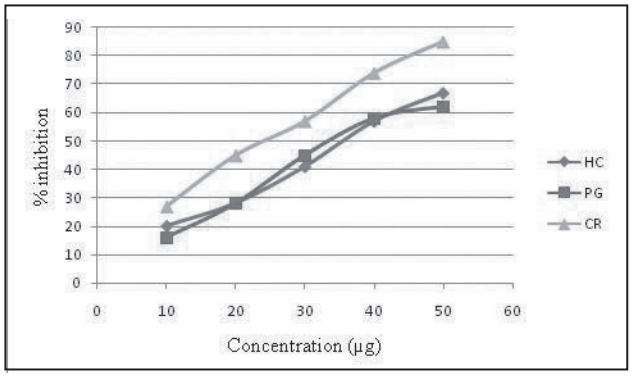

Figure 2. Plasma protease enzyme inhibition by plant extracts

Inhibition of acetyl cholinesterase activity:

Although all the three extracts were able to neutralize the acetyl cholinesterase activity, CR and PG exhibited better activity than HC. Fig.3 shows the pattern of neutralization by various extracts.

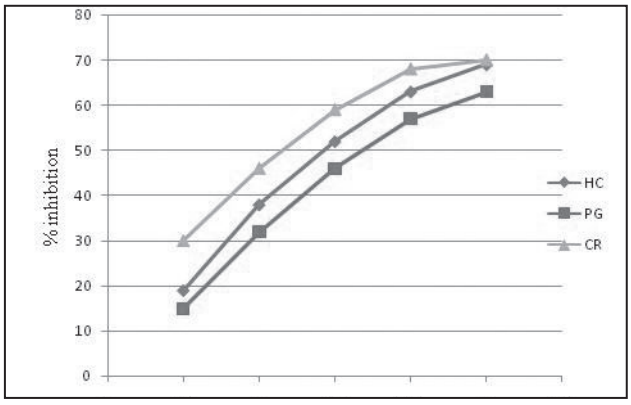

Figure 3. Graph showing acetyl cholinesterase activity inhibition by plant extracts

The ED values of HC, PG and CR in the neutralization of various enzyme activities is given in Table 2.

Table 2. Comparison of Effective doses of test extracts in the inhibition of various enzyme activities

Extract	Effective dose		
	A	B	C
HC	35	35.5	29
PG	45	34	33
CR	22	25	23

A-Neutralisation of venom induced myotoxicity, B- Neutralisation of venom induced acetyl cholinesterase activity, C -Neutralisation of venom induced plasma protease activity

DISCUSSION

Deaths following snakebite are very common in undeveloped areas of various states in India. The only effective treatment till date is the use of snake venom antiserum which is associated with serious side effects including anaphylactic shock. Over the last 20 years, more attention has been given to find a safer alternative for antiserum therapy9. Although many plants have been positively evaluated for venom neutralization capacity, no attempts have been made so far to develop herbal antivenom remedy10. Such an approach requires phytochemical and pharmacological and biochemical investigation of crude herbal extracts, their fractions or isolated constituents. Traditional healers of Kerala called "Vishavaidyas" effectively treat snakebites with plants and the success rate can be equated with the

antivenom therapy[11]. A few such plants were selected for evaluation of antivenom activity because they have been in use for many years and found effective. The very same method employed by Vishavaidyas was adopted for their extraction and the resultant extracts were used unmodified.

In the present research, we evaluated the efficacy of HC, PG and CR extracts in neutralizing the lethal as well as enzymatic activities of cobra venom. Significant neutralization of toxic enzymes is said to inhibit the lethality of venom as the pharmacological and toxicological properties of venom are mainly associated with proteins particularly enzymes[12, 13]. The protease enzyme has been reported to cause the local physiological effects like myonecrosis and muscular degeneration. Similarly, other enzymes like myotoxins and acetyl cholinesterase also has a role in the process of myonecrosis[14, 15]. All the three extracts under study presented excellent capacity in neutralizing various enzyme effects of cobra venom and in all the assays, CR showed superior activity compared to the other two. Enzyme inhibition may be the reason behind the efficacy of extracts in inhibiting venom induced lethality[16, 17]. However, detailed biochemical studies are needed to establish the correct mechanism of action[18]. It is important to note that these extracts have been prepared by the conventional methods of Vishavaidyas and were used as such in the crude form. No attempts were made to modify or refine their content so as not to lose any fraction with possible activity. The results of the study justify their traditional use in snake bite treatment and suggest further research for their development as antivenom remedy.

CONCLUSION

The present study establishes the presence of antivenom principles in the aqueous extracts of HC, PG and CR which in turn justifies their use for snake bite in traditional medicine. Since the extracts were used without any refining or fractionation, the activity cannot be attributed to the presence of any specific constituent. Further research using selected fractions or isolated compounds from the same extracts can find out the constituents responsible for the activity. Moreover, investigations at the biochemical level are needed to establish the mechanism of antivenom activity of these plants. Positive findings of such studies will support their traditional utility further and can help in introducing herbal remedy for snake bite.

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Evaluation of Antioxidant and anti Inflammatory Activity of *Hippeastrum puniceum*.(Lam.)Voss. Bulb Extract

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Received Date : 28.10.2013 Accepted Date : 13.12.2013

ABSTRACT

Inflammation is a major cause behind various chronic diseases like Rheumatoid arthritis, Ulcerative colitis, Atherosclerosis, Inflammatory bowel syndrome, Crohn's disease, Bronchial asthma etc. The acceptance level of many synthetic anti-inflammatory drugs in the market had been decreased over the years due to their unwanted effects mainly gastrointestinal problems. Thus many traditionally used medicinal plants were scientifically evaluated for the anti-inflammatory activity. Antioxidant activity being the prime factor in preventing inflammation. Thus most of the plants with antioxidant potency exhibits anti-inflammatory activity also. In the present study a traditionally used plant with anti- inflammatory activity, *Hippeastrum puniceum*.(Lam.) Voss(Amaryllidaceae) was selected for evaluating antioxidant and anti inflammatory activity by in-vitro models. Antioxidant activity was evaluated by iron chelating and total antioxidant assay. Protein denaturation and Proteinase inhibition methods were used for evaluating anti-inflammatory activity.A preliminary phytochemical analysis was carried out, which showed the presence of many active constituents like alkaloids, carbohydrates, flavonoids, phenolics, terpenoids amino acids and saponins.The results of the study revealed the antioxidant and anti-inflammatory potential of aqueous bulb extract which could be attributed to the presence of flavonoids and phenolics.

Keywords : *Anti-inflammatory, Antioxidant, Hippeastrum puniceum, Preliminary phytochemical screening*.

INTRODUCTION

Avery large population of developing countries still relay on herbal medicine and India has a rich tradition of herbal medicine as evident from Ayurveda[1].Over the years the acceptance of synthetic drugs had declined drastically due to their unwanted side effects, toxicity and inefficiency. In addition to the emergence of new infectious diseases, proliferative disorders such as cancer and growing multidrug resistance in pathogenic microorganisms have prompted renewed interest in the discovery of potential drug molecules from medicinal plants[2].Traditional medicine, including tribal medicine is to be studied and documented, as a part of preservation of our ancient knowledge and culture.

Antioxidants are our first line of defence against free radical damage, and are critical for maintaining optimum health and wellbeing. Apart from the dietary sources, Indian medicinal plants also provide antioxidants. Most of the plants with anti oxidant potential do prevent inflammation, thus the antioxidant potential is an essential factor in curing inflammatory disorders. As the demand of herbal medicine is increasing in both developing and developed countries, many studies have been conducted on various medicinal plants and their antioxidant and anti-inflammatory potential were successfully proven.

The present study was undertaken as a small step in scientifically proving the claimed pharmacological use of the plant Hippeastrum puniceum in preventing inflammation. The bulbs of the plant were traditionally used in curing tumours and various inflammatory disorders. Some of tribal community used the bulbs in healing wounds and in treating piles.It is a bulbous perennial ornamentalplant; belonging to Amaryllidaceae family distributed worldwide (Figure 1). Although this plant has been used in the tribal and folkloric medicine for many decades, no attempts were so far made to scientifically evaluate its therapeutic utility. The present study is undertaken to evaluate the in-vitro antioxidant

and anti-inflammatory potential of the aqueous extract of the plant Hippeastrum puniceum.

MATERIALS AND METHODS

Plant material :

Plant used in the present study, Hippeastrum puniceum(Lam.)Voss was collected from Ettumanoor, Kottayam, Kerala, authenticated by Dr. Jomy Augustine, St. Thomas College, Pala.The voucher specimen of the plant (No:2252) was deposited in Department of Pharmacognosy, University College of Pharmacy, Cheruvandoor, Ettumanoor, Kottayam.

Preparation of the extract :

About 500g of fresh bulbs with auniform size was sliced into very small pieces and subjected to hot aqueous extraction for 6 h. The juicy solution obtained was filtered through muslin cloth and marc was discarded. The solution was concentratedat a temperature below 60^0C. The dried Hippeastrum puniceum (HP) extract so obtained was then stored in a refrigerator for further use.

Preliminary Phytochemical Analysis[3] :

Preliminary phytochemical analysis was conducted onHP extract to detect the presence of various phytoconstituents such as alkaloids, carbohydrates, phenolics, flavonoids, glycosides, saponins, proteins,steroids and triterpenoids. Analysis was carried out using various chemical reagents.

Antioxidant activity by Iron chelating assay[4] :

In the assay, 2ml of various concentrations of test extract were incubated with 1ml o-phenanthroline solutionand 2ml 2M ferric chloride solution at ambient temperature for 10 min.The absorbance of solution was measured at 510 nm against blank (mixture of methanol and distilled water). A control was also prepared in similar manner by omitting the sample.

Ascorbic acid was used as standard. The antioxidant potential of test extract was determined and reported as IC50 value.

Antioxidant activity by Total Antioxidant Assay[5] :

0.3 ml of HP extractwas mixed with 3 ml of reagent solution containing 1 ml each of Ammonium molybdate (4mM), Sodium phosphate (28mM), and Sulphuric acid (0.6M) and kept at 90^0C for 90 min. After cooling to room temperature, absorbance was measured at 695 nm. The reducing capacity of the test extractwas expressed as the ascorbic acid equivalents.

Anti-Inflammatory activity by Protein denaturation method[6]:

The sample and standardmixtures were prepared by adding 0.45 ml bovine serum albumin (5% aqueous solution) to 0.05 ml of HP extract and diclofenac (100-500 µg/ml of final volume) to different tubes at pH 6.3 The tubes were incubated at 37°C for 20 min and then heated at 57°C for 3 min. 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm. For control tests 0.05 ml distilled water was used instead of extracts while product control tests lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated using standard formula.

Anti-Inflammatory activity by Proteinase inhibitory action[6]:

1ml of various concentration of HP extract (100-500µg/ml) was added, 1ml of 25 mM tris HCl buffer (pH 7.4) containing 0.06 mg trypsin, 25 mM tris HCl buffer (pH 7.4). The mixtures were incubated at 37°C for 5 minutes. Then 1.0 ml of 0.8% (w/v) casein was added. The mixtures were incubated for an additional 20 min. 2.0 ml of 70% Perchloric acid was added to terminate the reaction and the resultant suspension was centrifuged

and the absorbance of the supernatant was read at 280 nm against blank. Diclofenac was used as standard and the similar procedure was carried out. The percentage inhibition was calculated using standard formula.

Statistical analysis :

Results of in-vitro studies were reported as Mean ± SD. The mean of three observations were taken for the analysis. In the case of in-vitro anti inflammatory studies paired student's t - test analysis was done to ascertain the significance.

RESULTS

Preliminary Phytochemical Analysis :

The preliminary phytochemica[1] analysis of HP extract showed the presence of carbohydrates, alkaloids,tannins,terpenoids saponins, flavonoids and aminoacids, recorded in table 1.

Table No.1- Preliminary phytochemical analysis of HP extract

Sl.No	Phytoconstituents	Observation
1.	Alkaloids	+++
2.	Carbohydrates	+++
3.	Flavonoids	+
4.	Tannins	++
5.	Glycosides	-
6.	Terpenoids	++
7.	Saponins	+++
8.	Proteins & Amino acids	+++

Antioxidant activity by Iron chelating assay :

The sample exhibited moderate activity when compared to the standard.The IC50values of both standard and the extract are given in table 2.The IC50 values were calculated from individual calibration graph of standard and extract. (Figure 2 & 3).

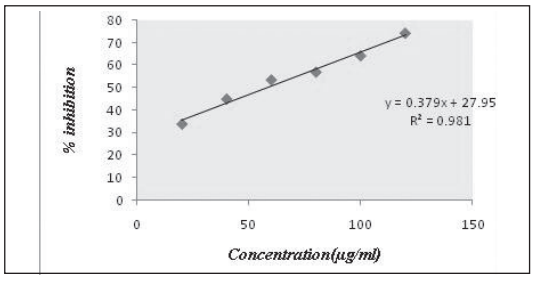

Figure - 2 : Calibration curve of Ascorbic acid

Table No. 2 - Comparison of IC50 values of Herbal extract and Ascorbic acid

Sl.No	Sample	IC50 Values(µg/ml)
1.	Ascorbic acid	58.02
2.	HP	160.84

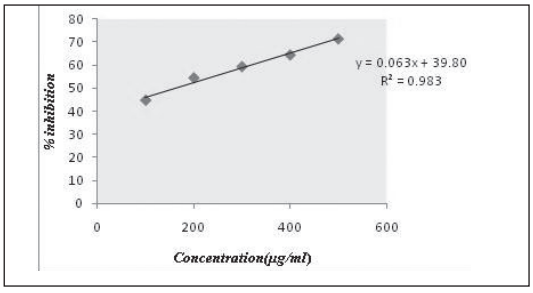

Figure - 3 : Calibration curve of HP extract

Anti oxidant activity by Total antioxidant assay :

The total anti-oxidant potential of the test extract was determined using standard graph of ascorbic acid (Figure 4).The test extract exhibited very good antioxidant activity which was equivalent to 0.3762 mg / gof ascorbic acid and the results were depicted in table no.3.

Table No. 3 - Total antioxidant activity of HP

Sample (1mg/ml)	Absorbance (695nm)	Concentration from graph(mg/g)
HP	0.193±0.0057	0.355

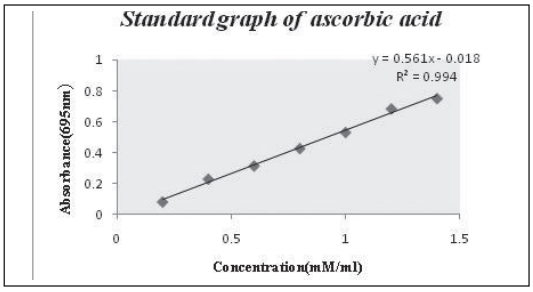

Figure - 4 : Standard graph of Ascorbic acid

Anti Inflammatory activity by Protein denaturation

The HP extract showed significant inhibition ($P<0.05$) of protein denaturation and the percentageinhibition was found to be 75.32% at a concentration of 500µg/ml. A comparison of the anti inflammatory activity of HP extract and standard diclofenac sodium was given in figure 5.

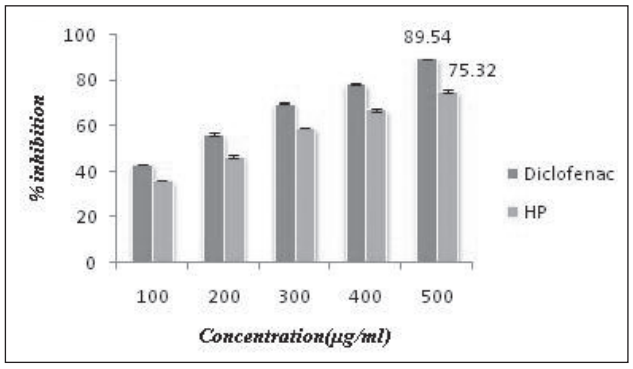

Figure - 5 : Inhibition of protein denaturation

Anti Inflammatory activity by Proteinase inhibitory action

The in vitro anti inflammatory activity of HP extract was calculated and compared with standard Diclofenac. A significant inhibition ($P<0.05$) was shown by the test extract. At 500µg/ml the test extract showed apercentage inhibition of 70.95% and displayed in figure 6.

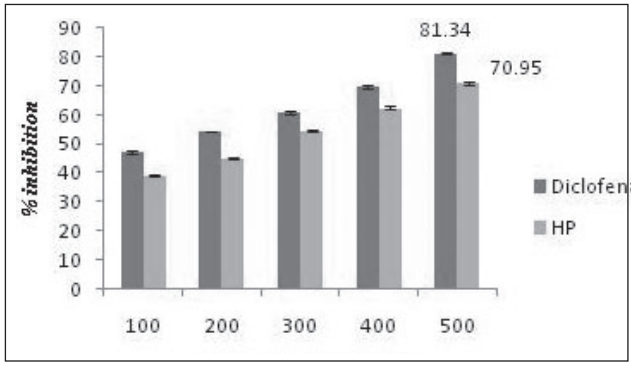

Figure - 6 : Proteinase inhibitory action

DISCUSSION AND CONCLUSION

The present study was designed to evaluate the anti inflammatory and anti oxidant potential of HP extract. The antioxidant potential was evaluated by two in-vitro models i.e. iron chelating assay and total antioxidant assay. In iron chelating assay, the ferric ions are reduced in presence of antioxidants to ferrous ions. O-phenanthrolineforms chelates with ferrous ions to form orange red complex whose intensity is measuredat 510nm. Thus the percentage inhibition at different concentration was calculated from absorbance obtained[7]. The standard used was Ascorbic acid and the IC50 value of test was one-third of that of standard. The total antioxidant assay based on reduction of Phosphate-Molybdenum (VI) to Phosphate-Molybdenum (V) and subsequent formation of a green complex at acidic pH. As the reducing capacity increases the extent of reduction of Mo(VI) increases and can be measured by the increase in green complex formation[8].The antioxidant potential of the extract was measured as mg/g of ascorbic acid equivalent, which was found to be equivalent to 0.3762.

The anti-inflammatory activity was evaluated by in-vitro models i.e. Proteinase inhibition and protein denaturation. The in-vitro anti inflammatory activity of test with respect to proteinase inhibition and protein denaturation was calculated and compared with standard Diclofenac. The results showed a significant inhibition by the test sample which was comparable with the standard at a concentration of 500µg/ml.Neutrophils are known to be a source of proteinase which carries in their lysosomal granules many serine proteinases. It was previously reported that leukocytes proteinase play important role in the development of tissue damage during in inflammatory reactions and significant level of protection was provided by proteinase inhibitors[8, 10]. Proteinase enzyme causes the breakdown proteins and also stimulates the release of many inflammatory mediators thereby causing tissue damage and inflammation. Thus by inhibiting proteinase inflammation can be controlled. In the proteinase inhibition assay, trypsin causes the breakdown of protein i.e., casein which will cause inflammation, but this effect was controlled by the test extract which inhibited the action of trypsin. The percentage inhibition was calculated at different concentration of test extract and compared with standard Diclofenac. The results showed an inhibition of 70.95% at 500µg/ml. Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat[8,9]. In the inhibition of protein denaturation assay, an external stress was applied in the form of heat to the bovine serum albumin, which results in the loss of its tertiary structure, but this process was inhibited by the extract to about 75.32% at a concentration of 500µg/ml.Preliminary phytochemical analysis of the aqueous extract, showed the presence of alkaloids, flavonoids and phenolics, saponins, carbohydrates, amino acids and triterpenoids. Phytochemical constituents like flavonoids and phenolics have been found to offer protection from oxidation. The anti inflammatory activities of

alkaloids, saponins and triterpenoids also have been proved by earlier research. Thus the presence of these constituents in the HP sample may be the reason behind its antioxidant and anti-inflammatory potential.

HP is a common ornamental plant in India and so should be promoted as an anti-inflammatory remedy. However, further research in animals is needed to establish its therapeutic utility.

ACKNOWLEDGEMENT

The authors are thankful to the Principal, University College of Pharmacy, Cheruvandoor, MG University Campus, Kottayam, Kerala, for providing all the necessary facilities to carry out this research work.

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Dissertation:- KaplanSJ. Post-hospital home health care; the elderly's access and utilization [dissertation]. St. Louis (MO): Washington Univ.; 1995.

Patent:- Nilani. P, Sankar. V, Chandrasekharan. A. K. Herbal Ant Repellant. INDIA patent: 2007-09-14, filed 2004-09-29.

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Published by : Principal, PSG College of Pharmacy, Peelamedu, Coimbatore
Printed at : Ace Data Prinexcel Private Limited, Peelamedu, Coimbatore, Ph:0422-2561500
Chief Editor : Dr. M. Ramanathan, PSG College of Pharmacy, Peelamedu, Coimbatore
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