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Original Article

INVESTIGATION OF *IN-VIVO* ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY IN RODENTS AND *IN-VITRO* ANTIOXIDANT ACTIVITY OF EXTRACTS OF WHOLE PLANT OF C*YATHOCLINE PURPUREA*

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ABSTRACT

Objective: Objective of the study was to evaluate *Cyathocline purpurea* (Buch-Ham ex D. Don.) Kuntze (Whole plant) for *in-vivo* analgesic and anti-inflammatory activity and *in vitro* antioxidant activity

Methods: Peripheral and central analgesic effect was evaluated by acetic acid and hot plate respectively in mice; anti-inflammatory effect was evaluated by carrageenan induced paw edema (acute) and cotton pellet induced granuloma (sub acute) methods in rats. Three extracts of different polarity i.e., petroleum ether extract of *Cyathocline purpurea* (PECP), methanol extract of *Cyathocline purpurea* (MECP) and aqueous extract of *Cyathocline purpurea* (AECP) were administered orally at doses of 100, 200 and 400 mg/kg b.w. Acute oral toxicity study, preliminary phytochemical screening and *in vitro* antioxidant potential of the extracts were also studied.

Results: Significant analgesic and anti-inflammatory activity was observed with MECP (400 mg/kg). MECP (100, 200 and 400 mg/kg) dose dependently reduced the writhing response, reduced paw edema in carrageenan study and decreased the weight of granuloma in cotton pellet model. PECP showed weak analgesic and anti-inflammatory activity while AECP was derived of activity. All the extracts did not show central analgesic activity. IC_{50} value of MECP showed significant antioxidant activity. All the extracts were found to be safe at the dose of 2000 mg/kg.

Conclusion: The results of the experimental study confirmed that MECP possesses significant analgesic, anti-inflammatory and antioxidant activity. The observed pharmacological effect may be due to the presence of phytoconstituents like flavonoids, steroids, phenols, saponins and terpenoids present in MECP.

Keywords: *Cyathocline purpurea*, Analgesic, Anti-inflammatory, Antioxidant.

INTRODUCTION

Pain is frequently associated with inflammation [1]. Inflammation is a complex pathophysiological process mediated by a variety of signaling molecules produced by leucocytes, macrophages and mast cells as well as activation of complement factors that bring about edema formation as a result of extravasation of fluid and proteins and accumulation of leucocytes at the inflammatory site [2]. Inflammation plays an important role in various diseases, such as rheumatoid arthritis, atherosclerosis and asthma, which all show a high prevalence globally [3]. Inflammation is one of the most important processes involved in the defense of an organism [4]. Pain is a pathophysiological response of living tissue to undesirable stimuli. The pharmacology of pain has become a complex field [5]. Non-steroidal anti-inflammatory drugs (NSAIDs) are used worldwide for the treatment of inflammation and pain. However, the side effects of currently available anti-inflammatory drugs include gastric ulcer, renal damage, bronchospasm and cardiac abnormalities have limited their use [6]. Therefore, development of newer and more substantial drugs with lesser side effects is still needed. India is a rich source of medicinal plants and a number of plant derived extracts are used against diseases in various systems of medicines such as Ayurveda, Siddha and Unani. Only few of them have been scientifically explored. Plants have served as the primary source of medicines for man, and they have continued to provide mankind with new, novel therapeutic remedies to date [7]. *Cyathocline purpurea* (Buch-Ham ex D. Don.) Kuntze. Fam. Asteraceae is a seasonal Indian medicinal plant commonly found in moist habitats such as along watercourses and in rice fields throughout most of peninsular and northern India at an elevation of 1300 m [8]. Traditionally *Cyathocline purpurea* has anticancer [9]; antimicrobial, anthelmintic, hypotensive properties and the roots are reported to have stomach relieving properties [8]. Literature survey revealed the presence of chemical constituents cythoclol [10]; eudesmanolide, guaianolide, sesquiterpene lactones, isoivangustin and guaianolide, 6 α-hydroxy-4(14), 10(15)-guainadien-8α-, 12-olide [11]. Sesquiterpene lactones containing alpha-methylene-gamma-lactone moiety are reported to possess anti-inflammatory activity [12]. The presence of sesquiterpene lactones containing alpha-methylene-gamma-lactone moiety like santamarin, 9 β-acetoxycostunolide and 9 β-acetoxyparthenolide have been reported to be present in *Cyathocline purpurea* [13]. However till now no pharmacological studies about the analgesic, anti-inflammatory and antioxidant activity of *Cyathocline purpurea* have been reported. The objective of the study was to investigate the analgesic and anti-inflammatory activities of extracts of *Cyathocline purpurea* (Whole plant) in different animal models, and *in vitro* evaluation of antioxidant activity of the extracts.

MATERIALS AND METHODS

Procurement and authentification of plant

The whole plant *Cyathocline purpurea* (Buch-Ham ex D. Don.) Kuntze. Fam. Asteraceae was collected in the month of January 2012, from Pune, Maharashtra. The plant was then identified and authenticated by J. Jayanthi, Scientist C, Botanical Survey of India and voucher specimen (No. BSI/WRC/Tech/2013/1094) was deposited at that institute.

Drugs and chemicals: Carrageenan, DPPH (1, 1-diphenyl -2-picylhydrazyl), TBA (Thiobarbituric acid) (Sigma-Aldrich, St. Louis, MO, USA), Acetic acid (Pure Chem. Ltd., India), Petroleum ether, TCA

(Trichloracetic acid) (Merck), Methanol (Molychem, India), 2-Deoxy-D-Ribose (Sisco laboratories, Mumbai), Acetylsalicylic acid -100 (Cipla Pharmaceuticals), Pentazocine (Fortwin, Ranbaxy), Diclofenac (gift sample from Emcure pharmaceuticals Ltd., Pune) were obtained, and all other chemicals and solvents used were of analytical grade.

Preparation of extracts

The whole plant was shade dried and powdered. Dried powder (100 g) was subjected to successive extractions by maceration using petroleum ether followed by methanol and then water. The extracts were filtered and concentrated on a rotary evaporator (Medica Instrument, India) and stored in desiccator. The percentage yields of petroleum ether extract of *Cyathocline purpurea* (PECP), methanol extract of *Cyathocline purpurea* (MECP) and aqueous extract of *Cyathocline purpurea* (AECP) were 3.3 %, 4.7 % and 6.7 % respectively.

Experimental animals and approval

Female Wistar rats weighing (180-220 g) and female Swiss albino mice (25-30 g) were purchased from National Toxicology Centre, Pune, India. They were housed in the animal house at a ambient temperature of 25 ± 1 °C and relative humidity of 45 to 55% under 12-h light : 12-h dark cycle. The animals had free access to food pellets (Manufactured by Pranav Agro Industries Ltd., Sangli India) and water *ad libitum*. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA), India (Approval No. CPCSEA/28/2014).

Phytochemical screening

Preliminary phytochemical studies of all the extracts were performed for glycosides, proteins, carbohydrates, amino acids, steroids, alkaloids, flavonoids, tannins, triterpenoids, phenols and saponins using standard procedures [14].

Acute oral toxicity study

Healthy female Swiss albino mice were subjected to acute oral toxicity studies as per OECD guideline- 425. The animals were fasted overnight and divided into 3 groups with 5 animals in each group. Extracts (PECP, MECP and AECP) were administered orally at one dose level of 2000 mg/kg body weight. The mice were observed continuously for behavioral and autonomic profiles for 2 hrs and for any signs of toxicity or mortality up to 48 hr [15].

Analgesic activity

The acetic acid (chemical) and hot plate (thermal) analgesic test methods were used.

Acetic acid induced abdominal writhing in mice.

Swiss albino mice were treated according to the method described by Colier *et al*, 1968 [16]. Mice were pretreated orally with PECP, MECP, AECP and acetylsalicylic acid, 60 min before administration of acetic acid solution at a dose of 10 ml/kg (0.6%, i.p.). The number of abdominal constrictions (full extension of both hind paws) was cumulatively counted over a period of 15 min.

The mice were divided into eleven groups of 6 mice each.

Group 1: - Vehicle control (2% Tween 80).

Group 2: - Standard (Acetylsalicylic acid 100 mg/kg p.o.).

Group 3, 4 and 5: - PECP (100, 200 and 400 mg/kg, p.o.), respectively.

Group 6, 7 and 8: - MECP (100, 200 and 400 mg/kg, p.o.), respectively.

Group 9, 10 and 11: - AECP (100, 200 and 400 mg/kg, p.o.), respectively.

The percent inhibition of writhing was calculated as follows:

% Inhibition = (VC-VT/VC) * 100

Where, VT, number of writhes in drug treated mice.

VC, number of writhes in control group of mice

Hot plate method in mice

Swiss albino mice were treated according to the method described by Eddy and Leimback, 1953 [17]. Mice were screened by placing them on hot plate (UGO Basile, Italy. Model No. DS-37) maintained at 55 ± 1 °C and the reaction time was recorded in seconds. The time for paw licking or jumping on the hot plate was considered as a reaction time. The responses were recorded before and after 30, 60, 90, 120, 150 and 180 min after the administration of PECP, MECP, AECP and Pentazocine. A cut-off time of 15s was used to avoid injury to the animals.

The mice were divided into eleven groups of 6 mice each.

Group 1: - Vehicle control (2% Tween 80).

Group 2: - Standard (Pentazocine 5 mg/kg s.c.).

Group 3, 4 and 5: - PECP (100, 200 and 400 mg/kg, p.o.), respectively.

Group 6, 7 and 8: - MECP (100, 200 and 400 mg/kg, p.o.), respectively.

Group 9, 10 and 11: - AECP (100, 200 and 400 mg/kg, p.o.), respectively.

Anti-inflammatory activity

Carrageenan induced rat paw edema

Female Wistar rats were treated according to the method described by Winter *et al*, 1962 [18].

Female Wistar rats were divided into eleven groups of 6 rats each.

Group 1: - Vehicle control (2% Tween 80).

Group 2: - Standard (Diclofenac 10 mg/kg p.o.).

Group 3, 4 and 5: - PECP (100, 200 and 400 mg/kg, p.o.), respectively.

Group 6, 7 and 8: - MECP (100, 200 and 400 mg/kg, p.o.), respectively.

Group 9, 10 and 11: - AECP (100, 200 and 400 mg/kg, p.o.), respectively.

Inflammation was produced by injecting 0.1ml of 1% lambda carrageenan (Sigma Chemical Co., USA) in sterile normal saline into the sub plantar region of the right hind paw of the rat. Rats were pretreated orally with PECP, MECP, AECP and diclofenac 1h before the carrageenan injection. The paw volume was measured from 0-6 h, at an hourly interval using pleythsmometer (Ugo Basile, Italy, Model No. 7140). The mean changes in injected paw volume with respect to initial paw volume were calculated. Percentage inhibition of paw volume between treated and control group was calculated by the following formula,

% Inhibition = (1-VT / VC *100)

Where, VT and VC are the mean increase in paw volume in treated and control groups, respectively.

Cotton pellet induced granuloma in rats

Method described by *D'Arcy et al*, 1960 [19] was followed.

Female Wistar rats were divided into eleven groups of 6 rats each.

Group 1: - Vehicle control (2% Tween 80).

Group 2: - Standard (Diclofenac 10 mg/kg p.o.).

Group 3, 4 and 5: - PECP (100, 200 and 400 mg/kg, p.o.), respectively.

Group 6, 7 and 8: - MECP (100, 200 and 400 mg/kg, p.o.), respectively.

Group 9, 10 and 11: - AECP (100, 200 and 400 mg/kg, p.o.), respectively.

Chronic inflammation was produced by implanting the pre-weighed sterile cotton pellets (50 mg) in the axilla region of the each rat through a small incision. PECP, MECP, AECP and diclofenac were administered orally for seven consecutive days after the cotton pellet implantation. Before implanting the cotton pellets, rats were anaesthetized with anesthetic ether. On the eight day animals were sacrificed by cervical dislocation and stomach was removed for histopathology study and cotton pellets were removed from animal's body, freed from the extraneous tissues, dried at 60 °C for 24 h and weighed.

In vitro* antioxidant activity of *Cyathocline purpurea

DPPH free radical scavenging assay.

The 1, 1-diphenyl -2-picylhydrazyl (DPPH) - free radical scavenging activity was determined using the method described by Blois M [20]. The antioxidant activity of the PECP, MECP, AECP and ascorbic acid were assessed on the basis of the radical scavenging effect of the stable DPPH free radical. 10-100 µl of each extract or standard was added to 2 ml of DPPH in methanol (0.33%) in a test tube. After incubation at 37°C for 30 minutes the absorbance of each solution was determined at 517 nm using spectrophotometer. The corresponding blank reading were also taken and the remaining DPPH was calculated by using the following formula,

DPPH radical scavenging activity (%) = [Abs $_{(control)}$ − Abs $_{(standard)}$ /Abs $_{(control)]}$ × 100.

Where, Abs $_{(control)}$: Absorbance of DPPH radical + methanol

Abs $_{(standard)}$: Absorbance of DPPH radical + extract/standard.

IC_{50} value calculated denotes the concentration of the sample required to scavenge 50% of DPPH radical.

Hydrogen peroxide radical scavenging assay

The ability of the PECP, MECP AECP and ascorbic acid to scavenge hydrogen peroxide was determined according to the method of Ruch *et al* [21]. A solution of hydrogen peroxide (2 mmol/l) was prepared in phosphate buffer (pH 7.4). Extracts (10–100 µg /ml) were added to hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. The percentage scavenging activity of hydrogen peroxide by PECP, MECP, AECP and ascorbic acid was calculated using the following formula,

% scavenging activity [H_2O_2] = [Abs $_{(control)}$ − Abs $_{(standard)}$ / Abs $_{(control)}$] × 100.

Where, Abs $_{(control)}$: Absorbance of the control and

Abs $_{(standard)}$: Absorbance of the extract/standard.

IC_{50} value calculated denotes the concentration of the sample required to scavenge 50% of hydrogen peroxide radical.

Hydroxyl radical scavenging assay.

The scavenging capacity for hydroxyl radical was measured according to the method of S. Ganapaty *et al* [22]. The assay was performed by adding 0.1 ml of 1mM EDTA, 0.01 ml of 10 mM FeCl3, 0.1 ml of 10 mM H_2O_2, 0.36 ml of 10 mM deoxyribose, 1.0 ml of different dilutions of the PECP, MECP AECP and ascorbic acid (10 – 100 µg/ml) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37 °C for 1 h. A 1.0 ml portion of the incubated mixture was mixed with 1.0 ml of 10% TCA and 1.0 ml of 0.5% TBA (in 0.025M NaOH containing 0.025% butylated hydroxyanisole to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the extracts is reported as % inhibition of deoxyribose degradation and is calculated as,

OH^- scavenged (%) = [Abs $_{(control)}$ − Abs $_{(standard)}$ / Abs $_{(control)}$] × 100.

Where, Abs $_{(control)}$: Absorbance of the control reaction and Abs $_{(standard)}$: Absorbance of the extract/standard. IC_{50} value calculated denotes the concentration of the sample required to scavenge 50% of hydroxyl radical.

Total phenolic content

The total soluble phenolic in the PECP, MECP, AECP and Gallic acid was determined with using Folin-Ciocalteau reagent [23]. 1 ml of extract solution containing 1 g extract in a volumetric flask was diluted with 46 ml of distilled water. 1 ml of Folin-Ciocalteau reagent was added and the content of the flask mixed thoroughly. 3 min later 3 ml of 2% sodium carbonate was added and the mixture was allowed to stand for 2h with intermittent shaking. The absorbance of the blue color that developed was read at 760 nm. Gallic acid was used as a standard. The concentration of total phenolic compounds in the extracts and gallic acid was determined as µg of gallic acid equivalent using an equation obtained from the standard gallic acid graph ($y= 0.025 x + 0.013$; $R^2= 0.964$), where y = absorbance and x =concentration.

Statistical analysis

Data was expressed as mean ± SEM and statistical analysis was carried out by using GraphPad 5.0 software (GraphPad, San Diego, USA) by applying one-way ANOVA with Dunnett's test or two-way ANOVA with Bonferroni test *p<0.05, **p<0.01 and ***p<0.001 was considered to be significant.

RESULTS

Phytochemical screening

Acute oral toxicity study: Administration 2000 mg/kg, p.o. of all the three extracts i.e. PECP, MECP and AECP did not produce any behavioral abnormalities and mortality. Therefore three doses (100, 200 and 400 mg/kg b.w) were selected for pharmacological studies.

Table 1: Phytochemical screening of *Cyathocline purpurea*.

Sr. No.	Test	PECP	MECP	AECP
1	Glycosides	-	+	+
2	Proteins	-	-	+
3	Carbohydrates	-	-	+
4	Amino acids	-	-	+
5	Steroids	-	+	-
6	Alkaloids	+	+	+
7	Flavonoids	+	+	-
8	Tannins	+	+	-
9	Triterpenoids	-	+	-
10	Phenols	+	+	+
11	Saponins	-	+	-

Key: (-) Absent, (+) Present.

Analgesic activity

Acetic acid induced abdominal writhing in mice: MECP (400 and 200 mg/kg) significantly (p<0.001 and p<0.05, respectively) reduced the number of wriths induced by 0.6% acetic acid at the dose of 10 ml/kg. Also PECP 400 mg/kg showed a significant (p<0.05) reduction in number of wriths when compared to vehicle control group. While MECP 100 mg/kg, PECP (100 and 200 mg/kg) and AECP (100, 200 and 400 mg/kg) showed non-significant results. The number of wriths in the acetic acid vehicle control group was found to be 68 ± 1.5. Acetylsalicylic acid (100 mg/kg) appears to be more effective in reducing the number of wriths, it significantly (p<0.001) reduced the number of wriths with 64.71 % inhibition. (Table 2)

Table 2: Effect of *Cyathocline purpurea* in acetic acid- induced abdominal writhing in mice.

Treatment	Dose (mg/kg, p.o.)	Number of writhing	Percentage inhibition
Vehicle control	-	68 ± 1.5	-
Acetyl salicylic acid	100	24 ± 2.1***	64.71
PECP	100	67 ± 2.0	1.47
PECP	200	64 ± 3.7	5.88
PECP	400	57 ± 3.4*	16.18
MECP	100	62 ± 2.6	8.82
MECP	200	56 ± 2.2*	17.65
MECP	400	44 ± 3.3***	35.29
AECP	100	67 ± 2.0	1.47
AECP	200	65 ± 2.5	4.41
AECP	400	65 ± 3.0	4.41

Values are expressed as mean ± SEM for six animals and analysed by One way ANOVA followed by Dunnett's test, *p<0.05, ***p<0.001 when compared to vehicle control.

Table 3: Effect of *Cyathocline purpurea* on carrageenan induced rat paw edema.

Treatment	Dose (mg/kg, p.o.)	Change in paw volume (ml)		
		1 h	3 h	5 h
Vehicle (Carrageenan) Control	-	1.43 ± 0.24	2.56 ± 0.10	2.78 ± 0.07
Diclofenac	10	1.10 ± 0.11 (23.14)	1.36 ± 0.04*** (46.84)	1.28 ± 0.04*** (53.99)
PECP	100	1.40 ± 0.04 (2.44)	2.47 ± 0.11 (3.45)	2.67 ± 0.11 (3.90)
PECP	200	1.34 ± 0.04 (6.63)	2.25 ± 0.12 (12.30)	2.37 ± 0.12* (14.59)
PECP	400	1.19 ± 0.05 (16.98)	2.09 ± 0.08* (18.48)	2.14 ± 0.07*** (22.76)
MECP	100	1.23 ± 0.06 (14.30)	2.15 ± 0.10* (15.94)	2.25 ± 0.09** (18.98)
MECP	200	1.22 ± 0.05 (15.23)	1.82 ± 0.09*** (28.82)	1.62 ± 0.07*** (41.50)
MECP	400	1.12 ± 0.06 (21.63)	1.51 ± 0.04*** (40.92)	1.35 ± 0.05*** (51.47)
AECP	100	1.39 ± 0.16 (3.02)	2.55 ± 0.18 (0.65)	2.74 ± 0.17 (1.14)
AECP	200	1.38 ± 0.09 (3.84)	2.56 ± 0.11 (0.13)	2.68 ± 0.12 (3.48)
AECP	400	1.40 ± 0.04 (2.44)	2.53 ± 0.12 (1.24)	2.66 ± 0.13 (4.20)

Values are expressed as mean ± SEM for six animals and analysed by Two way ANOVA followed by Bonferroni post-hoc test, *p<0.05, **p<0.01 and ***p<0.001 when compared to carrageenan control. The figures in parenthesis indicate the percent inhibition.

Table 4: Effect of *Cyathocline purpurea* on cotton pellet induced granuloma in rats.

Treatment	Dose (mg/kg, p.o.)	Increase in weight of cotton pellet (mg)	Percent inhibition
Vehicle Control	-	85 ± 3.1	-
Diclofenac	10	29 ± 1.2***	65.88
PECP	100	84 ± 3.5	1.18
PECP	200	81 ± 1.7	4.71
PECP	400	72 ± 2.5**	15.29
MECP	100	79 ± 2.1	7.06
MECP	200	67 ± 2.8***	21.18
MECP	400	44 ± 2.0***	48.24
AECP	100	84 ± 2.5	1.18
AECP	200	85 ± 2.4	0.00
AECP	400	84 ± 3.1	1.18

Values are expressed as mean ± SEM for six animals and analysed by One way ANOVA followed by Dunnett's test, **p<0.01, ***p<0.001 when compared to vehicle control.

Hot plate method in mice: All the three extracts (PECP, MECP and AECP) did not exhibit analgesic activity in hot plate model at all the doses tested. On the other hand, pentazocine (5 mg/kg, subcutaneously) showed significantly (P<0.001) increased pain threshold in mice when compared to the vehicle control group at 60 and 90 min.

Anti-inflammatory activity

Carrageenan induced rat paw edema: There was a gradual increase in paw edema volume of rats in the carrageenan control group. In the test groups, the MECP (200 and 400 mg/kg) showed a significant (p<0.001) reduction in paw volume in a dose dependent

manner at 3rd and 5th h. The inhibitory effect of the MECP at 400 mg/kg was recorded 40.92 % at 3rd h and 51.47 % at 5th h. However, PECP (400 mg/kg) showed significant (p<0.001) inhibition in paw volume at 5th h only with 22.76 % inhibition when compared to carrageenan control group. On treatment with AECP there was no significant inhibition at all the doses when compared to carrageenan control group. Diclofenac (10 mg/kg) caused significant (P<0.001) inhibition of increase in paw edema at 3rd and 5th h. The inhibitory effect of the diclofenac at 10 mg/kg was recorded 46.84 % at 3rd h and 53.99 % at 5th h (Table 3).

Cotton pellet induced granuloma in rats

MECP (200 and 400 mg/kg) significantly (p<0.001) inhibited the granuloma formation in a dose dependent manner with (21.18 and 48.24 % inhibition, respectively), when compared to vehicle control group. PECP 400 mg/kg also significantly (p<0.01) inhibited the granuloma formation with 15.29 % inhibition. However there was no significant inhibition in granuloma formation on treatment with AECP at all the doses tested. Diclofenac 10 mg/kg also significantly (p<0.001) inhibited granuloma formation with maximum inhibition of 65.88 % (Table 4). Histopathology of stomach of vehicle control group rats showed intact gastric mucosa, with no ulceration and no congestion. All the rats treated with (PECP, MECP and AECP) at dose of 400 mg/kg showed less ulcer but absence of congestion when compared to the standard group (diclofenac 10 mg/kg). Diclofenac treated rats showed ulceration and congestion (Figure 1).

In vitro* antioxidant activity of *Cyathocline purpurea

PECP, MECP and AECP showed promising scavenging effect in DPPH free radical assay, hydrogen peroxide assay and hydroxyl radical assay. IC_{50} value calculated indicates the concentration required to inhibit the radicals by 50 percent. MECP was found to be more active in inhibiting radicals than PECP and AECP. Although the scavenging abilities of the extracts were significantly lower than ascorbic acid used as reference standard.

The phenolic content of the extracts were obtained from the standard gallic acid graph. The phenolic content of MECP was found to be superior to PECP and AECP (Table 5).

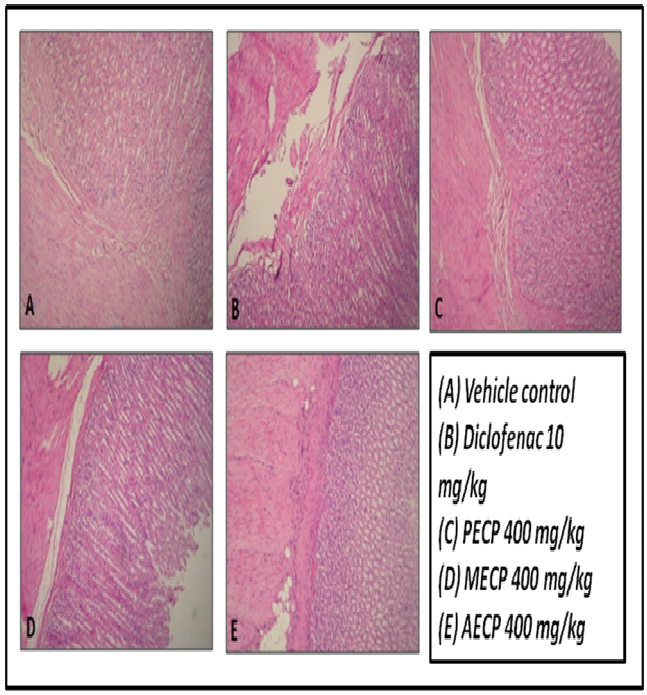

Fig. 1: Histopathology of stomach in cotton pellet induced granuloma in rats.

Table 5: *In vitro* antioxidant activity of *Cyathocline purpurea*

Antioxidant activity	DPPH free radical scavenging assay. IC_{50} µg/ml.	Hydrogen peroxide radical scavenging assay. IC_{50} µg/ml.	Hydroxyl radical scavenging assay. IC_{50} µg/ml.	Total phenolic content. µg/100 µg of GAE.
PECP	70	85	71	44
MECP	62	77	67	78
AECP	94	95	75	66
Ascorbic acid	58	65	52	-
Gallic acid	-	-	-	96

DISCUSSION

Pain and inflammation are associated with pathophysiology of various diseases like arthritis, cancer and vascular diseases. A number of natural products are used in various traditional medicinal systems to relief symptoms of pain and inflammation [24]. *Cyathocline purpurea* is reported to contain chemical constituents which may exert analgesic and anti-inflammatory effect; however till now there has been no investigation supporting the pharmacological properties of this plant. Acute oral toxicity study performed at the dose of 2000 mg/kg revealed the non-toxic nature of all the three extracts i.e. PECP, MECP and AECP.

There were no toxic reactions or mortality found with these extracts. The peripheral analgesic effect may be mediated through inhibition of cyclooxygenase and/or lipooxygenases, while central analgesic action may be mediated through inhibition of central pain receptors [25]. Therefore peripheral (acetic acid induced writhing) and central (hot plate test) models were selected to observe the analgesic effect of *Cyathocline purpurea*. Acetic acid induced writhing test is a simple, reliable and affords rapid evaluation of analgesic drugs [26]. The intraperitoneal administration of agents that irritate serous membranes provokes a stereotypical behavior in mice which is characterized by abdominal contractions, movements of the body as a whole, twisting of dorsoabdominal muscles, and a reduction in motor activity and coordination [27]. The abdominal constrictions induced in mice results from an acute inflammatory reaction with production of prostaglandins E2 and F2 in the peritoneal fluid [28]. MECP (400 mg/kg) significantly (p<0.001) inhibited the number of wriths with 35.29 % inhibition. PECP (400 mg/kg) and MECP (200 mg/kg) also significantly (p<0.05) inhibited the number of wriths compared to vehicle control group. Acetyl salicylic acid (100 mg/kg) showed maximum activity with 64.71 % inhibition. It has been reported that NSAID's prevent prostaglandin production, thus sensitization of pain receptors by prostaglandin at the inflammatory site is inhibited [29]. The mechanism of peripheral analgesic action of MECP, likewise other NSAID's, could probably be due to the blockade of the effect or due to the release of endogenous substances that excite pain nerve endings. The hot plate model has been found to be suitable for the evaluation of centrally acting analgesics [5]. Hence, the hot plate test was performed to check if *Cyathocline purpurea* would have any central analgesic effect. There were no significant results obtained in these test with *Cyathocline purpurea* on all the three extracts tested. On the other hand pentazocine (5 mg/kg, s.c.) showed a significant result by elevating the pain threshold. Hence it can be assumed that *Cyathocline purpurea* has no effect on central nervous system. Carrageenan induced rat paw edema is a suitable model for evaluating anti-inflammatory drugs [30]. Carrageenan has been widely used as an inflammagen capable of inducing

experimental inflammation for screening of compounds possessing anti-inflammatory effect [31]. The development of edema induced by carrageenan is a biphasic event; the early phase (90-180 min) of the inflammation is due to release of histamine, serotonin and similar substances. The later phase (270-360 min) is associated with the activation of kinin- like substances and the release of prostaglandins, proteases and lysosome [32]. Statistical analysis revealed that MECP (200 and 400 mg/kg) significantly ($p<0.001$) inhibited the development of paw edema induced by carrageenan from 3h onwards. Therefore it may be assumed that MECP is associated with inhibition of later phase regulated by prostaglandins, proteases and lysosome. PECP (400 mg/kg) also showed a significant ($p<0.001$) inhibition at 5 h, but was less active than MECP. Moreover, diclofenac (10 mg/kg) exhibited an enhanced effect of inhibiting the paw edema than MECP with 53.99 % inhibition at 5 h. (Table 3).

Chronic inflammation or proliferative phase is measured by methods for testing granuloma formation such as cotton pellet granuloma [33]. The cotton pellet induced granuloma model is a widely used method to evaluate the transudative and proliferative components of chronic inflammation [34]. The repair phase of inflammation starts as proliferation of fibroblasts, as well as multiplication of small blood vessels. Such proliferating cells penetrate the exudates producing a highly vascularised reddened mass known as granulation tissue [4]. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma and the dry weight correlates well with the amount of granulomatous tissue formed [2]. MECP (200 and 400 mg/kg) were significantly ($p<0.001$) effective in both the models of inflammation, i.e. carrageenan induced rat paw edema as well as cotton pellet induced granuloma, therefore it can be assumed that it is effective in all phases of inflammation i.e. acute, sub acute, and proliferative phases (Table 4). Evaluation of the ulcerogenic effect of the three extracts (PECP, MECP and AECP) on the rat stomach revealed a lesser ulceration of the gastric mucosa and absence of congestion as compared to diclofenac. Ulceration of the gastric mucosa by anti-inflammatory drugs is a common side effect which usually indicates that prostaglandin synthesis inhibition may be involved in their mechanisms of action. Inhibition of the synthesis of prostaglandin, a group of prostanoid mediators of inflammation and intact gastric mucosa is largely responsible for the anti-inflammatory and gastric ulceration effects of NSAIDs.

Oxidation is essential in many organisms for the production of energy to fuel biological processes. However the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as rheumatoid arthritis, atherosclerosis and also cancer [23]. DPPH radical has been widely used as a model system to investigate the scavenging activities of extracts of plants. DPPH radical is scavenged by antioxidants through the donation of a hydrogen atom, forming the reduced DPPH-H [35]. Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. Thus, the removal of hydrogen peroxide is very important for antioxidant defense in cell or food systems [21]. Hydroxyl radical is the principal contributor for tissue injury [36]. The results for DPPH, hydrogen peroxide, and hydroxyl radical scavenging activity exhibited by the extracts were comparable with the standard compound, ascorbic acid. The IC_{50} value of MECP showed higher antioxidant activity while PECP showed moderate antioxidant activity in all the above three assays with less activity for AECP. The phenolic compounds contribute directly to the antioxidative action and play an important role in stabilizing lipid peroxidation [37]. The phenolic contents varied significantly among all the extracts.

Phytochemical analysis of the *Cyathocline purpurea* has mainly demonstrated the presence of flavonoids, steroids, phenols, tannins and saponins. Steroids, alkaloids and terpenoids have been reported to have analgesic and anti-inflammatory activity. Flavonoids and phenolic compounds have multiple biological effects such as antioxidant activity [38]. Flavonoids have also been reported to have anti-inflammatory effect [39]. Steroids can decrease inflammation and reduce the activity of the immune system, while triterpenoids impairs histamine release from mast cells and exerts anti-inflammatory effects [40]. Therefore peripheral analgesic and anti-inflammatory activity of *Cyathocline purpurea* might be attributed to phytoconstituents present in the extract like flavonoids, steroids, tannins, phenols and saponins.

CONCLUSION

The experimental findings in the study demonstrated the peripheral analgesic, anti-inflammatory and antioxidant activity of *Cyathocline purpurea*. Mainly MECP (200 and 400 mg/kg) was found to be highly effective. The results suggested that the mechanism of action of MECP seems to be similar to NSAID's rather than to steroidal drugs. The study justified and supported scientifically the ethno pharmacological use of the plant as an anti-inflammatory agent to treat pain and inflammation. Further attempts will be to isolate and define the active analgesic and anti-inflammatory fraction and its components.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest concerning this article.

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