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The Comparison of Hemodynamic Variations with Clonidine and Dexmedetomidine as Adjuvant with Bupivacaine in Spinal Anesthesia.

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ABSTRACT

We compared the heart rate and mean atrial pressure as hemodynamic variations after administration of intrthecaldexmedetomidine or clonidine with bupivacaine. Methodology: 90 patients were randomly divided into three groups, Bupivacaine-Clonidine group (BC) received 30mcg clonidine with 3.5ml of 0.5% bupivacaine, Bupivacaine –Dexmedetomidine group (BD) received 5mcg of Dexmedetomidine with 3.5ml of 0.5% bupivacaine& group (B) received 0.5% bupivacaine -3.5ml as intrathecal. The hemodynamic parameters MAP & HR were recorded by the help of standard monitor &standardized data entry format.The data collected were both descriptive and non-descriptive used for result analysis .The statistical constants arithmetic mean and standard deviation were computed to get valid interference about the data for comparison. In order to see whether the significant difference, the ANOVA test and chi square test were applied using SPSS. A p value of less than 0.05 was considered statistically significant.Results:we noticed that heart rate was slightly more decreasein BD than BC group after induction of drug totill 25 minutes but it was not statistical significance. In mean atrial pressure,after 5 minutes of intrathecaladministration, there was drastic decreased in MAP in BD group than other groups till 20 minutes. There was significant decrease in dexmedetomidine. When compared the hemodynamic variations among various groups, it concluded that there was more hemodynamic disturbance with dexmedetomidinethan other groups.

Key words: *Hemodynamic, Bupivacaine, Clonidine, Dexmedetomidine, HR, MAP*

INTRODUCTION

Spinal anaesthesia is an effective and safe alternative to general anaesthesia when the surgical site is located on the lower extremities and lower abdominal region. It has got the benefit of being, cost-effective, easy administration technique, quick onset of action, with relatively less adverse effects and most importantly patient remaining aroused throughout the procedure[1-2]. Surgical procedure that is often performed under general anesthesia have side such as postoperative nausea and vomiting, short-term cognitive impairment, prolonged sedation and early postoperative pain may be undesirable in outpatients, elderly and cardiovascular compromised patients[3].

Spinal anesthesia with bupivacaine is administered routinely for lower limb surgeries. Several authors have found that low prevalence rates of intraoperative hypotension with low dosages of intrthecal bupivacaine and levobupivacaine for hip surgery[4-5]. Bupivacaine used for spinal anesthesia are mostly available as hyperbaric solution and it is well established that addition of dextrose to increase specific gravity of the solution alters the anesthetic profile[6-7]. The α2 agonists dilate post-stenostic coronary vessels and attenuate the severity of perioperative hemodynamic abnormalities[8-9]. Clonidine,a selective partial α2 adrenergic agonists that cause a fall in the heart rate and blood pressure with

decreased systemic vascular resistance and cardiac output[10]. Dexmedetomedinehasan α1/α2 selectivity ratio which is eight times higher than the clonidine. It is highly potent and specific to α2 agonist with ashorter duration of action[11-12]. In current study we haveto compare hemodynamic variations between clonidine and dexmedetomidine.

METHODOLOGY

After obtaining approval from institutional ethical committee, (IEC No –NCP/IEC/2016/No.028), a total 90 patients with both genders, aged 18-70 years of physical status American society of Anesthesiologist I or II satisfying inclusion criteriawere accepted. On preanesthetic visit, the patients were explained about the study purpose and after informed consent were obtained. Patients were fasted for 8 hours and premedicated with ranitidine 150mg and metoclopramide 10mg in night before surgery. Patients with lower abdominal and lower limb surgery were randomly allocated in to three groups Bupivacaine -Clonidine group (BC) received 30mcg clonidine with 3.5ml of 0.5% bupivacaine, Bupivacaine -Dexmedetomidine group (BD) received 5mcg of dexmedetomidine with 3.5ml of 0.5%bupivacaine, 0.5%bupivacaine 3.5ml group (B) intrathecally. On arrival in the induction room, standard monitor, ECG, NIBP wereattached &baseline HR, MAP were recorded by using standardized data entry sheet. A Lumbar puncture was done at the L3-L4 level through the midline approach using a 25-gauze quinckebabcock needle with hole pointing upwards.The patients and the investigators were blinded to the treatment groups, and all recordings were performed by an anesthesiologist blinded to group allocation. Patients were then turned supine and 5 min after subarachnoid block. Hemodynamic parameters of the patient were noted before the intuition of the drug (basal), and then after every 5 minutes up to 30 min and then every 10 min up to 60 then later every 15 min until 2 hour. Any episode of hypotension or bradycardia during perioperative. Hypotension was defined a 20% reduction in systolic blood pressure from baseline value. Intravenous fluid was ready to treat hypotension and, whenever needed, atropine 0.3mg intravenous was prefilled when the heart rate dropped to 50 beats /min or < 20% of the basal value.

Statistics analysis

The data collected were both descriptive and non-descriptive used for result analysis. The statistical constants like arithmetic mean; standard deviation, etc. were computed to get valid interference about the data for comparison. In order to see whether the significant difference, the ANOVA test and chi square test were applied using SPSS. A p value of less than 0.05 was considered statistically significant.

RESULTS

Patients in all the three groups were comparable with respect to their gender, age, body mass index, baseline HR and MAP. The males were dominant in all the groups. The mean age group was midlife in all the groups but there were no significance difference among the groups

Table 1: Comparison of demographics of study population within groups

parameters	Group BC	Group BD	Group B	P Value
Age (yrs.)	45.27±15.45	46.9±19.57	45.93±13.40	0.927
Weight(kg)	57.67±8.69	56.17±12.05	56.53±9.41	0.837
Height (mtrs)	1.62±0.08	1.62±0.07	1.60±0.06	0.449
BMI (kg/m2)	21.86±2.46	21.32±4.21	22.03±3.36	0.699
Sex (male: female)	16:14	16:14	18:12	-
Baseline HR (beats/min)	77.37±9.87	78.27±7.37	75.93±12.72	0.673
Baseline MAP (mmHg)	91.53±9.55	92.93±9.50	90.77±10.88	0.697

In heart rate, from baseline to the induction of drug, there was no change in heart rate seen in any either groups but after 5 minutes, slightly decreased in BD and BC groups till 25 minutes then started rising and maintained.

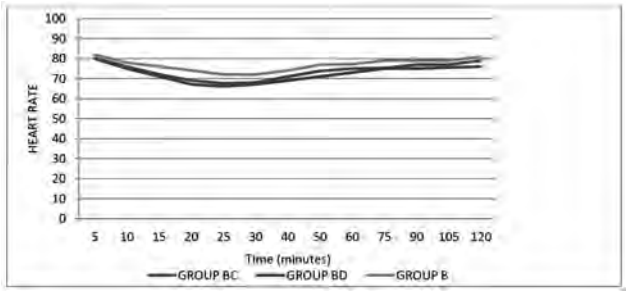

Figure 1: Comparison of heart rate during surgery

Mean heart rate of both the groups were above the 70/ min showing the hemodynamic stability in clonidine and dexmedetomidine groups at given doses

In mean atrial pressure, from induction of drug to 5minutes, there was not much difference seen in all the groups. But after 5minutes, we obtained drastic decreased in MAP in BD group than other groups till 20 minutes. After that, MAP started rising till the end of surgery

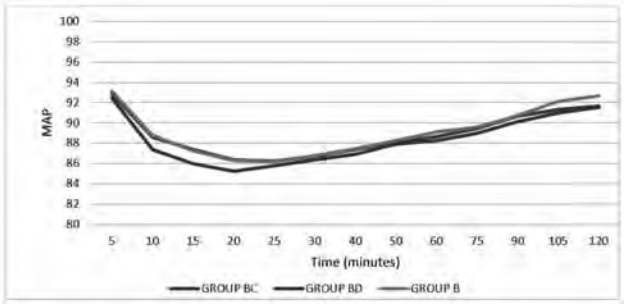

Figure 2: Comparison of mean atrial pressure during surgery

Table 2: Comparison of heart rate during surgery

INTERVAL	GROUP BC		GROUP BD		GROUP B		Between groups difference (p value)		
	Mean	SD	Mean	SD	Mean	SD	BC vs BD	BC vs B	BD vs B
Baseline	78.13	8.27	78.93	7.21	76.06	12.61	0.75	0.42	0.26
5 min	75.00	8.08	76.23	7.05	75.76	12.01	0.62	0.75	0.85
10 min	74.53	8.02	74.81	7.23	75.66	10.43	0.90	0.61	0.69
15 min	73.83	7.91	74.06	7.22	75.33	9.96	0.91	0.48	0.55
20 min	73.21	7.89	73.16	7.22	75.56	9.86	0.98	0.08	0.07
25 min	72.03	7.80	72.56	7.29	76.46	9.88	0.95	0.07	0.06
30 min	72.56	6.54	73.13	7.92	76.76	9.88	0.79	0.16	0.09
40 min	73.00	6.67	73.13	7.91	76.00	9.34	0.80	0.11	0.24
50 min	73.72	6.67	74.81	7.06	76.86	8.93	0.57	0.14	0.26
60 min	74.23	6.88	75.92	7.19	77.13	8.63	0.39	0.41	0.53
75 min	75.16	6.77	76.03	6.90	76.73	8.37	0.65	0.50	0.71
90 min	75.44	7.13	76.53	6.89	76.76	8.28	0.58	0.48	0.90
105min	75.56	7.2	76.96	6.78	76.93	8.64	0.47	0.60	0.98
120min	76.23	7.5	77.41	6.73	77.26	8.56	0.60	0.43	0.91

BC(Bupivacaine + clonidine), BD (Bupivacaine+ dexmedetomidine), B (plain bupivacaine)

Table 3: Comparison of heart rate during surgery

TIME INTERVAL	GROUP BC		GROUP BD		GROUP B		Between groups difference (p value)		
	Mean	SD	Mean	SD	Mean	SD	BC vs B D	BC vs B	BD vs B
Baseline	94.23	9.10	94.89	9.41	94.34	8.35	0.841	0.875	0.966
5 min	92.89	9.02	92.50	8.77	93.11	7.55	0.649	0.256	0.495
10 min	88.56	8.73	87.35	8.05	86.76	7.38	0.566	0.049*	0.013*
15 min	87.36	8.35	86.00	8.73	88.00	6.98	0.818	0.013*	0.002**
20 min	86.36	8.07	85.76	7.97	86.30	6.98	0.840	0.013*	0.002**
25 min	86.23	8.14	86.34	7.43	86.20	6.88	0.547	0.025*	0.002**
30 min	86.70	8.33	86.90	7.24	86.10	6.80	0.335	0.031*	0.015*
40 min	87.40	8.50	87.90	6.86	87.43	6.06	0.319	0.057	0.056
50 min	88.13	9.11	88.23	7.10	88.79	5.85	0.400	0.066	0.058
60 min	88.66	7.31	89.00	7.31	89.12	5.59	0.646	0.071	0.059
75 min	89.45	7.49	90.10	7.49	89.60	5.50	0.672	0.074	0.061
90 min	90.63	8.05	91.18	7.86	90.73	5.23	0.641	0.410	0.198
105min	91.33	7.99	91.33	7.97	92.11	5.03	0.483	0.611	0.273
120min	91.66	8.11	91.55	8.40	92.06	4.95	0.765	0.632	0.329

*significance, **highly significance

DISCUSSION

The α2 adrenergic agonists including clonidine and dexmedetomidine, lower central sympathetic outflow by acting like a brake and modify intraoperative cardiovascular and endocrine responses surgical stimuli[13]. Clonidine is very well evaluated, established and widely used α2 agonist as an adjunct to regional anesthesia in last decade but there has been need for clinical studies related to dexmedetomidine to prove its hemodynamic stability safety, efficacy and suitable dose for intrathecal administration[14-15]. Most studies using very low doses of intrathecal clonidine such as 15-30 mcg in humans found no hemodynamic instabilityand using 37.5-150 mcg of clonidine intrathecally reported significant hypotension and bradycardia[16-17]. Dexmedetomidine has been used as intravenous in doses ranging from 0.1 to 10 μg/kg/h but higher doses have associated with a significant incidence of bradycardia and hypotension[18].

In our study, heart rate was decreased more by addition of dexmedetomidine to bupivacaine as compared to clonidine, although it was not statically significant to other groups. Agrawal A et al in their study found that a fall in heart rate was seen in all groups compared to baseline value. The values were lowest in Group 1 at all observations. One patient in group 1 and two patients in group 2 had bradycardia[19]. In our study, we didn't observe heart rate below 60 beats or any episode of bradycardia. Reddy VS et alfound that trend of mean HR in the dexmedetomidine groups appeared to lower than that of clonidine and placebo groups, but there was statistically significance difference among the groups except at 5mins after spinal anesthesia[20]. MS

saravanaBabu et al in their study found that there was no significant difference of heart rate in both groups at the time of administration of drugs, but it decreasedat 30 min post -injection, there was a fall in both the groups. And this decrease was significant in the RC group compared to RD group but none of the patient showed bradycardia at any time[21].This was similar to the findings of Dobrydnjov et al, where only one patient each in 15 μg and 30 μggroups needed vasopressor or atropine[22]. Kumar SK et alobserved the trend of mean heartrates in the dexmedetomidine group appears to be lower than that of clonidine and control groups, but there is no significant difference among the groups except at 5 min after spinal anesthesia where the mean heart rate was significantly lower[23] (P = 0.0299).Lee HM et al found that the loading dose was infused over 10 min, and there were no significant differences (1 vs3 vs3) among the three groups[24]. This decrease in heart rate may be related to decreases in plasma catecholamine concentrations and the sympathetic outflow caused by α2-adrenergic activation[24].

MS saravanaBabu et al in their study found that there was no significant difference of mean arterial blood pressure in both groups at the time of administration of drugs, but it decreasedat 30 min post -injection, there was a fall in both the groups. There was a decreasing trend of mean arterial pressure post -injection in both group and this decrease was significant in the RC group compared to RD group but none of the patient showed bradycardia or hypotension at any time[21].

In present study we noticed that there was no significant drop in MAP among the groups before administration of study drugs but a significant decreased in MAP between BC group & B group, and BD group & B group at 10 minutes to 30 min intervals, but there was no statically significant between BD and BC groups (Table No. 3). We didn't get any episode of hypotensive patient in any of our groups. The mean atrial pressure didn't drop lower than 85mmHg. Reddy VS et al studiedthe trend of MAP showed no significance difference in MAP among the group before administration of premedication but both dexmedetomidine and clonidine had significantly lower MAP after premedication up to 5min after spinal anesthesia when compared saline. Dexmedetomidine and clonidine were not differing significantly after premedication but after 2 to 5 minutes of administration of spinal anesthesia,MAP was lower in Dexmedetomidine group[20].

Khanzi et al also claimed that addition of dexmedetomidine or clonidine to bupivacaine didn't cause a significant decrease in the blood pressure intra-operatively or post-operatively[25]. In Kumar SK et al study, the trend of MAP, showed no significant difference in MAP among the groups before administration of premedication but both dexmedetomidine and clonidine group had a significantly lower MAP after premedication[23]. The decrease in mean arterial pressure may suggest that intrathecal local anesthetics block the sympathetic outflow and reduce the blood pressure[26].

CONCLUSION

The addition of dexmedetomidine to intrathecal bupivacaine decreases more heart rate and mean atrial pressure when compared to clonidine with bupivacaine or plain bupivacaine. Thereason that dexmedetomidine is highly specific to α2 agonist with a shorter duration of action and decreases plasma catecholamine concentrations and the sympathetic outflow caused by α2-adrenergic activation.

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Pharmacological Validation of Dhanavantram Kwatham for Analgesic, Anti-Inflammatory and Anti-Arthritic Activity Using Experimental Animal Models

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ABSTRACT

Emerging evidences are indicating that Ayurvedic medicines are effective against many chronic disease conditions with less side effects and also exhibiting cost-effectiveness. Currently, Dhanavantram kwatham (DKM), a poly herbal formulation is well known for treatment for pain management, chronic inflammation associated disorders, paralysis and arthritis like conditions in south part of India. Though it is showing beneficial effects in Ayurvedic practice, but the pharmacological validations for DKM has not been elucidated. Thus, the present study is planned to validate the analgesic, anti-inflammatory and anti-arthritic effect of DKM in experimental animal models. The analgesic activity was carried out using acetic acid induced writhing model in mice. The inflammation in rats was induced by administration of carrageenan in rat hind paw and adjuvant arthritis in rats was induced by administration of sub-plantar injection of Freund's complete adjuvant (FCA). The results indicate that in comparison to DKM (250 mg/kg), DKM (500 mg/kg) treated group has shown significant reduction in number of writhing, paw volume and paw-edema and also this effect was comparable with diclofenac treated group. Hence DKM is exhibiting analgesic, anti-inflammatory and anti-arthritic effect. This poly herbal formulation could be an effective formulation in clinical conditions like rheumatoid arthritis where the pain and inflammation play a major role.

Key words: *Ayurveda, Inflammation, Analgesic, Poly herbal, Rheumatoid arthritis*

INTRODUCTION

Pain and inflammation are major events in the several pathological conditions like arthritis, trauma, tissue damage and injury. Inflammation is a protective response that involves immune cells, blood vessels, and molecular mediators, more over inflammation and pain are interlinked[1]. Tissue injury is initiating the inflammation and onset the various biochemical reactions which sensing the pain via stimulating nervous system. The chronic inflammation triggering the adaptive changes in the nervous system which will be exaggerated the pain sensation[2]. Rheumatoid arthritis (RA) is a chronic progressive disease resulted from autoimmune reactions in the body leads to inflammation in the joints and painful deformity and immobility, especially in the fingers, wrists, feet, and ankles. RA is more common in women and the prevalence various from 0.3% to 1% in developed countries[3]. Currently there is no effective treatment available for RA and existed agents for RA is exhibiting more drugs related side effects. Ayurvedic, Siddha, Unani and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurvedic is most developed and widely practiced in India. Reports are implicating that Ayurvedic medicines are effective against many chronic disease conditions like RA with less side effects and also minimizing the treatment expenses with better outcome[4]. Recently, Dhanavantram Kwatham (DKM), a poly herbal formulation is well known for treatment for multiple disorder viz. pain, chronic inflammatory diseases, paralysis, arthritis and abdomen pain in south part of India. It contains Himalayan Cedar, Cardamom- Lesser Cardamom, Cinnamon-Ceylon Cinnamon, Liquorice –

Licorice, Arjuna, Bael Tree, Country Mallow, Indian Gooseberry- Emblic Myrobalan, Indian Sarsaparilla and Indian Valerian as constituents. Though it is showing beneficial effects in several pathological conditions, but the pharmacological validations for DKM has not been elucidated. Hence this present study is planned to validate the analgesic, anti-inflammatory and anti-arthritic activity of DKM using experimental animal models. Validating and interlinking analgesic, anti-inflammatory and anti-arthritic activity of DKM may bring new therapeutic knowledge in the Ayurvedic practice for the therapy of pain management, chronic inflammation which contribute major role in RA like chronic conditions.

2. METHODOLOGY

2.1. Experimental Animals

Swiss albino male mice (25-35 g) were used in the study for analgesic activity. Adult male wistar rats (180-250 g) were used in this study for anti-inflammatory and anti-arthritic activity. The animals were supplied from the central animal house facilities, JSS College of Pharmacy, Ooty. All the animals were housed in a separate polypropylene cage in a good ventilated room and were maintained at 25±2°C temperature and 55% relative humidity (RH) conditions with a 12h light/ dark cycle. The animals had free access to food and water ad libitum. All the experimental animals were acclimatised at least for two weeks to adapt to the laboratory conditions before starting the experiments. All the animal experimental procedures were carried out according to the "Guide for the Care and Use of Laboratory Animals" (Indian Council of Medical Research). Institutional Animal Ethical Committee (IAEC), JSS College of Pharmacy, Ooty has approved the study protocol (Proposal No. JSSCP / IAEC / M.PHARM / PH. COLOGY / 09/ 2014-15).

2.2. Chemicals

Dhanavantram Kwatham (DKM) was procured from Arya Vaidya Sala, Kottakkal, Kerala, India. Carrageenan and Freund's complete adjuvant (FCA) was purchased from from Sigma Aldrich, USA. All other chemicals, reagents and solvents were of analytical grade unless mentioned.

2.3. Treatment groups

This study includes five groups which are as follows, the first and second groups were control and vehicle treated, the third group received DKM (250 mg/kg), whereas the fourth group was treated with DKM (500 mg/kg). The fifth group was administered with standard Diclofenac (10 mg/kg). The same groupings were followed for all three activities and each group was kept with 6 animals. The dose selections were made according to traditional practice.

2.4. Drugs preparation and administration

DKM (250 & 500 mg/kg), diclofenac (10 mg/kg) were suspended in 0.3% carboxymethyl cellulose (CMC) and administered orally 30m prior to the experimentations in respective groups, whereas control and vehicle groups received normal saline. The dose calculations and administrations were made in each animal according to surface ratio method.

2.5. Analgesic activity-Acetic acid induced writhing model

After 30m of drugs administration, 1% acetic acid is administered through intraperitoneally and then the number of writhing response was calculated for 10 minutes[5].

2.6. Anti-inflammatory activity- Carrageenan induced paw edema model

After 30m of drugs administration, 0.1% of Carrageenan (0.1ml) was administered into the sub plantar tissue of right hind paw of all the animals. After administration, 1h, 2h, 3h and 4h the paw volume was measured by the digital plethysmograph[6]. The volume of mercury displaced (mm3) while keeping edematous paw is indicating the volume of paw edema formation.

2.7. Anti-arthritic activity- Freund's complete adjuvant (FCA) model

The adjuvant arthritis in rats was induced by administration of sub-plantar injection of 0.1ml of Freund's complete adjuvant (FCA). The sub-plantar injection of FCA produces local edema after few hours with a progressive increase in size then it reached its maximum on 21st day. On 1, 7, 14 and 21st day after FCA injection, the paw-edema volume of each rat was measured thoroughly using Digital Plethysmometer

(UGO Basile)[7]. The changes in the body weight were measured and the volume of mercury displaced (mm3) while keeping edematous paw is indicating the volume of paw edema formation followed by reduction upon treatment.

2.8. Statistical Analysis

Data were expressed as mean ± SEM. Statistical significance between the groups were analyzed by one way ANOVA, followed by Dunnett multiple comparison tests using Graph Pad Prism, 4.03 (San Diego, USA). Alteration in the body weight of FCA administered rats between first and 21st day was analyzed by unpaired student t-test. Probability levels less than 0.05 ($p<0.05$) were fixed as the criterion for statistical significance.

3. RESULTS AND DISCUSSION

3.1. Analgesic activity

Administration of acetic acid in mice has significantly increased ($p<0.001$) the number of writhing response in comparison to control mice indicates the induction of pain stimulus. Treatment with DKM (500mg/kg) has remarkably decreased ($p<0.001$) the writhing response in comparison to vehicle treated group. Interestingly the activity exhibited by DKM (500mg/kg) is comparable with standard diclofenac ($p<0.001$). Treatment with DKM (250mg/kg) did not show any significant analgesic activity (Table No.1). The results emphasized that DKM (500mg/kg) is exhibiting analgesic activity mainly through attenuating peripheral prostaglandins release, because earlier reports indicate that acetic acid induced pain signals transmitted to central nervous system through release of prostaglandins which contributes to the increased sensitivity to nociceptors resulted writing response[8].

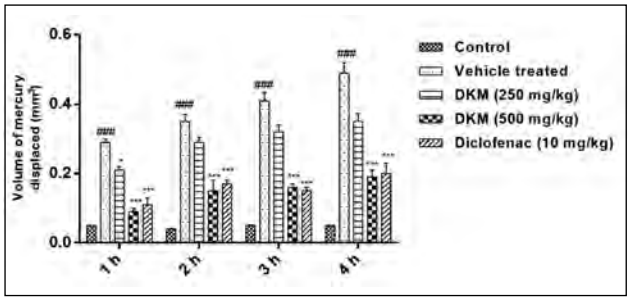
Table No. 1: Effect of DKM on acetic acid induced writhing response in mice

S. No	Treatment Groups	Number of writhing	% decrease in writhing response
1	Control	0.00±0.0	--
2	1% Acetic acid	73.17±1.68###	--
3	DKM (250 mg/kg)	52.31±1.75	28.50%
4	DKM (500 mg/kg)	38.17±0.62***	47.83%
5	Diclofenac (10 mg/kg)	34.83±0.87***	52.39%

Data are expressed as mean ± SEM. Superscript ### denotes $p<0.001$ vs control; *** denotes $p<0.001$ vs acetic acid administered group respectively significance with one way ANOVA followed by Dunnett-t test.

3.2. Anti-inflammatory activity

Fig. 1: Effect of DKM on Carrageenan induced paw edema volume in rats



Time in hours after Carrageenan administration

Data are expressed as mean ± SEM. Superscript ### denotes $p<0.001$ vs control; *, *** denotes $p<0.05$, $p<0.001$ vs carrageenan administered group respectively significance with one way ANOVA followed by Dunnett-t test.

Fig. 1 represents that administration of carrageenan in hind paw region of rats has significantly increased the paw volume gradually from 1h ($p<0.001$), 2h ($p<0.001$), 3h ($p<0.001$) to 4h ($p<0.001$) in comparison to control rats indicates the formation of edematous and induction of inflammation. Treatment with DKM (250 mg/kg) has significantly decreased the paw volume in 1h ($p<0.05$) and fail to decrease the paw volume in 2h, 3h and 4h. Administration of DKM (500 mg/kg) has remarkably decreased the paw edematous formation in 1h ($p<0.001$), 2h ($p<0.001$), 3h ($p<0.001$) and 4h ($p<0.001$) indicates the intensity of anti-inflammatory activity and this effect is similar like diclofenac standard treatment in all time intervals ($p<0.001$).

Carrageenan-induced paw edema consists two phases, the first phase (1-2h) inflammation is mediating through release of histamine or serotonin and the second phase of edema is occurred because of release of prostaglandin[9]. In the present study, treatment with high dose of DKM is inhibiting the paw edema formation in 1, 2, 3 and 4th hours after carrageenan administration indicate that

DKM might have decreased the release of histamine, serotonin and prostaglandins in both the phases resulted in anti-inflammatory activity.

3.3. Anti-arthritic activity

a. Body weight

The FCA administered rats has shown significant reduction ($p<0.01$) in the body weight indicates the incidence and occurrence of arthritis in the rats. Interestingly treatment with DKM (500 mg/kg), Diclofenac (10 mg/kg) did not decrease the body weight of FCA administered rats, but DKM (250 mg/kg) treated rats shown slight reduction in the body weight which is represented in table no. 2.

Table No. 2: Effect of DKM on body weight of FCA administered rats

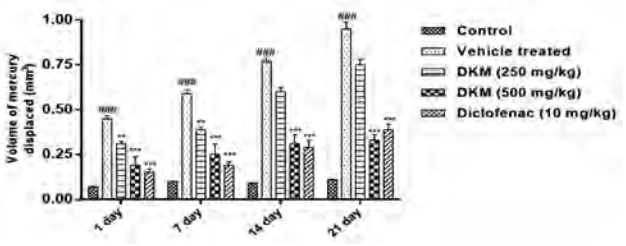
S. No	Treatment groups	Body weight (g)		% alterations in body weight
		1st day	21st day	
1	Control	223.20±2.57	229.11±1.98	2.62%
2	1% Acetic acid	219.15±1.47	196.23±1.54##	-10.50%
3	DKM (250 mg/kg)	210.19±2.87	200.77±2.25	-4.76%
4	DKM (500 mg/kg)	236.05±2.54	239.21±3.15	1.25%
5	Diclofenac (10 mg/kg)	229.25±1.99	233.67±2.23	1.17%

Data are expressed as mean ± SEM. Superscript ## denotes $p<0.01$ significance with unpaired student t-test

b. Paw volume

Induction of FCA in rat paw has significantly increased the paw volume gradually from 1st day ($p<0.001$), 7th day ($p<0.001$), 14th day ($p<0.001$) to 21st day ($p<0.001$) in comparison to control rats indicates the induction of arthritis. Treatment with DKM (250 mg/kg) has significantly decreased the paw volume in 1st day ($p<0.01$), 7th day ($p<0.001$) and fail to decrease the paw volume in 14th and 21st day. Administration of DKM (500 mg/kg) has remarkably decreased the paw edematous formation in 1st day ($p<0.001$), 7th day ($p<0.001$), 14th day ($p<0.001$) to 21st day ($p<0.001$) indicates the efficacy of anti-arthritic activity and this effect is comparable with diclofenac standard treatment in all days ($p<0.001$) which is summarized in fig. 2.

Fig. 2: Effect of DKM on FCA induced paw edema volume in rats



Number of days after FCA administration

Data are expressed as mean ± SEM. Superscript ### denotes $p<0.001$ vs control; **, *** denotes $p<0.01$, $p<0.001$ vs FCA administered group respectively significance with one way ANOVA followed by Dunnett-t test.

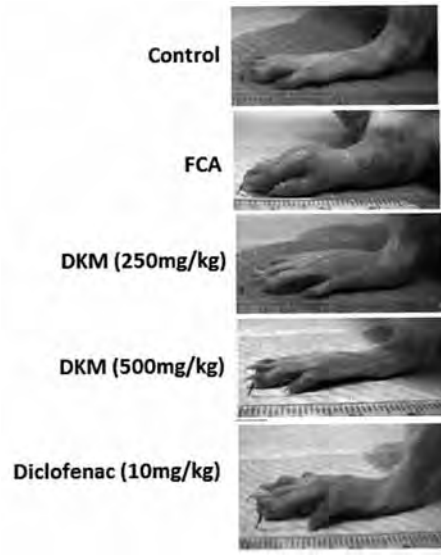
DKM at 500 mg/kg showed significant anti-arthritic activity, and the activity was comparable with that of diclofenac. DKM significantly increased the body weight of animals compared with the arthritic controls evidenced the anti-arthritic activity of DKM. Freund's complete adjuvant induced arthritis model is exactly mimics the pathogenesis of rheumatoid arthritis for testing various agents and this model is characterized by a very rapid erosive disease. The bacterial peptidoglycan and muramyl dipeptide present in the FCA are responsible to enhance the cytokines in the joints resulted induction of adjuvant arthritis[10]. The treatment with DKM (500 mg/kg) has remarkably decreased the paw volume and paw edematous formation in 1, 7, 14 and 21st days after FCA administration suggest that DKM might have antagonized the cytokines like IL-6, IL-1β and TNF-α thereby it may exhibits anti-arthritic activity in animal models

c. Paw size

The FCA administered rats has shown increased paw size in comparison to control rats whereas DKM (250 mg/kg) has shown slightly reduction in the paw size. Interestingly, treatment with DKM (500 mg/kg) has significantly reduced the paw size indicates the intensity of anti-arthritic activity and standard drug diclofenac also reduced the paw size similar like DKM (500 mg/kg) after 21st day of FCA administration which are depicted in Fig. 3. Adjuvant induced arthritis is non-specific immune response within the joint can also result in inflammatory and erosive disease. DKM is

significantly reduced the paw size in high dose indicates that DKM is potent anti-arthritic formulation, because paw swelling is an index of measuring the anti-arthritic activity[11].

Fig. 3: Represents the effect of DKM on paw size in FCA administered rats on 21st day



4. CONCLUSION

The present study was designed to investigate and validate the Dhanavantram kwatham (DKM), a poly herbal Ayurvedic formulation for analgesic, anti-inflammatory and anti-arthritic activity in experimental animal models. The results have shown that promising analgesic, anti-inflammatory and anti-arthritic activity of Dhanavantram Kwatham in experimental animal models. In general, drugs which could control the inflammation as well as pain may be a potential agent for the treatment of chronic disease conditions like rheumatoid arthritis and osteoarthritis where the inflammation and pain play a major role. Present study finding supports the traditional claims and provides a scientific basis for analgesic, anti-inflammatory and anti-arthritic effect of DKM (500 mg/kg). So it is hoped that these studies will support the intake of DKM for the treatment of arthritis and this poly herbal formulation could be an effective formulation in clinical conditions. The mechanism of DKM may be attributed through attenuating prostaglandins, serotonin, histamine and cytokines in chronic inflammatory conditions like arthritis and further clinical validation of the DKM is needed.

Conflict of interest

The authors declare no conflicts of interest in this research

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Antibiotic Susceptibilities of *Nocardia asteroides* Isolated from Ocular Specimens

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ABSTRACT

Nocardia, an actinomycete, is becoming increasingly important as a cause of ocular infection in south India. A total of 39 isolates of *Nocardia asteroides* were obtained from four different ocular complications such as corneal ulcer (n=25), endophthalmitis (n=8), preoperative cases (n=5) and other infections (n=1). In the disk diffusion test 98%, 96.67% and 94.74% of *Nocardia* isolates were sensitive to amikacin, moxifloxacin, and gentamycin & vancomycin, respectively. Based on the MIC values, it is recommended that levofloxacin, amikacin, moxifloxacin, tetracycline and vancomycin as suitable antibiotics for the treatment of ocular infections caused by *Nocardia* spp.

Key words: *Ocular infection, Nocardia, Disk diffusion test, Minimum Inhibitory Concentration (MIC).*

INTRODUCTION

The actinomycetes comprise a group of prokaryotes that have the ability to form Gram-positive, branching filaments of less than 1 μm in diameter. The main animal pathogens in the actinomycetes are the genera Actinomyces, *Nocardia and Dermatophilus*. Clinically, infection by the *Nocardia* is rare despite their widespread distribution; the most common being pulmonary infections although primary lesions of brain and kidney have been described. Ocular nocardiosis is an uncommonly reported clinical entity. In the eye, *Nocardia* have been described as a cause of suppuration in the lacrimal sac; in lesions of the conjunctiva and in cases of keratitis (Bruce et al., 1942). They also cause scleritis, conjunctivitis, canaliculitis, dacryocystitis, orbital cellulitis, and endophthalmitis. Corneal infection is by far the most common ocular infection caused by *Nocardia* and is reported after accidental and surgical trauma including refractive surgery (Garg et al., 2012). Infection may develop after minor trauma to the eye in healthy individuals, following ocular surgery such as cataract extraction, or following hematogenous dissemination in immuno-compromised patients. Ocular pathology of nocardiosis includes uveitis, exudative choroiditis, retinal abscess, retinal detachment and keratitis. Nocardial endopathalmitis is associated with a high mortality, and survivors have invariably had total blindness in the involved eye (Brown-Elliot et al., 2006). *Nocardia* keratitis generally runs a prolonged course. The usual presenting symptoms are pain, photophobia, blepharospasm, and lid swelling (Parsons et al., 1989), ulcer often has a grey, sloughing base and undermining overhanging necrotic edges (Duke et al., 1965). Among the various species of *Nocardia* responsible for ocular infection, N. asteroides has been found to be the most common causative species of keratitis. *Nocardia* spp. are also capable of evading the host's bactericidal mechanisms (Prajna et al., 2007). The dry, dusty, and often windy conditions areas may

facilitate the aerosolization and dispersal of the *Nocardia* and thus enhance their acquisition via inhalation of the fragmented cells (Brown et al., 2006). An understanding of the epidemiological features, risk factors and etiological agents that occur in a specific region are important in rapid recognition, timely institution of therapy, optimal management and prevention of ocular disease due to *Nocardia* sp. *Nocardia* are capable of establishing resistance to this lysozyme subsequently helping for pathogenesis. The virulence factors also include enzymes catalase and superoxide dismutase (that catalyzes the conversion of superoxide to hydrogen peroxide and oxygen), as well as a cord factor (CF), is a cell wall glycolipid seen in Mycobacterium, *Nocardia*, Rhodococcus, and Corynebacterium (which interferes with phagocytosis by macrophages by preventing the fusion of the phagosome with the lysosome) (Spargo et al., 1991). Beaman et al. (1979) reported that *Nocardia* are able to grow within macrophages, and is resistant to phagocytic attack. Host neutrophil mobilization can inhibit *Nocardia* but does not kill them. Infection progresses after the initial inhibition by neutrophils unless antimicrobial therapy or cytotoxic lymphocytes take over (Bennett et al., 2009).

In order to start specific therapy, it is necessary to do meticulous laboratory investigations, and this includes microscopy and culture of corneal scrapings for identification of the microbial agent. Antibiotic susceptibility testing is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection in vivo. Minimum inhibitory concentration (MIC) is the lowest concentration of antimicrobial agents that will inhibit the visible growth of microorganisms. Evaluation of MIC is indispensable to confirm resistance of microorganisms to an antibiotic. Mostly, *Nocardia* are susceptible to cefamandole, cefotaxime, ceftriaxone, amikacin, and imipenem and resistant to ciprofloxacin, ampicillin, amoxicillin, clavulanic acid, erythromycin (which presumably applies to clarithromycin), and ciprofloxacin. Treatment of *Nocardia* keratitis is very prolonged. Since their discovery, sulphonamides were widely used to treat *Nocardia* in the early years. Sulphacetamide, sulphamethoxazole, and trimethoprim were considered to be the treatment of choice (Lee et al., 2001), until it was discovered that the minimum inhibitory concentrations of amikacin was much lower than the sulphonamides when used to treat ocular nocardial infections (Sridhar et al., 2001). Third generation fluoroquinolones such as gatifloxacin and moxifloxacin have a spectrum of activity against most of the common ocular pathogens, including *Nocardia* (Callegan et al., 2003). Against this background the present study was employed with the following objectives: collection of ocular samples from a tertiary eye care centre and processing the same, so as to isolate *Nocardia* spp.; identification of *Nocardia* to species level by employing standard biochemical tests and to determine the antimicrobial susceptibility pattern for *Nocardia* by disc diffusion method and minimum inhibitory concentrations analysis.

MATERIALS AND METHODS

Ocular specimens and primary processing

All the patients referred to the Ocular Microbiology Department at Aravind Eye Hospital and Post Graduate Institute of Opthalmology, Coimbatore, from September 2013 to August 2014 were included in the present study. The corneal scrapings were collected from suspected cases of keratitis after a thorough examination by an ophthalmologist. The specimens were collected by using a sterile Kimura's spatula aseptically under slit lamp illumination after administrating a local anesthetic such as lignocaine under the supervision of a trained laboratory technician. Specimens from cases of conjunctivitis and endophthalmitis were collected as conjunctivital swab and vitreous & aqueous tap, respectively and processed. Corneal button, eye ball, contact lens and contact lens solution were collected from infected patients aseptically. A part of the specimen was subjected to direct microscopy after Gram staining / KOH wet mount. The specimens were also inoculated on blood agar, chocolate agar & brain heart infusion broth and incubated at 37°C for 24 h and observed for growth after 24 – 48 h. The colonies were also subjected for Gram staining using the standard procedures (Sreekumar et al., 2016)

Characterization of *Nocardia* spp.

The following tests were performed so as to characterize the presumptively identified actinomycete isolates:

lysozyme resistance, casein hydrolysis, xanthine hydrolysis, tyrosine hydrolysis, esculin hydrolysis, urease production, nitrate reduction, carbohydrate (mannitol, rhamnose & sorbitol) fermentation & acetamide utilization (Brown-Elliott et al., 2006) and resistance to tobramycin, cephalosporin & erythromycin (Biehle et al., 1996).

Antibiotic susceptibility testing of *Nocardia* spp. by disk diffusion method

Antibiotic susceptibility testing was performed Muller Hinton (MH) agar supplemented with 5% sheep blood in accordance with the procedure outlined by Clinical and Laboratory Standards Institute [M100-S15, (CLSI, 2011)]. For this experiment, a total of twelve antibiotics (HiMediaTM, Mumbai, India) belonging to eight different classes viz., penicillin (ampicillin, AMP; 2 µg), cephems/ cephalosporins (cefazolin, CFZ; 30 µg), glycopeptides (vancomycin, VAN; 30 µg), aminoglycosides (amikacin, AMK; 30 µg; tobramycin, TOB; 10 µg & gentamicin, GEN; 10 µg), fluroquinolones (ciprofloxacin, CIP; 5 µg, ofloxacin, OFX; 5 µg, gatifloxacin, GAT; 5 µg, moxifloxacin, MOX; 5 µg), sulfonamide (co-trimoxazole, CTM; 25 µg) and phenicols (chloromphenical, CHL; 10 µg), at varying concentrations were used. Exactly, four or five test colonies were selected and transferred aseptically into sterile Muller Hinton (MH) broth. The broth was incubated at 35 - 37°C for 2 to 5 h. The broth culture turbidity was adjusted to turbidity equivalent to that of 1.0 McFarland's standard. A sterile cotton swab was dipped into the culture broth and excess of inoculum was removed by pressing the saturated swab against the inner wall of the culture tube. The entire surface was streaked using swab horizontally, vertically and around the outer edge of the plates to ensure a heavy growth over the surface. The plates were allowed to dry for 5 min. With sterile forceps, the antibiotics were placed at on the agar surface. The discs were gently pressed down with the end of the cotton swab to ensure that the disc adheres to the surface of the agar. The plates were incubated for 24 - 48 h at 37°C. After incubation, the plates were observed for the zone of bacterostatis.

Determination of minimum inhibitory concentrations (MICs) of antibiotics for *Nocardia* spp. by broth micro dilution method [M24-A2, CLSI (2011)]

The test isolates were prepared as mentioned previously. The following antibiotics were used: amikacin, chloramphenicol, cephazolin, cefotaxime (CTX), ciprofloxacin, gentamycin, levofloxacin (LVX), moxifloxacin, ofloxacin, tetracycline (TET), tobramycin and vancomycin. The antibiotic powders were checked for stated potency, expiry date and were stored at 4°C until use. An analytical balance was used for the weighing purpose. The following formula was used to determine the amount of powder or respective diluent for a standard solution.

$$\text{Weight of the powder} = \frac{\text{Volume of the solvent} \times \text{concentration (mg/L)}}{\text{Potency of powder (mg/g)}}$$

The antimicrobial stock solution was prepared at concentration of 10 times the highest concentration to be tested (for example: 128 µg/ml of working standard as desired, a stock solution of 1280 µg/ml was prepared). The antibiotic dilution was prepared for required range as per the range CLSI guidelines.

Table 1: Preparation of antibiotics with their prescribed ranges for MIC analysis from stock solution concentration of 1280 mg/L and water as diluents

S. No.	Antibiotics	Working standard dilution range (mg/L)	Solvent
1	Amikacin	0.008-128	Water
2	Chloramphenicol	0.5-32	Ethanol
3	Ciprofloxacin	0.06-128	Water
4	Cephazolin	0.03-64	Water
5	Cefotaxime	0.-128	Water
6	Gentamycin	0.008-128	Water
7	Levofloxacin	0.03-128	Water
8	Moxifloxacin	0.12-128	Water
9	Ofloxacin	0.12-128	Saturated NaHCO3
10	Tetracycline	0.06-128	Water
11	Tobramycin	0.008-128	Water
12	Vancomycin	0.06-32	Water

For the broth micro dilution procedure, sterile 'U' bottomed 96 well microtiter plates were used for the test. The plates were appropriately labeled and the freshly prepared antibiotic dilution was aliquated (100 µl) in

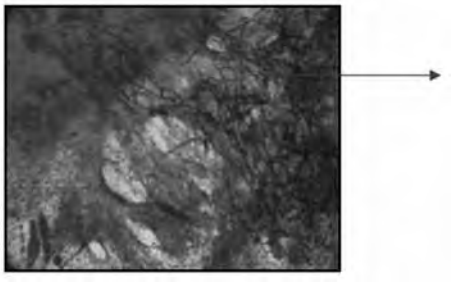
to the designated microtitre well. Exactly, 100 µl of the sterile MHB supplemented with 5% sheep blood and 100 µl of the inoculum was dispensed in to each well. Separate wells were maintained for growth control (100 µl sterile MHB medium supplemented with 5% sheep blood + 100 µl inoculum) and sterility control (sterile MHB supplemented with 5% sheep blood). For quality control, Staphylococcus aureus (ATCC 29213) was used in every batch of MIC analysis. The plates were incubated at 37°C for 24 - 48 h.

The minimum inhibitory concentration is the lowest concentration of an antimicrobial agent at which no visible growth could be detected by visual inspection. The MIC50 was taken as the MIC median value; the MIC90 was the 90th percentile value and represented the concentration of drug that would inhibit 90% of the isolates tested.

RESULTS AND DISCUSSION

Direct microscopic examination by Gram staining revealed the microscopic morphology of the causative agent. The positive smears exhibited branching filaments. These causative agents were further confirmed after the sample processing and subjecting the isolates for Gram staining. Nocardia were seen microscopically as beaded Gram-positive, thin, branching, filamentous organisms

Plate 1: Gram reaction of Nocardia spp. (100 ×)



(Plate 1). Gram staining is the most sensitive method by which used to visualize and recognize Nocardia in clinical samples (Saubolle et al., 2003). In the present study, of a total of 539 ocular specimens processed, 312 fungal isolates and 188 bacterial isolates were obtained. Based on colony characteristics and microscopic morphology a total of 39 isolates were identified as Nocardia spp.

Table 2: Cultural characteristics of Nocardia spp. from ocular infections

Isolate number	Incubation period	Colony morphology
NA 1	24-48 h	White powdery colonies
NA 2	24-48 h	White powdery colonies
NA 3	12-24 h	Yellow mucoid colonies
NA 4	More than 2 days	Transparent white mucoid colonies
NA 5	More than 2 days	Transparent white mucoid colonies
NA 6	More than 2 days	Small rounded mucoid colonies
NA 7	More than 2 days	White colored Tiny mucoid colonies
NA 8	12-24 h	White mucoid colonies
NA 9	More than 2 days	White mucoid colonies
NA 10	12-24 h	Off white mucoid colonies
NA 11	24-48 h	Off white colored rounded mucoid colonies
NA 12	24-48 h	Off white colored partially mucoid colonies
NA 13	More than 3 days	White powdery dried colonies
NA 14	12-24 h	Creamy white mucoid colonies
NA 15	12-24 h	Creamy white colonies having spreaded growth
NA 16	12-24 h	Off white dried colonies
NA 17	12-24 h	Off white mucoid colonies
NA 18	More than 3 days	White colored tiny powdery colonies
NA 19	24-48 h	White colored colonies
NA 20	12-24 h	Yellow colored mucoid colonies
NA 21	12-24 h	Creamy white mucoid colonies
NA 22	12-24 h	Off white colonies having spread growth
NA 23	12-24 h	Off white colored partially mucoid colonies
NA 24	12-24 h	Off white colored partially mucoid colonies
NA 25	12-24 h	White colored dried powdery colonies
NA 26	12-24 h	Cream colored moderate slimy mucoid colonies
NA 27	12-24 h	Cream colored moderate slimy mucoid colonies
NA 28	12-24 h	Light orange colored slightly mucoid colonies
NA 29	12-24 h	White colored powdery colonies
NA 30	12-24 h	White colored powdery colonies
NA 31	12-24 h	White colored powdery colonies
NA 32	12-24 h	White colored powdery colonies
NA 33	2 days	White colored mucoid colonies

Isolate number	Incubation period	Colony morphology
NA 34	24-48 h	White colored powdery colonies
NA 35	24-48 h	Off white colored colonies
NA 36	24-48 h	White colored dried powdery colonies
NA 37	24-48 h	White colored dried powdery colonies
NA 38	24-48 h	White colored dried powdery colonies
NA39	24-48 h	White colored dried powdery colonies

Plate 2: *Nocardia* spp. from ocular infections isolated on blood agar

These 39 isolates were obtained from different ocular complications such as corneal ulcer (n = 25), endophthalmitis (n = 8), preoperative cases (n = 5) and other cases (secondary infections, pus, sclera abscess, intra ocular lens culture, buccal space infection etc.; n=1). Although Nocardia are known to cause pulmonary infections, these are also identified as rare but important cause of ocular infections (Garg, 2012). Corneal infection is by far the most common ocular infections caused by Nocardia and is important after trauma including refractive surgery (Decroos et al., 2011; Bharathi et al., 2003; Garg et al., 2010). Decroos et al. (2011) isolated Nocardia from cases of both exogenous and endogenous endolphthalmitis. Ramakrishnan et al. (2009) and Lalitha et al. (2005) have reported large number of these cases from south India. In patients with suspected nocardial infections and a compatible clinical picture, a definitive diagnosis usually depends on the demonstration of the organisms in smears examined microscopically together with isolation and identification by microbiologic culture.

Of these 39 patients positive for Nocarida ocular infections, 30 and 9 were found to be males and females respectively, suggesting male preponderance. The age groups of the target patients ranged between 2 and 81 years. The majority of the patients (n = 14) were between the age group of 60 and 70 years followed by 50 and 60 years of age (n = 9). Our study proved that ocular nocardial infections were higher among those aged more than 50 years, as reported by Manikandan et al. (2007). Although they did not have any underlying immunodeficient condition, which may predispose to nocardial infection, their general immune status may be low in these age groups, which make them vulnerable to nocardial infection. Nocardia isolates (n = 39) were subjected to various biochemical tests so as to identify the isolates till species level. The results of the biochemical tests are tabulated.

Table 3: Speciation tests employed for the identification of Nocardia asteroides from ocular infections.

Biochemical test	Result
Lysozyme resistance	Resistant
Casein hydrolysis	-
Xanthine hydrolysis	-
Tyrosine hydrolysis	-
Esculin hydrolysis	-
Urease production	+
Nitrate reduction	-
Mannitol fermentation	-
Sorbitol fermentation	-
Rhamnose fermentation	-
Acetamide utilization	-

(+ - Positive reaction, - - Negative reaction)

The species were identified based on the standard biochemical test results (Brown-Elliot et al., 2006; Land et al., 1991). All the 39 isolates were identified as N. asteroides after performing the biochemical tests.

In vitro antibiotic susceptibility testing by disk diffusion method

In the disk diffusion test 98%, 96.67% and 94.74% of Nocardia isolates were sensitive to AMK, MOX, and GEN & VAN, respectively. Exactly 91.9%, 80% and 76% of Nocardia isolates sensitive to CTM, TOB and CIP & OFX respectively. Further, 75%, 72%, 64.29% and 62% of the isolates were sensitive to AMP, GAT, CFZ and CHL, respectively. Precisely, 36% of isolates were resistant to CFZ. The treatment of Nocardia keratitis is quite well established, with AMK emerging as the best drug (Boiron et al., 1988). Among the antibiotics tested, it was found that AMK was most effective followed by MOX, GEN and CTM. Here, based on the antibiogram analysis AMK is recommended as the most suitable antibiotic for treating nocardial ocular infections. Similar to our findings, Reddy et al. (2010) and Lalitha et al. (2007) reported that nocardial isolates have good susceptibility to AMK and sulphonamides.

Table 4: Antibiogram pattern of Nocardia spp. (n = 39)

S. No.	Antibiotics with disc content	Sensitive	Intermediately resistant	Resistant
1	Amikacin (30 µg)	98 %	2 %	-
2	Ampicillin (2 µg)	75 %	25 %	-
3	Cephazolin (30 µg)	64.29 %	-	35.7 %
4	Chloramphenical (10 µg)	62.5 %	12.5 %	25 %
5	Cortimoxazole (25 µg)	91.9 %	8.2 %	-
6	Ciprofloxacin (5 µg)	76 %	24 %	-
7	Gentamycin (10 µg)	94.74 %	-	5.26 %
8	Gatifloxacin (5 µg)	72 %	28 %	-
9	Moxifloxacin (5 µg)	96.67 %	3.33 %	-
10	Ofloxacin (5 µg)	76.34 %	18.4 %	5.3 %
11	Tobramycin (10 µg)	80 %	10 %	10 %
12	Vancomycin (30 µg)	94.12 %	5.89 %	-

Table 6: MIC50 and MIC90 values for *Nocardia asteroides*

Antibiotic	MIC50 (µg/ml)	MIC90 (µg/ml)
Amikacin	2	16
Chloramphenicol	16	32
Cephazolin	8	64
Cefotaxime	4	64
Ciprofloxacin	4	32
Gentamicin	8	64
Levofloxacin	4	8
Moxifloxacin	2	16
Ofloxacin	4	32
Tetracycline	2	16
Tobramycin	8	32
Vancomycin	2	16

MIC50 – minimum inhibitory concentration median

MIC90 – minimum inhibitory concentration 90th percentile

MICs are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agent. Majority of the isolates (n=14) had the AMK MICs of 2 µg/ml and 5, 3 & 3 isolates were inhibited at the AMK concentration of 4, 8 and16 µg/ml, respectively. The MIC50 and MIC90 of AMK were noted to be 2 µg/ml and 16 µg/ml, respectively for N. asteroides. Most of the isolates were inhibited at the concentration of GEN 2 µg/ml (n=12). The MIC50 and MIC90 of GEN were noted to be 8 µg/ml and 64 µg/ml, respectively for N.

Table 5: Minimum inhibitory concentration of antibiotics for Nocardia asteroides from ocular infections

Antibiotics	Antibiotic	MICs (µg/ml)						
	Ranges (µg/ml)	2	4	8	16	32	64	128
Amikacin	2–128	23	7	-	6	1	1	1
Chloramphenol	2–128	10	1	2	8	4	2	2
Cephazolin	2–128	14	5		3	9	4	1
Cefotaxime	2–512	18	6	5	4	5	1	-
Ciprofloxacin	2 –128	18	8	5	1	5	1	1
Gentamycin	2–128	12	3	9	4	6	2	3
Levofloxacin	2–16	18	4	13	5	-	-	-
Moxifloxacin	2–128	20	10	2	3	2	2	-
Ofloxacin	2–32	15	10	3	3	8	-	-
Tetracycline	2–64	23	5	4	5	-	2	-
Tobramycin	2–64	13	5	7	6	5	3	-
Vancomycin	2–16	28	2	1	8	-	-	-

asteroides. The MIC of TOB ranged between 2 µg/ml and 64 µg/ml and most of the isolates were inhibited at TOB 2 µg/ml (n=13). Exactly, VAN 2 µg/ml inhibited most of the isolates (n=28), followed by 16 µg/ml (n=8). More number (n=14) of isolates were inhibited at the CFZ 2 µg/ml, 9 isolates had CFZ MIC 32 µg/ml. CTX showed MIC range of 2 µg/ml - 512 µg/ml. The MIC50 and MIC90 of CTX were noted to be 4 µg/ml and 64 µg/ml , respectively for N. asteroides. A good number of the isolates were inhibited at CIP 2 µg/ml (n=18), followed by 4 µg/ml (n=8), 8, 32 µg/ml (n=5). Exactly, 18 isolates were inhibited at LVX 2 µg/ml. Most of the isolates growth were inhibited at the MOX MIC 2 µg/ml (n=20). Most of the isolates were inhibited at the MOX 2 µg/ml (n=15), followed by 4 µg/ml (n=10) and 32 µg/ml (n=8). Each 3 isolates had MOX MIC value of 8 µg/ml and 16 µg/ml. The MIC50 and MIC90 of MOX were noted to be 4 µg/ml and 32 µg/ml, respectively. TET MIC ranged between 2 µg/ml and 64 µg/ml. Precisely, 2 µg/ml TET inhibited most of the isolates (n=23). Rest of the isolates had TET MIC value of 4 µg/ml (n=5), 8 µg/ml (n=4), 16 µg/ml (n=5) and 64 µg/ml (n=2). Greater part of the isolates (n=18) were inhibited at CHL 16 µg/ml, followed by 2 µg/ml (n=10).

From the present findings, based on the MIC90 value, it is highly recommended that LVX, AMK, MOX, TET and VAN as suitable antibiotics for the treatment of ocular infections caused by *Nocardia* spp. Treatment of nocardial keratitis is very prolonged. Since their discovery, sulphonamides were widely used to treat *Nocardia* in the early years. Sulphacetamide, sulphamethoxazole, and trimethoprim were considered to be the treatment of choice (Lee et al., 2001), until it was discovered that the minimum inhibitory concentrations of amikacin was much lower than the sulphonamides when used to treat ocular nocardial infections (Sridhar et al., 2001). Ever since, amikacin in the concentration of 2–2.5% is the treatment of choice in the monotherapy of nocardial keratitis (Denk et al., 1996). A case of nocardial keratitis resistant to amikacin has been reported by Pandya et al., 2008. It was treated successfully by adding 0.3% ciprofloxacin to the regimen. Many fluoroquinolones have emerged after the launch of ciprofloxacin. Third generation fluoroquinolones such as gatifloxacin and moxifloxacin have a spectrum of activity against most of the common ocular pathogens, including *Nocardia* (Callegan et al., 2003). However, Donnenfeld et al. (1985) reported the failure of suphonamide therapy in the case of *Nocardia* keratitis. Bharathi et al. (2003) had reported that approximately 85% of the *Nocardia* strains were resistant to sulphonamides. Manikandan et al. (2007) reported that 53% of the nocardial isolates were resistant to sulphamethaxazole and trimethoprim. In the present study AMK was found to be the most effective drug against nocardial (98.7%) isolates. Sridhar et al. (1998) and Husain et al. (1995) had reported 100% sensitivity to AMK by *Nocardia asteroides*.

According to Garg (2011), although keratitis and scleritis cases respond very well to AMK therapy, the outcome of endophthalmitis management is not encouraging. There are many investigations reporting that with the exception of N. transvalensis, all *Nocardia* species have low MIC values to AMK in vitro (Dalovisio et al., 1978; Wallace et al., 1988). In the present study, majority (n=14) of the isolates had AMK MIC as 2 µg / ml. The present analysis revealed that most quinolones listed has good activity against the majority of the isolates. A total of 23 of 39 isolates were inhibited at 2 µg/ml concentration of TET. Various reports show that AMK is the drug of choice for nocardiasis (Sridhar et al., 2001; Denk et al., 1996; Reddy et al., 2010; Lalitha et al., 2007). Other drugs which are shown to be effective are trimethoprin-sulphamethoxale combination and sulphacetamide (Sridhar et al., 2001).Warren et al. (2010) reported that *Nocardia* isolates have 100% susceptibility to CTM, AMK and linezolid.

Visual outcomes of treatment for *Nocardia* keratitis were described by Lalitha et al. (2007). Based on their observations, patients who present within 15 days of the onset of the infection show the highest recovery rate and are more likely to respond to topical antibiotics. Any delay in initiating appropriate therapy can lead to severe complications including progressive corneal thinning, resulting in perforation, endophthalmitis, and extension to the adjacent sclera (Sridhar et al., 2001).

CONCLUSION

It is concluded that, even though *Nocardia* are rare, but they are also important cause of ocular infection. The infection caused by these organisms pose several diagnostic and therapeutic challenges. Appropriate diagnosis and antibiotic susceptibility analysis will help in the institution of appropriate therapy. The MIC analysis thus proves that, LVX, AMK, MOX, TET and VAN as suitable antibiotics for the treatment of ocular infections caused by *Nocardia asteroides*.

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Chemotherapy Induced Adverse Drug Reactions In Cancer Patients

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ABSTRACT

The aim of the present study was to monitor adverse drug reactions in cancer patients receiving chemotherapy. The present study was carried out on 135 patients for six months. The causality assessment was done by using Naranjo's scale. The severity of ADR's was assessed using Hartwig's severity scale. The mean age of patients in sample was 49.31± 12.69 years. In our present study females (63%) were affected most commonly than males (37%). Monotherapy with Paclitaxel found to have highest number of ADRs (39). 5-FU+ cisplatin combination therapy had highest number of ADRs (104) followed by doxorubicin+ Cyclophosphamide (88) and doxorubicin+cyclophosphamide+5-FU (63). According to naranjo's algorithm, around 76% reactions were probable in causality, 20% were possible and 4% were definite in causality. According to Schumock and Thornton preventability criteria, more than half of the reactions are definitely preventable in nature (62%). Modification of dose of the drug and appropriate treatment measures should be implemented to minimizing the effects after chemotherapy and improving the patient's outcomes. In majority of post chemotherapy cases emesis is uncontrolled, even the anti-emetics are prescribed as prophylaxis. In general, myelosuppression is dose limiting ADR but it is observed even dose adjustment has been done.

Key words: *Chemotherapy, Causality, Paclitaxel, Severity, Adverse drug reaction.*

INTRODUCTION

Chemotherapy has broadened to include, as well as conventional cytotoxic drugs (which act on all cells, and rely on a small margin of selectivity to be useful as anticancer defective cell cycle controls that underlie malignancy[1]. Chemotherapy may be given after surgery (adjuvant chemotherapy) or before surgery (neoadjuvant chemotherapy). A few anticancer agents given orally or injected into the muscle or fat tissue below the skin, but most are injected into a vein. Treatment can be given at home, at the doctor's office or in hospital – it depends on the type of chemotherapy. Chemotherapy usually starts within 4 to 12 weeks after surgery. It is commonly given on a 21 or 28 day cycle. Drugs are generally given weekly or once every third week, with a rest period to allow patients to recover. The length of the cycle depends on the type of drugs used. The length of the period will vary, but it often lasts from 3 to 6 months[2].

2. MATERIALS AND METHODS

The study was carried out on 135 patients in Chemotherapy ward, Department of Radiotherapy, Government General Hospital, Kakinada for duration of six months i.e. April to September 2017. Our study was approved by the Institutional Ethical Committee. The study does not require any investigations or interventions to be conducted on patients. New and old patients of chemotherapy, any patient who developed at least one ADR during the treatment period were included in the study. The patients who did not develop any ADRs were excluded from the study, patients who are upon radiotherapy were excluded. Patient related information (demographic details clinical and treatment data) were collected in a specially designed data collection form. Patients age, sex, diagnosis, suspected drugs causing ADRs, treatment details (dose, frequency, date of starting and stopping), description of the event, onset

and ablation of adverse event, information on challenge and dechallenge, duration of hospital stay, type of ADRs, system affected by the ADRs, outcome of the ADRs, and drugs used to manage the ADRs were analysed.

Naranjo's probability scale was used to evaluate the relationship between suspected ADR and the drug. The scale consists of a questionnaire which contains 10 questions with the options yes, no, and do not know, and the score was given for each option. The total score calculated from this questionnaire defines the category as >9: Definite, 5-8: Probable, and 1-4: Possible. The severity of the ADRs was determined using Hartwig's Severity Scale. According to this scale, ADRs were assessed as mild (level 1, 2), moderate (level 3, 4, 5), and severe (level 6, 7). The modified Schumock and Thornton criteria were used for determining the preventability of the ADR. Data was analysed using descriptive statistics and results were expressed as percentage.

3. RESULTS AND DISCUSSION

Fig.1: Age Distribution of Patients

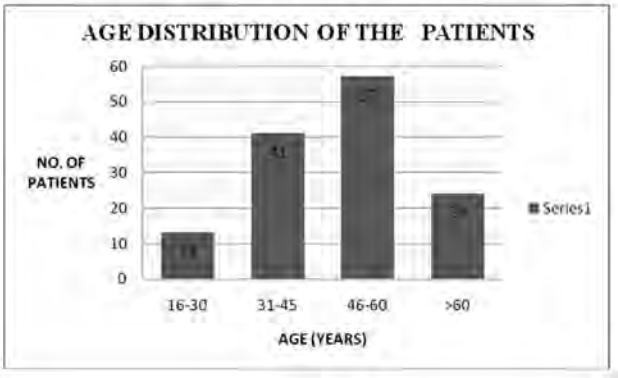


Fig.2: Gender Distribution of patients

Table 1: No. of ADRs Due to Monotherapy

INDIVIDUAL DRUGS	No. of ADRs
Paclitaxel	39
Docetaxel	17
Nab paclitaxel	9
Capecitabine	9
Oxaliplatin	6
5-Fu	4
Cisplatin	3
Zoledronic acid	2
Etopside	2

Table 2: No. of ADRs Due to Doublet Therapy

DRUGS	No. of ADRs
Cisplatin + 5-FU	104
Doxorubicin + Cyclophosphamide	88
Gemcitabine + Oxaliplatin	35
Cyclophosphamide + Cisplatin	26
Paclitaxel + Carboplatin	23
Doxorubicin + Cisplatin	20
Oxaliplatin + Capecitabine	20
Etoposide + Cisplatin	10
Gemcitabine +Cisplatin	10
Paclitaxel + Cisplatin	10
Irinotecan + Capecitabine	6
Doxoubicin + Dacarbazine	6
Docetaxel + Cyclophosphamide	5
Oxaliplatin + 5FU	4
Pemetrexate + Cisplatin	4

Table 3: No. of ADRs Due to Triplet Therapy

DRUGS	No. of ADRs
Doxorubicin + Cyclophosphamide + 5-FU	63
Docetaxel + Cisplatin + 5-FU	20
Cyclophosphamide +Vincristine + Doxorubicin	14
Cisplatin + 5-FU + Paclitaxel	7
Etoposide + Cisplatin + Bleomycin	7
Doxorubicin + Cisplatin + Cyclophosphamide	5
Paclitaxel + Carboplatin + Zoledronic acid	4
Paclitaxel + Doxorubicin + Cisplatin	4
Decarbazine + Vinblastin + Cisplatin	3
Oxaliplatin + 5-FU + Leucovorine	2

Table 4: Distribution of ADRs Based on the Organ System in Males Vs Females

Organ System	Adverse Drug Reactions	Males	Females	Total
Blood system	Anemia	8	19	27
	Neutropenia	3	6	9
	Thrombocytopenia	1	7	8
	Leucopenia	5	12	17
	Febrile Neutropenia	1	3	4
GI System	Nausea& Vomiting	29	56	85
	Constipation	4	10	14
	Diarrhea	12	14	26
	Mucositis	2	10	12
	Gastritis	3	2	5
	Stomatitis	1	0	1
	Abdominal Pain	1	1	2
	Metallic Taste	8	11	19
	Loss of Appetite	26	43	69
CNS&PNS	Peripheral Neuropathy	17	21	38
	Headache	5	6	11
	Somnolence	0	3	3
	Confusion	4	3	7
Musculoskeletal system	Backache	3	0	3
	Myalgia	3	2	5
	Arthralgia	1	3	4
Skin& Appendages	Injection Site of Extravasation	5	14	19
	Pigmentation Of Skin &Nails	6	31	37
	Alopecia	25	69	94
	Rashes	2	0	2
	Nail damage	1	1	2
	Maculopapular eruption	0	3	3
	Dermatitis	1	0	1
	Hand-Foot syndrome	2	4	6
Respiratory system	Breathless ness	2	2	4
	Cough	2	1	3
Renal system	Urinary Urgency	0	1	1
	Red- Orange discolouration of urine	1	12	13
Immunologic	Hypersensitivity reaction	5	1	6
Ophthalmic	Vertigo	1	0	1
	Blurred vision	1	0	1
	Conjunctivitis	0	1	1

Fig. 3: Causality Assessment Criteria

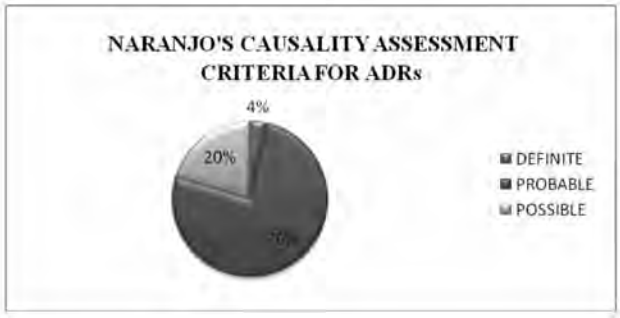


Fig.4: Severity of ADRs

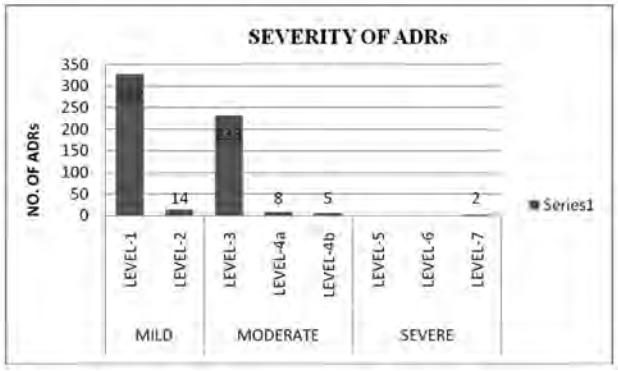
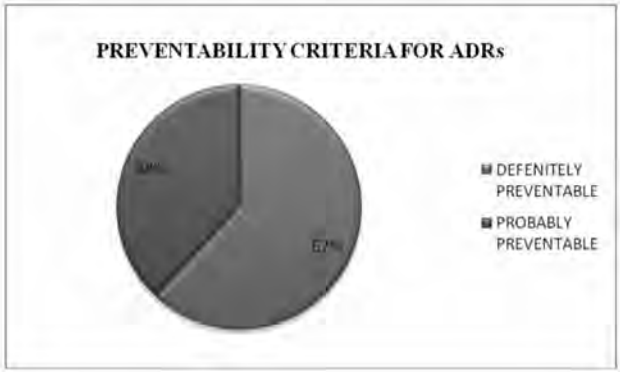


Fig.5: Preventability Criteria of ADRs



The mean age of patients in sample was 49.31± 12.69 years. Similar findings regard to age group was reported in some studies[3-14]. ADR's were most common in adults and elderly age group patients. This may be due to the low metabolizing capacity and reduced excretory functions leading to accumulation of drugs in the body[15] and thus increasing the risk of ADRs and also associated co- morbidities like diabetes mellitus & hypertension.

In our present study females (63%) were affected most commonly than males (37%). Similar findings regard to gender wise distribution was reported in some studies[3, 5, 6,11,12]. On the contrary, there are studies where males were found to have more number of ADRs as compared to females[8, 9]. The females were most commonly affected due to ADRs than males because of their smaller body size and mental make –up [16] and also result of different pharmacokinetic & pharmacodynamics responses to drugs [6,11,17]. Cancers pertaining to females were reported more in number. Hence, females were more commonly affected in our study.

Monotherapy with paclitaxel found to have highest number of ADRs (39). 5-Fu+ cisplatin combination therapy had highest number of ADRs (104) followed by doxorubicin+ cyclophosphamide (88) and doxorubicin+cyclophosphamide+5-Fu (63). Saini et al [18] reported high number of ADRs with paclitaxel and docetaxel monotherapy and 5-Fu+doxorubicin+cycloph osphamide combination therapy. Paclitaxel was used as a single agent therapy for breast cancer. Breast cancer was more predominant in our study. Hence, may be number of ADRs reported by paclitaxel was high in number. Paclitaxel as a single agent therapy was advised if failure of combination therapy for metastatic disease or relapse within 6months of adjuvant chemotherapy was observed [19].

Cisplatin and 5-FU were used in combination to treat cervical cancer, oesophageal cancer and anal cancer. Cyclophosphamide+doxorubicin+5-Fu and cyclophosphamide+doxorubicin, these drug combinations are used in treatment of breast cancer [20].

According to naranjo's algorithm, around 76% reactions were probable in causality, 20% were possible and 4% were definite in causality. Similar findings were reported in few studies [9, 10, 21]. A study done by Chopra et al[12] it was reported as possible followed by probable. According to Hartwig's and Seigel severity scale, most of the ADRs are mild in severity (58.03%), followed by moderate in severity (41.6%). Similar findings was reported in study conducted by Bairy et al[5]. This shows that ADRs are due to cancer chemotherapy are rarely life threatening with appropriate pre-medications and early detection. A study conducted by Chopra et al [12] shows 74.1% of the ADRs were moderate in severity followed by 17.9 % of reactions which are mild in

severity. The association between age and gender with severity of ADRs were not statistically significant.

According to Schumock and Thornton preventability criteria, more than half of the reactions are definitely preventable in nature (62%). A study conducted by Chopra et al [12] reported 51% of ADRs were classified as not preventable, 42% probably preventable and 7% definitely preventable.

4. CONCLUSION

Chemotherapy drugs have a narrow therapeutic index and the dosage needed to achieve a therapeutic response usually proves toxic to the body's rapidly proliferating cells. Cancer chemotherapy has a high potential to cause ADRs, measures need to be put at place to reduce the physical, emotional and economic burden on the patient due to ADRs. Modification of dose of the drug and appropriate treatment measures should be implemented to minimizing the effects after chemotherapy and improving the patient's outcomes. In majority of post chemotherapy cases emesis is uncontrolled, even the anti-emetics are prescribed as prophylaxis. In general, myelosuppression is dose limiting ADR but it is observed even dose adjustment has been done.

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Design and Evaluation of Buccoadhesive Tablets of Aceclofenac

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ABSTRACT

The present study is to develop and evaluate Buccoadhesive tablets of aceclofenac formulated to enhance bioavailability. Aceclofenac a non-steroidal anti-inflammatory agent with high protein binding, which makes it an ideal candidate for administration by buccal route. Tablets were prepared using various buccoadhesive polymers and evaluated for its characteristics. All the formulation showed compliance with standard limits. The formulations F3, F4, F5, and F6 showed considerable results indicating that Polymers HPMC K4M and Guar gum may be the choice for formulating mucoadhesive tablets. The release profile of F4 and F6 indicates that 15% concentration of HPMC K4M and Guar gum showed satisfactory results compared with 10% of HPMC , guargum and Xanthan Gum at various concentrations.

Key words: *Aceclofenac, Mucoadhesive, Tablets, Non-steroidal Anti-inflammatory agent.*

INTRODUCTION

Transmucosal drug delivery has gained importance in the recent years for systemic and local delivery of drugs and peptides that go through first pass effect and degrade in the gastrointestinal environment. Direct entry into systemic circulation can be achieved by delivering drugs in the buccal cavity, thereby avoiding gastrointestinal degradation of drugs and bypassing first pass metabolism.[1] Apart from this, there are many advantages of buccal drug delivery viz., easy accessibility, patient compliance, rapid cellular recovery following local stress and ability to withstand environmental extremes like change in pH, temperature etc. Many researchers have ascertained the absorption capability of buccal mucosa and that physicochemical parameters of drugs may favour buccal absorption. Recently, investigations are more focused on drugs that undergo first pass metabolism such as cardiovascular drugs, analgesics, beta blockers, and peptides[2]. In the present study Aceclofenac was chosen as model drug for the formulation of buccoadhesive tablets.

MATERIALS AND METHODS

Materials

Aceclofenac, Xanthan gum, HPMC, Guar gum, Lactose, Magnesium stearate, were from S.D fine chemicals. All other chemicals used in the study were of analytical grade.

Methods

Formulation of buccoadhesive dosage form

Buccoadhesive tablets were prepared by wet granulation technique using HPMC, Guargum, Xanthan Gum in various ratios. The blend of 150mg granules was then compressed using 6 mm diameter die in a single stroke multi-station tablet press[3].

Table No.1: Composition of various formulae

S. No	Ingredients per tablet	F1	F2	F3	F4	F5	F6
1.	Aceclofenac	50mg	50 mg	50 mg	50 mg	50 mg	50 mg
2.	Xanthan gum	10 mg	15 mg	-	-	-	-
3.	HPMC	-	-	10 mg	15 mg	-	-
4.	Guar gum	-	-	-	-	10 mg	15 mg
5.	Lactose	39 mg	34 mg	39 mg	34 mg	39 mg	34 mg
6.	Magnesium stearate	1 mg	1 mg	1 mg	1 mg	1 mg	1 mg

EVALUATION OF GRANULES:

The granules were evaluated for Flow properties, bulk density and Carr's index.

Evaluation of tablets

All the formulations of Buccoadhesive tablets were evaluated for its parameters according to monograph. The weight variation was determined for 20 tablets and hardness was determined for 6 tablets randomly from each batch. Friability was determined by testing 10 tablets in a friability tester for 4 minutes at 25 rpm[4].

Swelling studies

Buccal tablets were weighed individually(W1) and placed in 2% agar gel plates with the core facing the gel surface and incubated at 37± 0.1°C. The tablets were removed from the petridish and excess surface water removed from carefully using filter paper. The swollen tablets were reweighed (W2) and the swelling index was calculated using following formula. The degree of swelling of adhesive polymer plays an important role affecting tackiness[5, 6].

% Swelling Index = {(W2) – (W1)/ (W1)} x 100

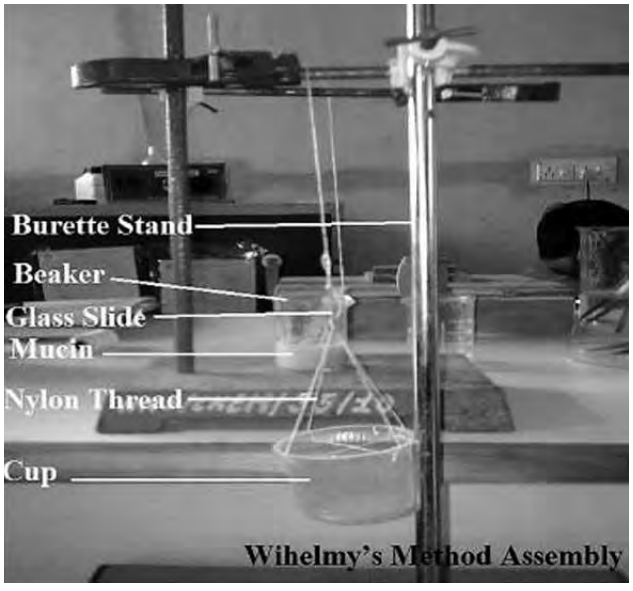

Figure No 1: Mucoadhesive Test Assembly

Ex-vivo residence time

The ex-vivo mucoadhesion time was studied by attaching the tablets wetted with 1 drop of phosphate buffer pH 6.8 on freshly cut sheep buccal mucosa by applying light force with a finger tip for 30 seconds and placed in the medium. The adhesion time was observed for about 7 hours. The time at which the tablet detaches from the buccal mucosa was recorded as the mucoadhesion time.

Figure No: 2 Measurement of ex-vivo residence time

In- vitro drug release study

In-vitro drug release studies were carried out using USP XXII dissolution apparatus type II (Lab India, Mumbai, India) at 100 rpm using 900 ml phosphate buffer (pH 6.8), maintained at 37.5 ±5ºC. The samples were collected at various time intervals and analyzed for drug release profile[7].

Stability studies

The stability studies were performed as per the ICH guidelines for 90 days. The formulations were kept under 40ºc and 75% RH for three months and evaluated for physical changes, tablet characteristics[8].

RESULTS AND DISCUSSION

The preformulation characteristics were studied for all the batches of granules. Formulation F1 to F6 showed good compressibility index with excellent flow characteristics and also hausner ratio indicating optimum granule property which is suitable for compression8. This is shown in Table No. 2

Table No.2: Powder flow properties

S. No.	Formulations	Flow Parameters			
		Angle of repose (θ)	Bulk density (g/cc)	Compressibility (%)	Hausner ratio
1.	F1	25o02±0.29	0.555±0.04	12.52±0.09	1.18±0.02
2.	F2	26o05´±0.74	0.404±0.02	14.73±0.14	1.42±0.05
3.	F3	24o88´±0.26	0.363±0.07	14.86±0.24	1.55±0.07
4.	F4	25o30´±0.14	0.418±0.01	15.44±0.14	1.12±0.02
5.	F5	26o71´±0.15	0.416±0.03	12.60±0.12	1.14±0.03
6.	F6	27o34´±0.12	0.420±0.05	15.87±0.20	1.10±0.03

The compressed tablets were found to be complied with Pharmacopeial standards for weight variation. The thicknesses of the tablets were recorded as 2.4 ± 0.05mm to 2.6 ± 0.05mm. The hardness of the mucoadhesive tablets varies from 6.0 ± 0.16 kg/cm2 to 5.5 ± 0.1kg/ cm2, shown in Table No. 3.

Table No.3: Evaluation of Mucoadhesive Tablets

S. No.	Formulations	Evaluations		
		Weight Variation %	Thickness (mm)	Hardness (Kg/Cm2)
1.	F1	2.0±0.3	2.4±0.05	5.5±0.12
2.	F2	2.4±0.8	2.43±0.03	5.6±0.20
3.	F3	1.7±0.9	2.47±0.02	5.5±0.48
4.	F4	1.3±0.3	2.52±0.05	5.7±0.17
5.	F5	2.1±0.4	2.56±0.05	5.7±0.24
6.	F6	1.9±0.1	2.6±0.02	6.0±0.16

All the formulations were subjected to evaluation of drug content. The results are shown in Table No. 4. The maximum percentage of drug content from all formulation was found to be 98.75 ± 0.09 and minimum was found to be 96.25 ± 0.35. Table No.4 shows the buccoadhesive strength of various formulations. The maximum mucoadhesive strength was absorbed in the formulation F4 and F6, where the concentrations of the polymers HPMC and Guar gum was more i.e. 15%w/w[9, 10].

Table No.4: Evaluation of Mucoadhesive Tablets

S. No.	Formulations	Specific Tests		
		Drug Content (%)	Mucoadhesive Strength (g)	Residence Time (mins)
1.	F1	97.51±0.48	11.25±0.95	100
2.	F2	97.11±0.26	15.59±1.2	270
3.	F3	96.25±0.35	12.25±1.3	300
4.	F4	97.5±0.19	16.75±0.8	360
5.	F5	98.50±0.15	14.90±0.9	305
6.	F6	98.75±0.09	16.45±1.0	300

Swelling index was represented in Table No. 5 & Figure No. 3. Increase in swelling was observed with increase in concentration of polymer[11]. The uptake of water by HPMC K4M was slower process when compare with xanthan gum and guar gum. The swelling was maximum in formulation F2 which causes more drug release as the swelling increase and leads the tablet to gets detach easily from mucosa since it may reduce adhesion. The swelling index of F5 and F6 were comparatively good. Therefore, they can sustain the release of drug better than other formulations[12]. The capacity of the formulation to take up water is an important parameter of the polymer system in view of release of drug at mucosal surface

Table No. 5: Swelling Index of Aceclofenac Buccoadhesive Tablets

S. No.	Time (Hrs)	% Swelling Index					
		F1	F2	F3	F4	F5	F6
1.	0.5	15.2±0.2	18.5±0.3	12.4±0.2	15.0±0.5	7.5±0.1	25.1±0.3
2.	1	30.6±0.4	35.7±0.6	20.5±0.6	25.8±0.2	27.5±0.9	39.3±0.5
3.	2	42.5±0.5	49.8±0.2	32.8±0.3	40.8±0.1	36.6±0.7	57.9±0.2
4.	3	59.0±0.1	61.1±0.3	40.5±0.8	45.6±0.3	65.8±0.6	66.0±0.3
5.	4	76.1±0.5	83.4±0.2	48.4±0.1	53.3±0.4	69.1±0.3	72.6±0.1
6.	5	98.2±0.3	119.8±0.5	60.4±0.5	69.1±0.2	76.6±0.7	98.8±0.3
6.	6	120.0±0.4	135.0±0.8	85.1±0.6	90.1±0.3	105.3±0.6	125.2±0.2

The formulations were tested for in-vitro drug release profile. The influence of polymer concentration was found to play a major role in regulating the drug release[13]. Drug release profiles of formulation F1 through F6 are shown in Figure No. 3.

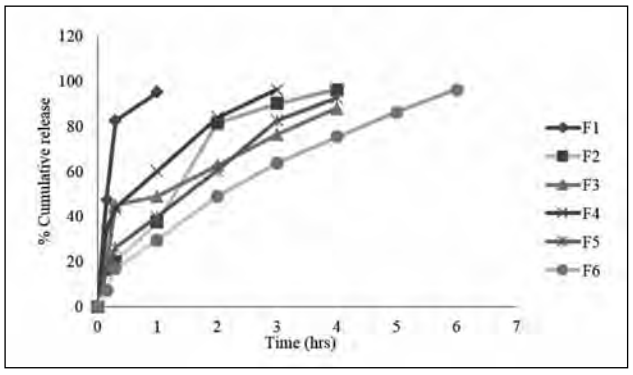

Figure No. 3: Drug dissolution profile of Formulations F1 to F6

Formulation F3 and F4 were found to release the drug at a controlled rate up to 6 hours in a steady rate[14]. Similarly F5 and F6 were also had a drug release up to 6 hours at a uniform rate. This may be because of the nature of HPMC and Guar gum and its controlled swelling property[15].

CONCLUSION

Formulation F3, F4, F5, F6 showed better results which Indicates that Polymers HPMC K4M and Guar gum may be the choice for formulating mucoadhesive tablets. The release profile of F4 and F6 indicates that 15% concentration of HPMC K4M and Guar gum showed better results compared with10% concentration and xanthan gum at various concentrations. The formulations were found to be stable .Aceclofenac buccoadhesive tablets can be prepared by using 15% of HPMC and Guargum.

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Efficient Synthesis of 2-Phenylbenzothiazole

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ABSTRACT

One pot effective conversion of phenolic ester to 2-phenyl benzothiazole by using different mild base catalyst was described. The 2-phenyl benzothiazole was obtained in high chemical yields and this transformation would facilitate synthesis by short reaction time, and rapid isolation of the products. An attempt to control process related impurities as per ICH guidelines was carried out .

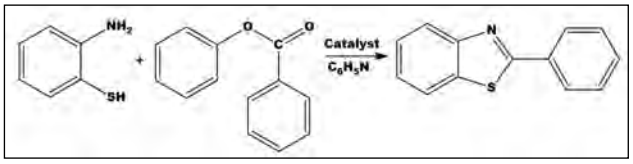
Key words: *2-Phenyl benzothiazole, Base catalyst, Quality control.*

INTRODUCTION

Benzothiazoles are an important heterocyclic compounds which exhibit diverse biological properties such as antitumor[1], antimicrobial[2], antiglutamate/antiparkinson[3], broad spectrum calcium channel antagonist[4], inhibition of enzymes such as aldose reductase[5], monoamine oxidase[6], lepoxygenase[7], cyclo-oxygenase[8], acetylcholine esterase[9], thrombine[10], proteases[11],

H+-K+ ATPase [12], carbonic anhyrase [13], HCV helicase[14] and plant growth regulation [15].

Many reports are available for preparation of 2-substituted benzothiazoles [16]. Over all general approaches involve one pot condensation and dehydration of 2-aminothiophenol with carboxylic acids [14, 17] or condensation with aldehydes under oxidative conditions [18]. The limitations of those procedures have limitations, such as drastic reaction conditions, low yields, tedious workup procedures and several side reactions. A novel method to overcome the limitations is still an important experimental challenge.



Scheme 1: Synthesis of 2-Phenly benzothiazole

The substitution of carboxylic acid or aldehyde component as alternative functional group, has not been explored so far. Therefore, the development of a simple and stable substitute for these aromatic acids or aldehydes by using phenolic esters would extend the scope of the reaction in organic synthesis. In continuation of our work on the development of useful synthetic methodologies, we wish to report the one-step conversion of phenolic esters to the corresponding benzothiazoles by using aminobenzenethiol. In the preliminary stage of investigation, we focused on the systematic study of different base catalysts for the model reaction of phenolic esters and o-aminobenzenethiols at reflux conditions in pyridine. A wide variety of base catalysts including t-BuOK, TEA, DABCO, K2CO3, Cs2CO3, and piperidine were employed to improve the yield for the specific synthesis of 2-phenyl benzothiazoles.

The chemicals and solvents used for the experimental work were commercially procured from E. Merck, India, S.D. Fine Chem, India and Qualigens, India. Silica gel G used for analytical chromatography (TLC) was obtained from S.D. Fine Chem, India. Melting points were determined in an open glass capillary using a Kjeldahl flask containing liquid paraffin and are uncorrected. Microwave oven reaction were done on Black & Decker, MX30PG 1000 W. The infrared

spectra of compounds were recorded in KBr on a FTIR-8400S, Fourier Transform (Shimadzu), Japan infrared spectrophotometer. The proton magnetic resonance spectra (1H NMR) were recorded on a Bruker 300 MHz instrument (Bruker, Germany) in DMSO/CDCl3 using TMS as internal standard. Chemical shifts (δ) are expressed in ppm. Mass spectra were recorded on LC-MS/MS (API-4000 TM), Applied Biosystems, MDS SCIEX (Canada).

A mixture of 2-aminothiophenol (1 mmol) and phenyl ester (1 mmol) dissolved in pyridine (5 ml) was added to 0.1 mmol of base catalyst, capped and irradiated in a microwave oven for the appropriate time (Table 1). The progress of the reaction was monitored by intermittent rapid cooling of the mixture to room temperature and analyzing by TLC (n-hexane/ethyl acetate: 2:8). After complete conversion of the substrate as indicated by TLC analysis, the reaction mixture was filtrated and then poured onto 50 g of crushed ice and stirring was continued for few minutes. The solid products were filtered, washed with cold water and recrystallized from ethanol.

IR Peaks was found to be at 1479 cm-1, 963 cm-1, 767 cm-1, 687 cm-1. 1H NMR (CDCl3) analysis reveals: d 7.37–8.12 (m, 9H, C6H5, C6H4).

Table - 1 Synthesis of 2-phenyl benzothiazole under optimized condition

Entry	Catalyst	Reaction Time (hr)	Yield* (%)
1	K2CO3	0.08	87
2	CS2CO3	0.07	88
3	t-BuOK	0.05	90
4	TEA	0.06	85
5	DABCO	0.05	89
6	Piperidine	0.08	82

*All the isolated products were characterized on the basis of their physical properties and IR, 1H NMR and 13C NMR spectral analysis and by comparison with authentic sample[19].

The synthesis of 2-phenyl benzothiazole was schematically synthesized and was authentically identified by their physical and spectral data [19]. Melting point was found to be 115 -116 ˚C.

13C NMR analysis data reveals (CDCl3): d 168.5, 154.5, 135.4, 134.0, 131.4, 129.4, 128.0, 126.7, 125.6, 123.6, and 122.0. HRMS: m/z (%, elemental composition), calculated for C13H9NS (M+) 211.0456 and 211.0462 for (M+, 100.0).

The synthesized 2-phenyl benzothiazole has been a versatile nucleus in wide pharmacological activities. But most of the earlier methodologies for the synthesis of 2-phenyl benzothiazole lead to process related impurities. The above discussed synthesis method was carried out by using different base catalyst devoid of process related impurities as per ICH guidelines. Optimization of conditions of reaction for choice the best of amount of catalyst was carried out with respect to best yield and time of reactions.

10 mol% of catalyst was chosen and pyridine has been selected as the best solvent.

This simple procedure of synthesis is an efficient methodology that has advantages such as shorter reaction time, high yield of product, mild condition, and easy work up without any complex purification.

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