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PHYTOCHEMICAL ANALYSIS AND *IN-VIVO* SCREENING OF *PSEUDODRYNARIA CORONANS* (WALL. EX. METT.) CHING ON ITS ANALGESIC AND ANTI-INFLAMMATORY PROPERTIES

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ABSTRACT: The methanolic rhizome extract of Pseudodrynaria coronans (MEPC) was assessed for its preliminary phytochemical constituents, anti-nociceptive and anti-inflammatory activity. Acute toxicity of the extracts was tested following the OECD – 425 guidelines and after giving MEPC at the dose of 2000 mg/kgbw indicating that the LD50 is higher than the given dose. Two doses 200 and 400 mg/kg bw. were selected for all the experiments. Hot plate and Tail immersion methods showed that the extract was statistically significant ($p < 0.05$ and $p < 0.01$). Different concentrations of the MEPC showed significant inhibition of protein denaturation and membrane stabilization. Also, the MEPC exhibited significant inhibition of carrageenan-induced paw oedema ($p < 0.01$) and cotton pellet-induced granuloma ($p < 0.05$ & $p < 0.01$) inflammatory responses in the rats.

INTRODUCTION: The advancement in pharmaceutical research makes natural products, mainly medicinal plants, a new hope for drug development. The pure compound(s) or standardized extracts derived from a wide pool of fauna become the greatest assets for researchers in their pursuit of combating different ailments ¹. Methods were developed to isolate the bioactive compounds from the highly acclaimed remedial plants instead of using the whole plant, thereby using them for developing drugs with higher efficacy and lower side effects ².

Pain may be defined as the uncomfortable sensation produced by nerve impulses produced by the nervous stimuli in the brain, the drugs used for terminating the pain or alter pain sensitivity are named 'Analgesic'.

Opiates and non-steroidal anti-inflammatory drugs are our only defense and remedy in our fight against pain, unfortunately leaving behind and adverse effects that are detrimental to our physiological system. Because of these side effects and the low potency of current analgesia-inducing drugs, there is an urgent call for the search for an alternative. Medicinal plants with their diverse phytochemical compounds may be the answer for the long-sought alternatives from which novel analgesic could be discovered ³. Man is equipped with a sturdy physiological system in which inflammation is one of the defense mechanisms in which pain is the primary associate followed by

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alteration in a membrane, vascular permeability, and increase denaturation of protein. Inflammation is classified into two categories, namely acute and chronic inflammation which is triggered by the release of kinins, prostaglandins, and histamine the inflammatory mediators. Steroidal and Non-steroidal anti-inflammatory drugs (NSAIDs) are clinically available treatments in the market, but due to their side effects, searching for phytochemicals alternative is the need of the hour⁴.

Pseudodrynaria coronans (Wall. Ex. Mett.) Ching is locally known as “Awmvel” among the Mizo. This epiphytic basket fern belongs to the Polypodiaceae family having a white rhizome that resembles a paw encircling the host plant. The plant is native to tropical Africa, South Asia, East Asia, Southeast Asia, Australia, and Oceania in which the Chinese use the rhizome for the treatment of traumatic injury, rheumatic arthritis, tinnitus, and toothache⁵. In Mizoram, the crushed rhizomes are applied externally for infection against herpes zoster and taken orally for an irregular heartbeat as traditional medicine. Unfortunately, this medicinally potential plant has not been studied intensively; therefore investigation of phytochemical constituents, acute toxicity, anti-nociceptive and anti-inflammatory studies have been carried out to study the true nature of this plant and to justify its traditional uses.

MATERIALS AND METHODS:

Collection and Identification of the Plant

Material: The rhizomes of *Pseudodrynaria coronans* were collected from Aizawl, Mizoram (India) in the month of September. The specimens were identified and authenticated by Botanical Survey of India (BSI), Kolkata (Reference No: BSI/ERC/TECH/2018-19/688) and herbarium was preserved in RIPANS library for future reference.

Preparation of Plant Extract: The rhizomes of the plants were washed, cut into pieces, dried in shade and powdered using mechanical grinder. The powdered material was then subjected to successive extraction using Soxhlet apparatus. The powdered sample was subjected to extraction with petroleum ether for the removal of fats, which was followed by the extraction with chloroform and methanol for 72 h each. Then the extracts were obtained by using rotary vacuum evaporator and stored in the refrigerator at 4 °C for further analysis.

Phytochemical Screening: The preliminary phytochemical screening of the methanolic extracts was carried out as per the standard protocol. In the phytochemical screening, methanolic extract of *Pseudodrynaria coronans* was screened for the presence of phytochemical constituents like alkaloids, carbohydrates, glycosides, tannins, phenols, steroids, flavonoids, proteins and amino acids, fats, and fixed oil.

Animals Care and Maintenance: Six to eight weeks old Wistar rats of both sexes were maintained in an institution animal house in RIPANS in simulated atmospheric conditions (temperature: 25±2 °C; relative humidity: 60-70%; 12 h of light-dark alternative cycles). Animals were acclimatized for 7 days in a sterile polypropylene cage using sawdust as bedding. Experimentation and methodology were approved by the Institutional Animal Ethics Committee of Regional Institute of Paramedical & Nursing Sciences (RIPANS), bearing Approval no. IAEC/RIPANS/34, 35 & 36.

Diet: All the animals were given water ad libitum and fed with a standard commercial rodent diet.

Acute Oral Toxicity Test: An acute oral toxicity test was performed as per the Organization for Economic Co-operation and Development guidelines 425 (OECD-425). A total of 10 animals (5 males and 5 nulliparous females) were selected and divided into two groups randomly. Group-I received methanol extract of *Pseudodrynaria coronans* (MEPC), and group - II received normal saline.

Administration of Dose: Animals were fasted overnight before oral administration. The weight of animals was measured, and dosage calculation was done accordingly. MEPC was administered at the dose of 2000 mg/kg body weights of the rat orally by feeding gavage. During the study, changes in body weight, a sign of toxicity, food and water intake were observed.

In-vivo Analgesic Activity:

Hot Plate Method: Analgesic activity of MEPC using hot plate method was determined by the method described by Hijazi *et al.* with slight modification⁶. Animals were divided into 4 groups consisting of 5 animals each (both sexes). The first

group (control group) was given normal saline, and group- II (Standard group) was given Morphine sulfate at the dose of 5 mg/kg b.w intraperitoneally. Group – II and group – IV were treatment groups receiving MEPC at the dose of 200 and 400 mg/kg b.w, respectively. The animals were placed on a hot plate (55 ± 1 °C), the reaction time before the treatment was taken, and four more readings were taken after drug administration at 30, 60, 90, and 120 min. The cut-off time of 15 seconds was maintained to avoid paw damage.

Tail Immersion Method: The tail Immersion method was performed as described by Malairajan *et al.* with slight modification (Malairajan *et al.*, 2011). The water bath was maintained at a temperature of 55 ± 1 °C, and the rat's tail about 3 cm was immersed in the water bath; the tail withdrawal time was noted as reaction time or tail-flick latency. Animals were divided into four groups (namely control, standard, 200 mg/kg b.w, and 400 mg/kg b.w) of 5 animals each. To avoid the injury of the tissues of the tail, the maximum cut-off time for immersion was taken as 15 sec.

The initial reading was taken before administration of the test and standard drugs and then at 30, 60, 90, 120, and 180 min after the administration. The reaction time was measured and calculated.

In-vitro Anti-Inflammatory Activity:

Anti-Denaturation Activity: Anti-denaturation assay was carried out using a method developed by William *et al.* with slight modification⁷. 0.2% BSA was prepared in Tris buffer saline, and the pH was adjusted using acetic acid to 6.8. Extract stock solution of 1000 µg/ml was prepared in methanol, and 4 different concentrations of 200, 400, 600, 800, and 1000 µg/ml were prepared from this stock solution. From these working concentrations, 50 µl each was taken in an Eppendorf tube, and 5 ml of 0.2% w/v BSA was added. The standard consisted of 10 µg/ml of Aspirin in methanol with 5 ml 0.2% w/v BSA solution. They were heated at 72 °C for 5 minutes and then cooled for 10 min. The absorbance of these solutions was determined by using UV/Vis Double beam spectrophotometer (Elico SL-196) at a wavelength of 660 nm. The % inhibition of precipitation (denaturation of the protein) was determined using the following formula:

$$\% \text{ inhibition of denaturation} = (\text{Absorbance of control} - \text{Absorbance of extract}) \times 100 / \text{Absorbance of control}$$

Inhibition of Albumin Denaturation: Albumin denaturation was determined following Chandra *et al.* method with slight modification⁸. The reaction mixture was made up of 0.2 ml of eggs albumin (Fresh hen egg), 2.8 ml of phosphate-buffered saline (PBS, pH 6.4), and 2 ml of varying concentrations (200, 400, 600, 800 & 1000 µg/ml) of MEPC. 2 ml of Aspirin was used as a reference drug instead of MEPC and double-distilled H₂O served as control.

The mixtures were incubated at 37 °C for about 15 min and then heated at 70 °C for 5 min. After cooling, their absorbance was measured at 660 nm, and the percentage inhibition of protein denaturation was calculated by using the formula;

$$\% \text{ Inhibition of denaturation} = (\text{Absorbance of control} - \text{Absorbance of extracts}) \times 100 / \text{Absorbance of control}$$

HRBC Membrane Stabilization Method: Blood samples were collected from healthy individuals who had not taken any NSAIDs drugs two weeks before the experiment. It was then mixed with an equal volume of sterilized Alsevers solution and centrifuged at 3000 rpm. The packed cells were washed with is saline solution, and a 10% v/v solution was prepared with normal saline and kept at 4 °C undisturbed before use.

Different concentrations of MEPC extract (200, 400, 600, 800, and 1000 µg/ml), Diclofenac sodium (standard), and control were separately mixed with 1 ml of phosphate buffer, 2 ml of hyposaline, and 0.5 ml of 10% HRBC suspension. All the mixtures were incubated at 37 °C for 30 min and centrifuged at 3000 rpm. The supernatant liquid was decanted, and the hemoglobin content was estimated spectrophotometrically at 560 nm. The percentage of HRBC membrane stabilization was calculated by using the formula⁹:

$$\text{Percentage stabilization} = \text{Absorbance of control} - \text{Absorbance of extract} \times 100 / \text{Absorbance of control}$$

In-vivo Anti-Inflammatory Activity:

Carrageenan-Induced Rat Paw Edema (Acute Model of Inflammation): Animals were divided into four groups, namely Control, Standard drug (Indomethacin 10 mg/kg, p.o.), MEPC 200 mg/kg

b.w, and MEPC 400 mg/kg b.w consisting of four rats each in a group. Treatments were given 1 h prior to the administration of carrageenan. Paw edema was induced by sub plantar injection of 0.1 ml Of 1%w/v carrageenan suspended in 0.9% normal saline into the right hind paw of each group of rats¹⁰. Paw edema was measured by taking the paw diameter using Vernier calliper at (0, 1, 2, 3, 4, 5, 6, and 24 h¹¹. The increase in paw thickness, as well as the percentage inhibition, was calculated by the following equations:

Increase in paw thickness in control

$$(P_c) = P_t - P_o$$

Increase in paw thickness in treated

$$(P_t) = P_t - P_o$$

$$\text{Percentage inhibition} = [(P_c - P_t)/P_c] \times 100$$

Where, Pt is the paw thickness at time t, Po is initial paw thickness carrageenan-Induced Paw edema in rats.

Cotton Pellet- Induced Granuloma in Rats (Chronic Model of Inflammation): Chronic inflammation was assessed through cotton pellet induced granuloma model according to D'Arcy *et al.* method with slight modification. Animals were divided into four groups consisting of four animals each in a group and were anesthetized, and sterile cotton pellets (10±1 mg) were implanted subcutaneously along the axillae of the rats. Control vehicle (0.5% CMC), MEPC (200 and 400 mg/kg p.o.), and standard drug Diclofenac sodium (10 mg/kg p.o.) were administered successively for 7 days starting from the day of cotton pellet implantation. The cotton pellets were surgically removed on the 8th day, and the weight of the wet pellets were measured immediately. After that, the wet pellets were dried in an oven at 60 °C for 24 h. An increase in the wet and dry weight of the pellets was recorded as a measure of granuloma formation, and also their percentage inhibition was calculated by using the formula.

$$\% \text{ inhibition} = \frac{\text{Mean granuloma weight of control (wet/dried)} - \text{Mean granuloma weight of test (wet/dried)}}{\text{mean weight of control}} \times 100$$

Statistical Analysis: For statistical analysis, GraphPad Prism 8.0.2 software was used. All the data were statistically analyzed using one-way

ANOVA followed by Dunnet's multiple comparison test. P values <0.05 were considered statistically significant.

RESULTS:

Phytochemical Analysis: The phytochemical investigation from the methanolic rhizomes extract of *Pseudodrynaria coronans* revealed the presence of steroids, flavonoids, tannin, saponin, triterpenoids, amino acids, and carbohydrates.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF METHANOL EXTRACT OF PSEUDODRYNARIA CORONANS

Phytochemical test	Different extract		
	Petroleum ether	Chloroform	Methanol
Alkaloids	-	+	-
Steroids	-	+	+
Flavonoid	-	-	+
Reducing sugar	-	+	-
Tannins	-	-	+
Saponins	-	-	+
Triterpenoids	-	+	+
Amino acid	-	-	+
Fats and Fixed oils	+	-	-
Carbohydrates	-	+	+
Glycosides	-	-	-

Acute Toxicity Studies: The acute oral toxicity study from the rhizomes of methanolic extract of *Pseudodrynaria coronans* showed no toxicity or mortality within 24 h, and there were no signs of behavioral changes within 14 days after administering 2000 mg/kg dose of the extract (**Table 2, 3 & 4**). Therefore 200 and 400 mg/kg, b.w were selected as doses for the study of analgesic activity.

In-vivo Analgesic Activity:

Hot Plate Method: The results of the hot plate method revealed that oral administration of the MEPC at the doses of 200 and 400 mg/kg, b.w., produced significant increases in the latency time, which can be compared to that of the standard drug (morphine, 5 mg/kg., b.w., p.o) as shown in **Table 5**. No significant difference in the thermal stimulus in rats treated with the vehicle (control) was observed throughout the different time points of the experiment. Administration of Morphine significantly increases the response time of the rats (8.34 seconds 90 min) and its analgesic effect decreased with time but remained significant even after 1 h and 30 min for both the extracts. In comparison, 400 mg/kg is more effective as

compared to the 200 mg/kg extract, and the results were found to be highly statistically significant ($P < 0.05$, $P < 0.01$, respectively).

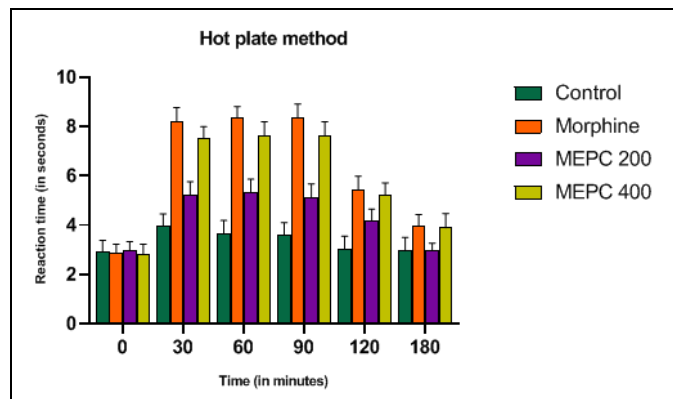


FIG. 1: ANALGESIC ACTIVITY OF MEPC AND MORPHINE ON HOT PLATE METHOD. THE ANIMALS WERE TREATED DAILY WITH THE EXTRACTS (P.O) OR MORPHINE (I.P). Each point represents the mean \pm S.E.M. of change of paw size. Both extracts and morphine were effective compared to control (normal saline), by one-way ANOVA, Dunnet’s multiple comparison tests

The results of the Tail immersion method presented in **Table 6** showed that oral administration of the methanolic extracts at the doses of 200 and 400 mg/kg, b.w., produced significant increases in the reaction time in comparison to that of the standard drug (morphine, 5 mg/kg, b.w., p.o).

When treated with the vehicle (control), no significant difference in the thermal stimulus in rats was observed throughout the experiment. The analgesic effect of Morphine decreased with time but remained significant even after an hour.

All doses of the extracts showed significant increases in the latency time of rats when compared to the control group. The analgesic activity in the

standard and extracts treated in animals is noted from 30 min onwards till 120 min in which response peaked at 90 min for Morphine and both the extracts.

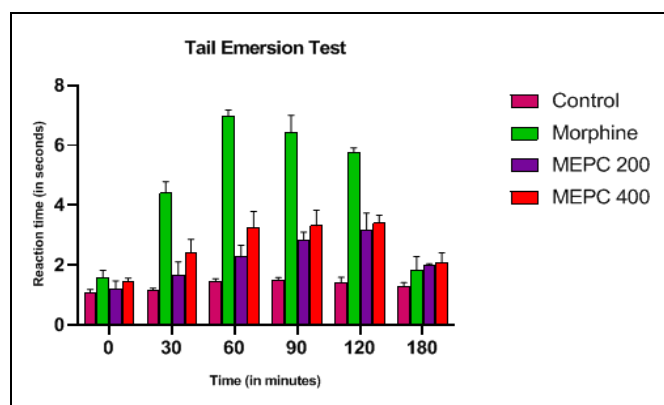


FIG. 2: ANALGESIC ACTIVITY OF MEPC AND MORPHINE ON TAIL EMERSION METHOD. THE ANIMALS WERE TREATED DAILY WITH THE EXTRACTS (P.O) OR MORPHINE (I.P). Each point represents the mean \pm S.E.M. of change of paw size. Both extracts and morphine were effective compared to control (normal saline), by one-way ANOVA, Dunnet’s multiple comparison tests.

In-vitro Anti-Inflammatory Activity:

Anti-denaturation Activity: The methanolic rhizomes extract of *Pseudodrynaria coronans* has the ability to protect Bovine Serum Albumin (BSA) against heat denaturation, as shown in **Table 7**. The methanolic extract has shown 60.36% and 82.02% anti-denaturation activity on BSA at a concentration of 800 $\mu\text{g/ml}$ and 1000 $\mu\text{g/ml}$, respectively.

This result indicates that the anti-denaturation action of MEPC increased with the increased concentration. The standard Aspirin had shown 87.0% of anti-denaturation effect at 1000 $\mu\text{g/ml}$.

TABLE 2: EFFECT OF MEPC AND ASPIRIN ON ITS ANTI-DENATURATION ACTIVITY BY INHIBITING THE HEAT DENATURATION OF BOVINE SERUM ALBUMIN (BSA)

Concentration (μml)	Aspirin		MEPC	
	Mean \pm SEM (Absorbance 660nm)	%Inhibition	Mean \pm SEM (Absorbance 660 nm)	% Inhibition
200	0.14 \pm 0.01**	35.2	0.16 \pm 0.01*	23.96
400	0.19 \pm 0.01**	49.76	0.14 \pm 0.01**	35.02
600	0.07 \pm 0.02**	66.82	0.12 \pm 0.01**	44.23
800	0.05 \pm 0.01**	76.49	0.08 \pm 0.02**	60.36
1000	0.02 \pm 0.01**	87.09	0.03 \pm 0.01**	82.02

*All the results presented here are Mean \pm SEM, compared with the standard by one-way ANOVA, Dunnet’s multiple comparison tests and the statistical significance taken at the level of $P < 0.05$ as significant and $p < 0.01$ as very significant

Inhibition of Albumin Denaturation: In this assay, the denaturation of egg albumin is induced by treatment of heat, and the denatured protein

expresses antigen associated with type III hypersensitive reaction¹². From the results, concentration-dependent inhibition of protein

albumin denaturation by the extracts and the standard drug Aspirin throughout the concentration range (200, 400, 600, 800, 1000 µg/ml) were highly prominent **8**. Maximum inhibition of

75.59% for MEPC and 83.82% for Aspirin standard drug was observed at the concentration of 1000 µg/ml.

TABLE 3: EFFECT OF MEPC AND ASPIRIN ON ITS ANTI-DENATURATION ACTIVITY BY INHIBITING THE DENATURATION OF EGG ALBUMIN

Concentration (µ/ml)	Aspirin		MEPC	
	Mean ±SEM (Absorbance 660 nm)	%Inhibition	Mean ± SEM (Absorbance 660 nm)	% Inhibition
200	0.31±0.02**	43.01	0.42±0.02**	21.5
400	0.22±0.02**	59.55	0.33±0.02**	38.23
600	0.14±0.01**	73.89	0.22±0.01**	58.63
800	0.12±0.01**	76.28	0.14±0.01**	73.34
1000	0.08±0.02**	83.82	0.11±0.01**	75.59

*All the results presented here are Mean±SEM, compared with the standard by one-way ANOVA, Dunnet’s multiple comparison tests and the statistical significance taken at the level of p<0.01 as very significant

HRBC Membrane Stabilization Method: As shown in **Table 9**, MEPC has shown significant anti-inflammatory activity when compared to the standard diclofenac sodium. The percentage of stabilization of MEPC and standard diclofenac at a

concentration of 1000 µg/ml was found to be 83.35% and 90.62%, respectively. The activity of the extracts increased with the increase in the concentration, which indicates the activity of the extract was concentration-dependent.

TABLE 4: EFFECT OF MEPC AND ASPIRIN ON ITS ANTI-DENATURATION ACTIVITY BY HRBS MEMBRANE STABILIZATION

Concentration (µ/ml)	Diclofenac		MEPC	
	Mean ± SEM (Absorbance 560 nm)	%Inhibition	Mean ± SEM (Absorbance 560 nm)	%Inhibition
200	0.11±0.028*	42.7	0.15±0.01 ^{ns}	20.31
400	0.07±0.010**	60.93	0.10±0.02*	45.31
600	0.04±0.01**	78.12	0.08±0.02**	58.33
800	0.03±0.01**	80.12	0.05±0.01**	71.35
1000	0.01±0.01**	90.62	0.03±0.01**	83.85

*All the results presented here are Mean±SEM, compared with the standard by one-way ANOVA, Dunnet’s multiple comparison tests and the statistical significance taken at the level of *p<0.05 as significant and **p<0.01 as very significant.

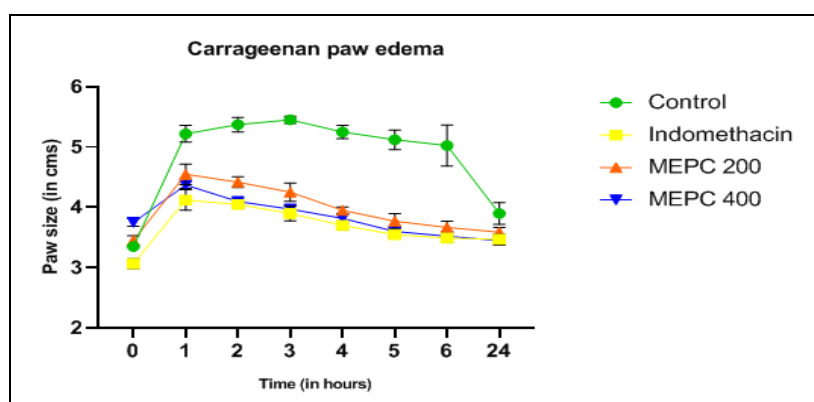


FIG. 3: EFFECT OF MEPC AND INDOMETHACIN ON CARRAGEENAN INDUCED PAW EDEMA. THE INFLAMMATION WAS PRODUCED BY SUBAPONEUROTIC. THE ANIMALS WERE TREATED DAILY WITH THE EXTRACTS (P.O) OR INDOMETHACIN (I.P). Each point represents the mean ± S.E.M. of change of paw size. Both extracts and Indomethacin were effective compared to control (normal saline), by one-way ANOVA, Dunnet’s multiple comparison tests

In-vivo Anti-inflammatory Activity: Carrageenan-Induced Rat Paw Edema (Acute Model of Inflammation): The results of the present finding shown in **Table 10** revealed that the MEPC (200 & 400 mg/kg) significantly inhibited

the carrageenan-induced edema, which is comparable with the standard drug Indomethacin (10 mg/kg). The anti-inflammatory activity of the extract was found to be dose-dependent.

Cotton Pellet- Induced Granuloma in Rats (Chronic Model of Inflammation): Based on the results shown in **Table 11**, the MEPC at the dose of 200 mg/kg and 400 mg/kg revealed a significant

anti-inflammatory activity by the reduction of wet and dry weight granuloma when compared with the standard drug Indomethacin (10 mg/kg).

TABLE 5: EFFECT OF MEPC AND INDOMETHACIN ON ITS ANTI-INFLAMMATORY ACTIVITY AGAINST COTTON PELLETT-INDUCED GRANULOMA

Groups	Treatment	Doses (mg/Kg)	Wet weight of cotton pellets (Mean \pm SEM)	Dry weight of cotton pellets (Mean \pm SEM)	% Inhibition
1	Control (1% carrageenan + Normal saline)	-----	63.97 \pm 1.97	25.75 \pm 0.85	-----
2	MEPC	200	56.1 \pm 1.08*	19.75 \pm 0.75**	23.3
3	MEPC	400	43.5 \pm 1.84**	14.75 \pm 0.47**	42.75
4	Indomethacin	10	42.75 \pm 1.79**	13.75 \pm 1.03**	46.6

DISCUSSION: In the present study, the methanol extract of the plant *Pseudodrynaria coronans* (MEPC) is analyzed for its analgesic and anti-inflammatory activities. Hot plate method and tail immersion methods were employed for the analgesic activity of MEPC. **Fig. 1** revealed that oral administration of the MEPC at the doses of 200 and 400 mg/kg, b.w., produced significant increases in the latency time which can be compared to that of the standard drug (morphine, 5 mg/kg, b.w., p.o). Hot plate method produces two measurable behavioral components in response to thermal pain (i.e., paw licking and jumping in rats which are considered supraspinally integrated) concerning their reaction times. Administration of Morphine significantly increases the response time of the rats to reach 8.34 secs (at 90 min). Its analgesic effect decreased with time but remained significant even after 1 h, 30 min for both the extracts. In comparison, 400mg/kg is more effective as compared to the 200 mg/kg extract, and the results were found to be highly statistically significant (P<0.05, P<0.01, respectively).

Similarly, the results of the Tail immersion method presented in **Fig. 2** showed that oral administration of the methanolic extracts at the doses of 200 and 400 mg/kg, b.w., produced significant increases in the reaction time which can be compared to that of the standard drug (morphine 5 mg/kg, b.w., p.o). Analgesic drugs which are centrally acting elevate the pain threshold of animals towards heat and pressure. Morphine administration significantly increased the response time of the animal to reach 6.994 seconds (at 60 min). Its analgesic effect decreased with time but remained significant even

after an hour. All doses of the extracts showed significant increases in the latency time of rats when compared to the control group.

Protein denaturation is a well-documented cause of inflammation and arthritic diseases. In certain arthritic diseases, the production of auto-antigens may be due to the denaturation of proteins (Sangita, 2012). The methanolic extract has shown 60.36% and 82.02% anti-denaturation activity on BSA at a concentration of 800 μ g/ml and 1000 μ g/ml respectively. This result indicates that the anti-denaturation action of MEPC increased with the increased concentration. The standard Aspirin had shown 87.0% of anti-denaturation effect at 1000 μ g/ml.

The denaturation of egg albumin is induced by treatment of heat, and the denatured protein expresses antigen associated with type III hypersensitive reaction¹². The conventional NSAIDs like phenylbutazone and Indomethacin were already proved that they not only act in the inhibition of endogenous prostaglandins production by blocking COX enzyme but also by prevention of denaturation of proteins¹².

Maximum inhibition of 75.59% for MEPC and 83.82% for Aspirin standard drug was observed at the concentration of 1000 μ g/ml. Since the erythrocyte membrane is similar to the lysosomal membrane and its stabilization suggests that the extract may also stabilize the lysosomal membrane, the HRBC method has been employed for the evaluation of *in-vitro* anti-inflammatory activity¹³. The percentage of stabilization of MEPC and

standard diclofenac at a concentration of 1000 µg/ml was found to be 83.35% and 90.62%, respectively.

The results of the present finding shown in **Fig. 3** revealed that the MEPC (200 & 400 mg/kg) significantly inhibited the carrageenan-induced edema, which is comparable with the standard drug Indomethacin (10 mg/kg). The anti-inflammatory activity of the extract was found to be dose-dependent. Through this result, it indicates that the extract was likely to act by hindering the release and/or action of prostaglandin 14 and 15.

Cotton pellet-induced granuloma represents a chronic inflammation model that is used extensively to assess the proliferative components of the inflammation by the development of granuloma in rodents 28 13. Based on the results of this study shown in **Table 5**, the MEPC at the dose of 200 mg/kg and 400 mg/kg revealed a significant anti-inflammatory activity by the reduction of wet and dry weight granuloma, which is found to be comparable with the standard drug Indomethacin (10 mg/kg).

It was reported that from the phytochemical screening, flavonoids, tannins, and saponins possessed analgesic and anti-inflammatory activities. Therefore, these phytochemical constituents found to be present in the methanolic extract of *Pseudodrynaria coronans* may be responsible for the observed analgesic effects and anti-inflammatory activity¹.

Based on the present investigation, it can be concluded that the methanolic rhizomes extract of *Pseudodrynaria coronans* possessed significant anti-nociceptive and anti-inflammatory activity, which may be due to the presence of various phytochemicals like flavonoids, triterpenoids, tannins, saponin, amino acids, carbohydrates, and steroids. These studies also support the traditional use of this plant in various ailments and also proved to be non-toxic through the findings of the Acute oral toxicity test. Further analysis is however required to isolate the phytochemical constituents responsible for the observed effects, which may be important for the discovery of lead compounds that can be used as an alternative for the new phase treatment of pain and inflammation.

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