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ANTI-INFLAMMATORY AND ANTINOCICEPTIVE ACTIVITY OF SOME ORCHIDS

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ABSTRACT: The present study was carried out to evaluate the anti-inflammatory activity assessed by Carrageenan induced rat paw edema model and HRBC membrane stabilization assay whereas Tail flick and Hotplate methods have been used to evaluate the antinociceptive property in selected orchid species *viz*. *Aerides maculosum* (AM), *Coelogyne breviscapa* (CB), *Dendrobium macrostachyum* (DM), *Pholidota pallida* (PP) and *Vanda testacea* (VT). The Soxhlet extracted (70% ethanol) orchids at 200 and 300 mg/kg bw were selected as a therapeutic dose. Results of anti-inflammatory by carrageenan induced paw edema revealed that the extracts treated animals have shown a significant decrease in paw volume reflecting a reduction of inflammation at 180, 240 and 300 min. HRBC membrane stabilization revealed that the extracts possess the highest percentage of inhibition at 10 mg/ml. From both *in vivo* and *in vitro* inflammatory activity, it was found that VT has maximum effect followed by DM, AM, CB and PP. Further, the results of analgesic methods showed that the extracts are contributing to the significant reduction of pain at 200 and 300 mg by increasing reaction time wherein DM recorded the highest effect followed by VT, AM, CB and PP. The results documents that orchid possess prominent anti-inflammatory and analgesic activities.

KEYWORDS: Orchids, anti-inflammatory, paw edema, HRBC stabilization, antinociceptive.

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1.INTRODUCTION

Inflammation and Analgesia (pain) are common nonspecific manifestations of many diseases [1]. The inflammation is a local response of animals towards chemical or physical injury or bacterial invasion characterized by the formation of edema, leucocytes infiltration and granuloma formation, tissue injury and repair in living tissues [2]. While analgesia is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage [3]. Analgesics are the drugs known as pain killers. The inflammation accompanied by chemical mediators include histamine, serotonin, slow reacting substances of anaphylaxis (SRS-A), TNF-α, interleukins, prostaglandins and some plasma enzyme systems undergo activation at the onset of inflammation to cause increased vasodilatation and permeability of blood vessels [4];[5]. Anti-inflammatory agents are capable of inhibiting the cyclooxygenase COX-1 and COX-2 pathway of arachidonic acid metabolism, which produces prostaglandins [5]. Prostaglandins are one of the important biomolecules, which play a key role in the induction of inflammatory response as their biosynthesis is significantly increased during inflammation [6]. The inflammatory and pain responses are elicited as a protective mechanism by an organism whenever any tissues are being damaged. However, sustained inflammation and pain can lead to undesired health effect [6]. Although the modern drugs used for the suppression and relief of pain and inflammation include non-steroidal anti-inflammatory drugs (NSAIDs), opiates, immunosuppressant, corticosteroids and histamines are associated with serious adverse side effects such as gastrointestinal disturbances, renal damage, respiratory depression, and possible dependence [7];[8];[9]. Hence the search for new, safe and effective analgesic and anti-inflammatory drugs from natural sources and medicinal plants has been an increasing interest possibly with fewer side effects. Orchidaceae is one of the largest family of plant kingdom comprising 25,000-35,000 species [10];[11]. They are a most extravagant group of flowering plants grown primarily as ornamentals and are valued as cut flowers [13]. Though orchids are grown primarily as ornamentals, it is well known that orchids have been used all over the world in traditional healing and treatment system of a number of diseases. They have been used variously in different diseases as antirheumatic, anti-inflammatory, antiviral, anticarcinogenic, anticonvulsive, diuretic, neuroprotective, relaxation, anti-aging, wound healing, hypoglycemic, antitumor and anticancer, antimicrobial, and many other activities [14]. Therefore the objective of the present study is to evaluate *in vivo* and *in vitro* anti-inflammatory and *in vivo* analgesic activity in some selected important medicinal unexplored orchids of South India.

2. MATERIALS AND METHODS

Collection of plant material

For the study, five species of wild orchids *viz. Aerides maculosum*, *Coelogyne breviscapa*, *Dendrobium macrostachyum*, *Pholidota pallida* and *Vanda testaceae* were collected from the forest area of Shimoga District, Karnataka and was identified and authenticated by Dr. Prashantha K.M,

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Department of Botany, Sahyadri Science College, Kuvempu University, Shimoga. Different parts of the orchid species were used for the extraction. The selection of different plant parts of orchid species was with respect to medicinal folk claims in various ethnobotanical studies [12],[14]. The parts selected from orchids for extraction were pseudobulbs in *Coelogyne breviscapa* (CB) and *Pholidota pallida* (PP), leaves in *Aerides maculosum* (AM) and *Vanda testaceae* (VT) and whole plant in *Dendrobium macrostachyum* (DM).

Preparation of extracts

The above-selected parts of each orchid were cleaned thoroughly, shade dried and pulverized mechanically. The exactly 100 g of powder was subjected to Soxhlet extraction using 70% ethanol. Further, the extracts were concentrated at low temperature and reduced pressure. The yield of crude extracts obtained was stored in desiccators for a maximum of 3 days; later preserved in the deep freezer (-20^{o}C) for further use.

Qualitative phytochemical analysis

The preliminary qualitative studies of all the ethanolic extracts of orchids were examined for the presence of various secondary metabolites using standard protocols [15],[16].

Experimental animals

Healthy adult Wistar strain of albino rats (150-200g) and mice (25-35g) of both sexes were used for the *in vivo* evaluation of anti-inflammatory and analgesic activity respectively. The animals were housed in separate polypropylene cages and were maintained under control conditions of 22 ± 2°C, with 12 h light/dark cycle. Food and water were provided *ad libitum* until the day prior to the study. All experimental procedures described were conducted according to CPCSEA guidelines with ethical clearance obtained by Institutional Animal Ethics Committee, KLE College, Huballi (Approval No. 04/KLEU' SCOPH/15).

Acute toxicity studies

The acute toxicity study by staircase method was carried out as per OECD guidelines [17]. Intraperitoneal administration of drugs was given up to 3000 mg/kg bw. They were observed continuously for the first 4 h and once daily following 14 days for general behavior, convulsions and mortality. The drug was found to be safe at the dose of 3000 mg/kg as no mortality or toxicity was observed. Based on the acute toxicity studies 1/15th and 1/10th of the maximum tolerated dose were selected *i.e*. 200 and 300 mg/kg ethanolic extract of orchids were used for the current study.

Anti-inflammatory activity

Carrageenan-induced paw edema

Anti-inflammatory activity of ethanolic extracts of orchids against carrageenan was studied according to the method given by Winter et al. [18]. Animals were divided into twelve groups comprising six rats in each dose for all the groups. Group I served as control received 0.1% carrageenan in physiological saline and Group II received Indomethacin (20 mg/kg bw, i.p.). Groups

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III to XII were administered with extracts AM, CB, DM, PP and VT (200 and 300 mg/kg bw. i.p.) respectively. All the animals were fasted overnight prior to the start of the experiment and only water was allowed *ad libitum.* Acute edema in left hind paws of the rats was induced by the subplantar injection of 0.1 ml of freshly prepared (1% w/v) carrageenan suspension in normal saline 30 minutes after the drug administration. The paw volume was measured by plethysmometer at 60, 120, 180, 240 and 300 min after the carrageenan injection. Mean decrease in the paw volume was measured. The percentage inhibition of paw edema was calculated by,

$$\text{Percentage inhibition of paw edema} = (1 - Vt/Vc) \times 100$$

Where,

Vc = increase in paw volume of the control group of rats at a given time; and

Vt = inflammation of the drug treated (i.e. treated orchid extracts) rats at the same time.

Human erythrocyte membrane stabilization assay

The HRBC membrane stabilization method was used to estimate the *in vitro* anti-inflammatory activity of orchid extracts [19]. Blood was collected from healthy volunteers and was mixed with equal volume of sterilized Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3000 rpm and the packed cells were separated. The packed cells were washed with an isosaline solution and a 10% v/v suspension was made with isosaline. This HRBC suspension was used for the estimation of anti-inflammatory property. Various concentrations of extract and standard were separately mixed with 1 ml of phosphate buffer, 2 ml of hyposaline and 0.5 ml of HRBC suspension. All the assay mixtures were incubated at 37 ^{0}C for 30 min and centrifuged at 3000 rpm for 20 min. The hemoglobin content in supernatant solution was estimated by a spectrophotometer at 560 nm. Indomethacin was used as the reference standard and a control was prepared by omitting the extracts. The hemolysis percentage was calculated by assuming the hemolysis produced by the control group as 100%. The percentage of HRBC membrane stabilization or protection was calculated using the formula,

$$\text{Percent protection} = 100 - (\text{OD of extract treated sample}/\text{OD of control}) \times 100$$

Antinociceptive activity

Tail immersion method

The antinociceptive (analgesic) activity was determined by measuring drug-induced changes in the sensitivity of the mice to heat stress applied to their tails [20]. The procedure is based on the observation that analgesic drugs are selectively capable of prolonging the reaction time of the typical tail-withdrawal reflex in mice induced by immersing the end of the tail in the warm water of 55±5°C. The reaction time is recorded in seconds by a stopwatch. Swiss albino mice weighing between 20-35g were used for evaluation of the analgesic activity. The selected mice were then divided into twelve groups of six mice for each dose. Group I served as control and received vehicle only and Group II received Diclofenac as standard (20 mg/Kg bw. i.p.). Group III-XII received ethanolic

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extracts of orchids, AM, CB, DM, PP and VT of dose 200 and 300 mg/kg respectively. All the animals in the groups were administered intraperitoneally. The initial reading was taken immediately before administration of drugs and then at 30, 60, 90, 120, 150 and 180 min after the administration. Tail flick latency difference or mean an increase in latency after drug administration was used to indicate the analgesia produced by test and standard drugs.

Hot Plate method

The paws of mice are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a stop-watch [21]. Swiss albino mice weighing between 20-35 g were divided into twelve groups, each group containing six animals for each dose. Group I served as the control with no protection. Group II animals received the standard drug of Diclofenac 20 mg/kg bw. whereas group III to XII animals were administered with ethanolic extracts of orchids AM, CB, DM, PP and VT at a concentration of 200 and 300 mg/Kg bw respectively intraperitoneally. The temperature of the hot plate was maintained 55±5 ºC, the reaction time in seconds (latency period) was observed for either the time taken for the mouse to react to the thermal pain by licking its paw or attempting to jump out. Observations were made before and after administration of respective drugs at an interval of 30 min for three hours.

Statistical analysis

Data analyzed using one way analysis of variance (ANOVA) followed by Dunnett's comparison test. Values are expressed as mean ± SEM (n = 6 in each group). The results obtained were compared with the control group. P value < 0.05 and < 0.01 were considered statistically significant.

3. RESULTS AND SISCUSSSION

Qualitative phytochemical analysis

The preliminary qualitative phytochemical investigation of all five orchids revealed the presence of many bioactive compounds *viz.* polyphenols, flavonoids, terpenoids, steroids, alkaloids and tannins. The results also revealed that saponins were present only in CB whereas absent in all the other extracts.

Anti-inflammatory activity

Carrageenan-induced paw edema

The inflammatory effect of orchid extracts and standard Indomethacin against paw edema induced by carrageenan are summarized in Table 1. Administration of carrageenan to the rats showed a rise in paw volume at different time intervals in the control group. Standard drug Indomethacin showed a reduction in paw volume of 1.08±0.04, 0.69±0.05, 0.62±0.05 and 0.40±0.03 which corresponds to 51.49, 70.37, 75.65 and 85.77% at 120, 180, 240 and 300 min respectively. Intraperitoneal administration of orchid extracts *viz*. AM, DM and VT at 200 mg exhibited significant reduction

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(P<0.01) in inflammation induced by carrageenan at 180 min was 2.05±0.06, 2.00±0.04, and 1.83±0.03 with percent inhibition of 12.30, 14.24, and 21.49, whereas CB showed a significant reduction (P<0.05) in paw volume of 2.10±0.07 and 10.02% at similar duration. However the extracts of AM, CB, DM, PP and VT at 240 min registered 1.92±0.03, 2.12±0.04, 1.75±0.05, 2.33±0.05 and 1.47±0.05 while it was 1.70±0.08, 2.08±0.07, 1.52±0.07, 2.50±0.05 and 1.25±0.09 at 300min (P<0.01) contributing a percent inhibition of 25.03, 16.57, 31.88, 9.52, 42.67% and 39.56, 25.78, 45.77, 11.06 and 55.55% respectively. The reduction in paw volume is more prominent at dose 300 mg by recording significant mean values (P<0.01) and percent inhibition at 180 min 1.57±0.10 (32.60%), 1.68±0.09 (27.98%), 1.38±0.10 (40.99%), 1.87±0.07 (19.46%) and 1.15±0.11 (50.35%), 240 min 1.36±0.11 (47.06%), 1.47±0.08 (42.78%), 1.11±0.07 (56.46%), 1.67±0.09 (34.99%) and 0.92±0.10 (64.40%) and at 300 min was 1.16±0.09 (58.58%), 1.30±0.10 (53.82%), 0.92±0.12 (67.17%), 1.47±0.07 (47.59%) and 0.75±0.07 (73.30%) respectively of AM, CB, DM, PP and VT. From the results, it is evident that the VT has maximum activity both at 200 and 300 mg dose followed by DM, AM, CB and PP in terms of the higher mean as well as inhibition percentage exhibiting higher inhibition of paw volume at 300 min of all the treatment under study. All the extracts at 200 and 300 mg/kg inhibited inflammation in a dose-related manner.

Human erythrocyte membrane stabilization assay

The protection of human blood erythrocyte membrane by five orchid extracts and standard against lysis is shown in Table 2. The extracts showed a concentration dependent anti-inflammatory activity, and the protection percent increased with increase in the concentration of the samples. The concentration of 2-10 mg/ml was evaluated in both standard and extracts. The maximum protection at 10 mg/ml of extracts were found to be 80.73±1.34 (AM), 70.00±0.60 (CB), 87.86±1.34 (DM), 57.56±1.12 (PP) and 95.16±0.36 (VT) whereas in standard Indomethacin it was found to be 92.48±0.83. It is clear from the results that VT possess the highest protection followed by DM, AM, CB and PP. Thus orchids exhibit notable protection of human RBC and significantly inhibit hemolysis.

Antinociceptive activity

Tail immersion Method

The antinociceptive activity of five orchid extracts assessed by the tail immersion method at different time intervals is shown in Table 3. The control group of animals showed the nearly steady state of reaction time. The mice treated with orchid extracts at 200 mg dose showed the extracts AM, DM and VT recorded significant increase (P<0.01) over the control in reaction time of 3.80±0.31 and 4.16±0.31, 4.50±0.22 and 5.83±0.48, and 4.00±0.37 and 4.83±0.40 at 120 and 150 min respectively, whereas CB at 120 and 150 min showed a significant increase (P<0.05) of 3.67±0.33 and 3.83±0.40, the extract PP has significant effect (P<0.05) of 3.83±0.40 only at 150 min. However the results at 300 mg recorded significant increase (P<0.01) in reaction time of

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extracts AM, CB, DM and VT of 5.17±0.40 and 7.00±0.63, 4.33±0.33 and 5.33±0.42, 8.00±0.58 and 11.33±1.14, and 7.33±0.50 and 9.50±0.56 while the extract PP showed a significant increase ($P<0.05$) of 4.00±0.37 and 4.83±0.70 at 120 and 150 min respectively. Standard drug Diclofenac (20 mg/kg) recorded the reaction time of 3.33±0.21, 4.67±0.21, 7.00±0.52, 9.50±0.34 and 14.60±0.21 at different time intervals. It is noted that the extracts in later phases of treatment are more effective. Further, it was also observed that the orchid extract DM has more effective analgesic property followed by VT, AM, CB and PP. The standard Diclofenac and extracts significantly prolonged the reaction time of animals with relatively extended duration of stimulation, confirming centrally mediated activity.

Hot plate Method

The analgesic activity of orchid extracts by hot plate method is given in Table 4. The onset of the reaction to thermal induced pain was significantly shorter in the control rats for any period of evaluation of pain. The reaction time of extracts response against thermal stimuli in hot plate at 200 mg dose showed a significant increase ($P<0.01$) in AM (9.50±0.43 and 10.17±0.48), DM (11.50±0.62 and 12.17±0.31), VT (10.33±0.49 and 11.67±0.49) whereas CB possess ($P<0.05$) of (7.67±0.61 and 7.83±0.42) respectively at 120 and 150 min and the extract PP even though showed analgesic effect it was not statistically significant. However the effective results were observed at dose 300 mg with a significant increase in reaction time of the extracts AM (13.17±0.54 and 15.55±0.48), CB (9.17±0.61 and 11.67±0.42), DM (15.83±0.54 and 17.00±0.31), PP (8.50±0.34 and 9.83±0.37) and VT (14.83±0.48 and 16.17±0.49) respectively at 120 and 150 min. The standard drug Diclofenac (20 mg/kg) showed the reaction time of 9.00±0.37, 12.17±0.31, 14.67±0.42, 17.17±0.48 and 19.5±0.22 at different time intervals. Thus from the results, it was evident that the extracts showed a dose dependent increase at different interval of time and the prominent increase was noted at 120 and 150 min, further the activity was found be inverse beyond 150 min. It was also found that among all the extracts DM has the highest analgesic activity followed by VT, AM, CB and PP. The present study evaluated the anti-inflammatory and antinociceptive effects of ethanolic extracts of five different orchids employing various experimental test models. Anti-inflammatory was tested both *in vivo* by carrageenan induced paw edema and *in vitro* by HRBC membrane stabilization. Cox-2 mediated increase in prostaglandin production in the central nervous system contributes to the severity of inflammation and pain responses following carrageenan injection in paw. Selective Cox-2 inhibitors that do not inhibit Cox-1 reduce levels of prostaglandin [22]. The results of the study showed an effective reduction of edema in all the orchids at 300 mg/kg bw with maximum inhibition at 180, 240 and 300 min. Among tested species of orchid, the VT has the highest effect in reduction of paw edema followed by DM, AM, CB and PP. The anti-inflammatory activity is more evident at later phases. Therefore it can be inferred that any plant extract that shows an effective reduction of edema in this model has to be effectively and selectively inhibiting Cox-2

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or PGE 2.In *in vitro* HRBC membrane stabilization assay, the tested extracts of orchids showed significant anti-inflammatory activity in a concentration dependent manner. The extracts at 10 mg/ml concentration showed the highest protection of HRBC in hypotonic solution with the highest percentage in VT followed by DM, AM, CB and PP. The extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane analogous to the lysosomal membrane and its stabilization during the inflammatory process. Stabilization of this membrane reduces inflammation by preventing the release of lysosomal constituents of activated neutrophil such as, bactericidal enzymes and proteases which causes further tissue inflammation and damage [19]. Membrane stabilization leads to the prevention of leakage of serum protein and fluids into the tissues during a period of increased permeability caused by inflammatory mediators [23]. The antinociceptive activity was performed in all the extracts were tested using tail immersion and hot plate method. The standard Diclofenac and extracts at 200 and 300 mg/kg showed a significant increase in pain threshold in both tail immersion and hot plate method. Maximum significance observed at 120 and 150 min was in the extract of DM, followed by VT, AM, CB and PP. The effectiveness of analgesic agents in the tail immersion pain model is highly correlated with relief of human pain [24]. Tail-immersion and hot plate method responses had been studied spinally mediated reflex and centrally acting analgesics respectively [25] In these tests, the nociceptors are sensitizing by sensory nerves and the involvement of endogenous substances such as prostaglandins are minimized [26]. Thus from the results, it can be concluded that the analgesic activity of orchid extracts may be fully mediated through the central mechanism and indicates a codeine-like mechanism by binding to opioid receptors. Different bioactive compounds are reported to be responsible for the anti-inflammatory and antinociceptive activity in plants. Recent literature reports that flavonoids possess potent antinociceptive and anti-inflammatory properties. Flavonoid has the ability to inhibit arachidonic acid metabolism and also enzyme prostaglandin synthetase involved in the production of chemical mediators of inflammation [27];[28];[29];[30]. Since prostaglandins are involved in the pain perception inhibition of their synthesis and distraction of synthesis of eicosanoids by flavonoids might be the possible reason for the analgesic activity of the extract [31]. Phytosterols and triterpenoid are attributed to reducing pain, swelling and inflammatory joint diseases with aging and stress [32]. Reports also suggest that flavonoids, phenolic compounds, tannins and saponins of some orchid showed anti-inflammatory and antinociceptive activity [33];[34];[35]. The ethanolic extract from the leaves of *Anoectochilus formosanus*h as shown delayed onset of anti-inflammatory activity starting from 4 hours post-carrageenan administration. Several compounds with anti-inflammatory activity were isolated from *Dendrobium monoliforme*. *Gastrodia elata* has also been a good source of compounds with anti-inflammatory, analgesic and anti-angiogenic activity [36]. In the present study phytochemical estimation of tested five orchid species reveals the presence of several bioactive compounds. Thus the presence of these compound

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and their synergistic properties might be rendering the possible mechanism for anti-inflammatory and antinociceptive activity in tested orchids. The current study reveals that the indigenous knowledge of medicinal orchids provides opportunities to validate their medicinal claims on scientific lines which are so far untapped from their potential.

4. CONCLUSION

On the basis of the results obtained it can be concluded that the ethanolic extracts of five orchids exhibit significant anti-inflammatory and analgesic activity. It can be predicted that more genera and species of orchid possesses the possibility of having medicinal properties. For therapeutic purpose, phytochemical investigation of these plants is an interesting area of research, leading to the isolation of several new compounds. Therefore, in recent years research is more oriented towards folk medicine or traditional practices, searching for new leads for the development of better drugs against infectious diseases and other common ailments.

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CONFLICT OF INTEREST

There is no any conflict of interest among the authors.

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SUPPIMENTARY FILES

Table 1: Anti-inflammatory effect of ethanolic extracts of five orchids on carrageenan induced paw edema

		Time Interval (min)				
Treatment	**Dose (mg/kg)**	**60 min**	**120 min**	**180 min**	**240 min**	**300 min**
Control		1.63±0.06	2.06±0.06	2.33±0.06	2.56±0.02	2.81±0.03
Standard	**20 mg**	1.22±0.06**	1.08±0.04**	0.69±0.05**	0.62±0.05**	0.40±0.03**
	%	23.10±3.19	51.49±2.16	70.37±1.80	75.65±2.11	85.77±1.29
AM	**200**	1.59±0.03	1.95±0.04	2.05±0.06**	1.92±0.03**	1.70±0.08**
	%	3.83±1.96	5.3±1.35	12.30±1.46	25.03±0.75	39.56±2.23
	300	1.47±0.03	1.6±0.03**	1.57±0.10**	1.36±0.11**	1.16±0.09**
	%	9.62±2.13	22.5±2.52	32.60±4.48	47.06±3.99	58.58±3.19
CB	**200**	1.60±0.04	1.98±0.06	2.1±0.07*	2.29±0.03**	2.08±0.07**
	%	2.40±1.32	4.30±0.68	10.02±1.32	16.57±1.93	25.78±2.89
	300	1.51±0.03	1.83±0.03*	1.68±0.09**	1.47±0.08**	1.30±0.10**
	%	8.06±1.69	11.38±2.28	27.98±3.27	42.78±3.37	53.82±3.56
DM	**200**	1.56±0.02	1.91±0.04	2.00±0.04**	1.75±0.05**	1.52±0.07**
	%	5.60±2.04	7.51±0.86	14.24±1.39	31.88±1.54	45.77±2.02
	300	1.45±0.04*	1.46±0.04**	1.38±0.10**	1.11±0.07**	0.92±0.12**
	%	11.06±1.76	29.11±3.07	40.99±4.39	56.46±3.04	67.17±4.38
PP	**200**	1.61±0.04	2.02±0.03	2.17±0.08	2.33±0.05**	2.50±0.05**
	%	1.90±0.90	3.47±1.52	6.97±1.59	9.52±1.31	11.06±1.27
	300	1.57±0.03	1.88±0.03	1.87±0.07**	1.67±0.09**	1.47±0.07**
	%	4.17±1.50	9.15±1.32	19.46±3.50	34.99±3.09	47.59±2.76
VT	**200**	1.54±0.03	1.89±0.03	1.83±0.03**	1.47±0.05**	1.25±0.09**
	%	6.03±2.45	8.18±1.19	21.49±1.38	42.67±1.86	55.55±2.93
	300	1.42±0.05*	1.36±0.05**	1.15±0.11**	0.92±0.10**	0.75±0.07**
	%	12.80±2.34	33.47±3.59	50.35±5.02	64.40±3.56	73.30±2.33

Values are expressed as mean ± SEM of six animals per group. Significance difference, $*p < 0.05$, $**p < 0.01$.

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Table 2: Anti-inflammatory effects of ethanolic extracts of five orchids on HRBC membrane stabilization assay

Percentage Inhibition						
Conc (mg/ml)	**Std**	**AM**	**CB**	**DM**	**PP**	**VT**
2	17.65±0.85	7.68±0.62	7.67±0.69	12.31±0.69	3.58±0.88	22.44±0.81
4	37.40±0.53	20.98±0.72	19.12±0.69	25.13±0.58	16.43±0.42	43.74±1.23
6	59.70±1.09	43.40±0.73	38.07±0.78	49.06±0.76	33.79±0.20	64.73±1.02
8	76.38±0.96	62.71±0.64	55.24±0.41	67.18±0.96	47.07±0.29	80.36±0.40
10	92.48±0.83	80.73±1.34	70.00±0.60	87.86±1.34	57.56±1.12	95.16±0.36

Table 3: Effect of ethanolic extracts of five orchids in tail immersion model

		Reaction time sec in different time intervals (minutes)					
Treatment	**Conc mg/kg**	**30 min**	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**
Control		2.33±0.21	2.33±0.21	2.33±0.21	2.33±0.21	2.33±0.21	2.33±0.21
Std	**20**	3.33±0.21	4.67±0.21**	7.00±0.52**	9.50±0.34**	14.60±0.21**	12.67±0.2**
AM	**200**	2.33±0.42	2.83±0.31	3.50±0.34	3.80±0.31**	4.16±0.31**	3.33±0.33
	300	2.83±0.48	3.50±0.22	4.50±0.43**	5.17±0.40**	7.00±0.63**	5.67±0.33**
CB	**200**	2.00±0.51	2.83±0.21	3.33±0.21	3.67±0.33*	3.83±0.40*	3.17±0.31
	300	2.50±0.5	3.33±0.21	3.83±0.40*	4.33±0.33**	5.33±0.42**	4.50±0.43**
DM	**200**	2.83±0.17	3.5±0.22	4.00±0.26**	4.50±0.22**	5.83±0.48**	5.00±0.37**
	300	3.33±0.21	4.33±0.33**	5.83±0.31**	8.00±0.58**	11.33±1.14**	8.50±0.43**
PP	**200**	1.67±0.21	2.67±0.42	3.17±0.31	3.50±0.34	3.83±0.40*	3.17±0.40
	300	2.33±0.42	3.33±0.42	3.67±0.33	4.00±0.37*	4.83±0.70*	4.00±0.37*
VT	**200**	2.67±0.33	3.17±0.4	3.67±0.33*	4.00±0.37**	4.83±0.40**	4.17±0.31**
	300	3.17±0.31	4.00±0.52**	5.33±0.42**	7.33±0.50**	9.50±0.56**	7.83±0.60**

Values are expressed as mean ± SEM of six animals per group. Significance difference, *$p < 0.05$, **$p < 0.01$.

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Table 4: Effect of ethanolic extracts of five orchids in Hot plate model of analgesia in mice

		Paw licking time in the sec in different Time (minutes)					
Treatment	**Conc mg/kg**	**30 min**	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**
Control		5.67±0.33	5.67±0.21	5.67±0.33	5.67±0.33	5.83±0.31	5.83±0.31
Std	**20**	9.00±0.37**	12.17±0.31**	14.67±0.42**	17.17±0.48**	19.5±0.22**	17.83±0.48**
AM	**200**	5.83±0.26	6.67±0.21	7.83±0.31**	9.50±0.43**	10.17±0.48**	8.00±0.58*
	300	6.50±0.43	7.67±0.42**	11.5±0.56**	13.17±0.54**	15.5±0.48**	13.67±0.80**
CB	**200**	5.50±0.22	6.50±0.22	7.33±0.21*	7.67±0.61*	7.83±0.42*	7.00±0.37
	300	6.00±0.45	7.00±0.45	8.00±0.52*	9.17±0.61**	11.67±0.42**	8.67±0.49**
DM	**200**	6.67±0.37	7.00±0.26**	9.67±0.56**	11.50±0.62**	12.17±0.31**	10.00±0.45**
	300	7.33±0.33*	9.5±0.43**	13.17±0.54**	15.83±0.54**	17.00±0.31**	15.33±0.49**
PP	**200**	5.17±0.31	5.83±0.31	6.83±0.31	7.17±0.48	7.80±0.37	6.95±0.42
	300	5.83±0.31	6.50±0.43	7.67±0.42*	8.50±0.34**	9.83±0.37**	7.83±0.6*
VT	**200**	6.33±0.48	6.83±0.31*	8.00±0.37**	10.33±0.49**	11.67±0.49**	8.5.0±0.56**
	300	7.17±0.31*	8.33±0.42**	12.33±0.67**	14.83±0.48**	16.17±0.49**	14.17±0.31**

Values are expressed as mean ± SEM of six animals per group. Significance difference*$p < 0.05$, **$p < 0.01$.

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