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EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF ENHYDRA FLUCTUANS ON MALE WISTAR RATS

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ABSTRACT
Enhydra fluctuations an edible semi-aquatic vegetable plant are widely used in the traditional system of medicine. Ethanolic extract of *Enhydra fluctuans* was screened for analgesic & anti-inflammatory activity. Analgesic activity was evaluated by the hot plate method and anti-inflammatory activity was evaluated by formalin induce paw edema in Wistar rats (male). Ethanolic extract dose of 300 mg/kg reduce formalin induce paw inflammation and also increased the pain threshold in rats evidenced by hot plate method. The experimental results concluded that *Enhydra fluctuans* have significant analgesic and anti-inflammatory activity may due to flavonoid and phenolic compound content.

INTRODUCTION

Enhydra fluctuans is a medicinal plant that contains some active medicinal component which can be used for medicinal purposes [1]. It is a semi-aquatic herbaceous plant that grows abundantly all over India [2]. It grows over water body land within the temperature of 27^0C-35^0C and the growth rate gradually declines above 38^0C [3]. The stems are fleshy, 30cm or more in length. Branched, rooting at the lower nodes, and possess hair [3]. The leaves taste bitter and they are stalk less [4]. linear-oblong, 3-5cm in length [5]. It is used to treat inflammation [6], skin diseases [7], and smallpox [8], liver diseases [9,10] nervous diseases, etc [11,12].According to pathology the meaning of inflammation is an injury that takes place in living tissues which may be caused by bacterial attacks, parasitic attacks, or various physical and chemical attacks. It is beneficial to repair the damaged part of the tissue by removing the infection to destroy the evil agents [1]. The response of inflammation is a protective mechanism that evolved in higher organisms to protect them

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from infections and injury [13. Its main motive is to target and remove the injurious agents and to remove damaged tissues so that it can begin to heal [14]. The response causes changes in blood flow [15], increases the permeability of blood vessels, and helps in traveling of fluid potions and white blood cells from the circulation site to the site of tissue damage [5,6].

Inflammatory diseases like asthma, gout, allergy, glomerulonephritis hepatitis, inflammatory bowel disease, perfusion injury include various types of disorders and conditions that are characterized by inflammation [16-19]. In India inflammatory diseases gradually increased because of environmental causes such as global warming, physiological causes, hereditary (genetic), microorganism etc [20,21]. During this research work, emphasis has been given to investigate and explore the analgesic and anti-inflammatory activity of ethanolic extract of *Enhydra fluctuans*.

MATERIALS AND METHOD

Plant material

Fresh leaves and stems of that plant (Fig 1) were collected locally in the rural area of Sashadanga, North 24 Parganas,West Bengal, India in the month of September 2020 and identified by Botanical Survey of India, Acharya Jagadish Chandra Bose Indian Botanical Garden, Shibpur, Howrah, West Bengal, India. Collected plant material was shade dried and milled in a dry format in a mixture grinder for further studies.

Chemicals and Reagents

All necessary chemicals (petroleum ether, ethanol) used were of analytical grade and were purchased from Modern Chemical Laboratory, Garia, Kolkata, West Bengal, India.

Extraction

The coarse powder of *Enhydra fluctuans* was successively extracted with petroleum ether (60-80^{o}C) and ethanol (99.99% pure) using a Soxhlet apparatus. Solvents were evaporated to collect the extracts and packed into Eppendorf tube and kept in desiccators for further investigation [22].

Phytochemical screening

Ethanolic extract was subjected to preliminary phytochemical analysis for identification of different phytoconstituents present in the plant [23].

Animal

Adult male Wistar rats weighing 150-180 gm were used for this research purpose. They were kept in clean polypropylene cages and were fed with a standard diet and water as necessary with light and dark cycle [24]. All studies were approved by the Institutional Animal Ethics Committee of Bharat Technology, Uluberia, Howrah.

Experimental design

Acute Toxicity Studies

Acute toxicity studies were performed followed by OECD guidelines 425. Wistar rats were selected by random technique. The animals were fasted for 4 hr. with free access to water only. The ethanol extract administered orally at a dose 2000 mg /kg bw p.o. The behavioral changes, mortality, abnormalities of animals were observed next 24 hrs to 14 days [24].

Selection of dose

Acute toxicity study confirmed there was no mortality found at the dose 2000mg/kg. Therefore dose optimization was done and 300mg/kg bw was selected as experimental study.

Evaluation of analgesic activity [25]

Analgesic activity was performed in male Wistar rats by hot plate apparatus (Eddy's hot plate).

Animals were divided into three groups (6 animals in each) randomly.

i. Group I (Normal control) Received clean water and normal food.

ii. Group II (standard) Received Standard drug (Diclofenac sodium 10 mg/kg, ip).

iii. Group III (Test): Treated with ethanolic extract of *Enhydra fluctuans* (300 mg/kg, ip).

Evaluation of anti-inflammatory activity [25]

Male Wistar rats were selected for the research. Formalin-induced paw oedema was performed according to the process. Paw volume was measured by Plethysmometer.

Animals were divided into four groups (6 animals in each).

i. Group I (Normal control): Received clean water and normal food. No drug given.

ii. Group II (Formalin control): Received formalin (0.01ml/2.5%, i.p.) after 7 days for one time.

iii. Group III (Standard): Received formalin (0.01ml/2.5%, i.p.) and Standard drug (Diclofenac sodium 10mg/kg, i.p).

iv. Group IV (Test): Received formalin (0.01ml/2.5%, i.p.) and extract of *Enhydra fluctuans*.

Statistical Analysis [24]

Result were expressed as mean ± SEM, (n=6). The statistical significant between groups was determined by one way analysis of variance (ANOVA), followed by Dunnett's multiple comparison among groups by Graphpad Instat 3 & Prism Graphpad 9. Difference of P<0.001 and p<0.05 were considered statistical significant.

RESULT

Phytoconstituents reported from ethanolic extract of the plant are flavonoids, carbohydrates, phenolic compounds and tannins. Ethanolic extract of *E. fluctuans* induced parenteraly and significantly increased of pain threshold and act as an analgesic drug (Table 1 and 2). Ethanolic extract of *E. fluctuans* produce significantly anti-inflammatory in formalin induce paw oedema in male wistar rats (Table 3), (Fig 2).

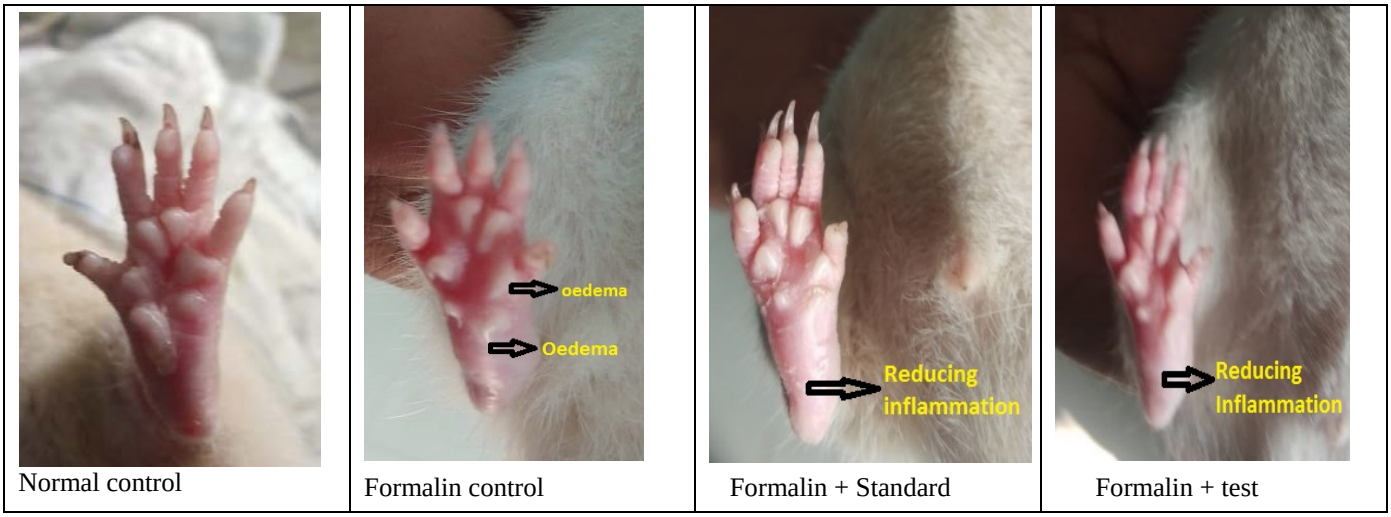
TABLE-3 Effect of ethanolic extract of *Enhydra fluctuans* on Formalin induced paw oedema in male Wistar rats.

Group	Treatment	Reaction time(hr.) after drug administration					
		0 hr.	1 hr.	3 hr.	12 hr.	36 hr.	72 hr.
I	Normal Control	19.63 ±0.18	19.63 ±0.18	19.63 ±0.18	19.63 ±0.18	19.63 ±0.18	19.63 ±0.18
II	Formalin Control (0.01ml, ip 2.5%)	19.43 ±0.21##	22.16 ±0.12##	24.56 ±0.12##	25.46 ±0.9##	26.36 ±0.14##	23.45 ±0.12##
III	Formalin control + Diclofenac Sodium (0.01ml, ip 2.5% + 10mg/kg, ip)	19.80 ±0.13	21.20 ±0.13**	23.10 ±0.12**	22.0 3±0.80**	21.13 ±0.12**	20.96 ±0.11**
IV	Formalin+ control +Test drug (0.01ml, ip 2.5%+ Extracted drug 300 mg/kg, ip)	19.75 ±0.12	21.35 ±0.29*	23.33 ±0.12**	22.33 ±0.12**	21.91 ±0.10**	21.15 ±0.04**

*Values are mean± SEM (n=6), ##p<0.01 considered statistically significant as compare to normal control group; *p<0.05, **p<0.01 considered statistically significant when compared to Formalin control group.*

Fig. 1 Whole plant of *Enhydra fluctuans*

Fig. 2 Effect of ethanolic extract of *Enhydra fluctuans* on formalin induced paw oedema in male Wistar rats.



Normal control | Formalin control | Formalin + Standard | Formalin + test

DISCUSSION

Narcotic receptor and cholinergic receptor were responsible for physiological activity like paw licking and jumping. The ethanolic extract of the *Enhydra fluctuans* was impedance with narcotic receptor or with focal cholinergic framework and gave the restorative impact, thus resulting increase the threshold of the rats and change the physiological reaction (paw licking and Jumping). Formalin was induced in male Wistar rats of paw at different dose the lower portion induced the advancement of a prompt oedema. The paw oedma which occurred in two stages related with two times of expansion in vascular porousness. Formalin induced at a low dose induces an oedema which mainly results from a neurogenic inflammation mediated by neuropeptides such substance P [25].

At higher doses, formalin induced an oedema which mainly depends on the release of substance P, prostanoids, 5-hydroxytryptamine and histamine. Bradykinin plays no significant role in the vascular changes whereas this peptide has been reported to participate in the stimulation of nociceptive afferent neurons. This discrepancy could be explained by a difference in the threshold of stimulation of the nociceptive neurons and that of the cells of the vascular walls, or by a formation of kinins in close contact of the neurons [26, 27].

Ethanolic extract of *Enhydra fluctuans* improves the paw oedma by various pathway. It is improves at low dosage inflammation by neurogenic inflammation mediated with neuropeptides such as substance P way. It also improve the higher dose way such as substance P, Prostanoids, 5-hydroxytryptamine and histamin.

CONCLUSION

The present study reveals that the ethanolic extract of *Enhydra fluctuans* was significantly improved the paw oedema in formalin induce paw inflamed rats. It was also increased pain thresholds. Thus it concluded that the ethanolic extract of *Enhydra fluctuans* is potential against formalin induce paw inflammation and also have a well analgesic effect may be due to present of flavonoids and phenolic compounds in the plant .

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Kishor Kumar Roy and Md. Kabirul Islam Mollah designed the executed the experimental work. Md. Masud Reja and Ranjan Kumar Maji collected the contents and performed the literature survey. Dibyendu Shil contributed to drafting the manuscript and critical revision of the article. All the authors framed the final manuscript.

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