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In vivo Anti- inflammatory and Antipyretic activity of cow urine distillate, Hygrophila spinosa extract and their combination in Albino rats

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ABSTRACT
Treatment of inflammatory conditions and pyrexia mainly involves Non Steroidal anti-inflammatory drugs (NSAIDs), which are synthetic in nature and associated with many adverse effects. The use of traditional medicines is rapidly increasing in the world being economic and with least side effects. Various herbs and cow urine were used extensively in Indian system of medicine since time immemorial. In this study, Kosli cow urine distillate (CUD) @ 0.5 ml orally per animal, hydroalcoholic extract of Hygrophila spinosa (HSE) @ 400mg/kg body weight orally and their combination were evaluated for anti-inflammatory activity in rat paw edema model and antipyretic activity in Brewer's yeast induced pyrexia in rats. Thirty adult Albino rats of any sex were used in five groups of six animals each. Rat paw edema was produced by injecting 1% suspension of carrageenan into plantar aponeurosis of right hind paw of the rat and paw volume was measured at 0 and after 3 hours of carrageenan injection. Pyrexia was induced by subcutaneous injection of 20 % (w/v) brewer's yeast suspension at the dose rate of 10 ml/kg into the animal's dorsal region below the nape of the neck. The rectal temperature of each animal was recorded before injection of yeast (-18h), at 18 hour after injection of yeast (0h) and then at 1h, 3h, 6h and 18h. H. spinosa extract alone and in combination of Kosli cow urine distillate showed significant anti-inflammatory and antipyretic activities. Cow urine distillate showed lower anti-inflammatory activity whereas antipyretic activity was not significant.

KEYWORDS: Traditional, H. spinosa, distillate, combination, anti-inflammatory, antipyretic.

I. INTRODUCTION

Inflammation is a defensive mechanism of body against numerous stimuli such as physical, chemical, infectious agents, environmental, physiological, pathological factors, Ag-Ab reactions etc. The cardinal signs of inflammation involve erythema, swelling, pain, heat and loss of function. Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of most widely used drugs for reduction of pain and inflammation. These drugs act by inhibition of arachidonic acid-metabolising activity of cyclooxegenase enzyme and synthesis of prostaglandins. Unrestricted use of NSAIDs poses several side effects such as ulcers, hemorrhage (Hajhashemi et al, 2009), liver and kidney toxicities (Pannu and Nadim, 2008) and even contribute for drug related death. Inhibition of the synthesis of prostaglandin is expected to result in pain relief and reduce inflammation in inflamed tissues. Many medicinal plants with anti-inflammatory therapeutic effects are available with low or no side effects. Development of oedema by carrageenan injection involves various mediators and prostaglandins are strongly linked to the processes involved in inflammation and sensation of pain and redness associated with inflammatory reaction. Pyrexia is most common sign of many ailments. Yeast induced fever is considered to be pathogenic in nature. Antipyretic action involves inhibition of prostaglandin (mainly PGE_2) production in the hypothalamus. Antipyretic agents reduce the elevated body temperature by acting centrally on thermoregulatory centre i.e., hypothalamus. Antipyretic drugs like Aspirin, Paracetamol etc. are used for management of fever but these synthetic drugs are associated with many side effects.

Alternative medicine including herbal medicine and cow urine therapy are gaining interest of people worldwide. Traditionally many plants and their formulations are used for various ailments because of their extensive biological and medicinal activities, easy availability, higher safety and lesser cost. Hygrophila spinosa is an herbaceous, medicinal plant of family Acanthaceae that grows

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in marshy places- on the sides of tanks, ditches, and in paddy fields. It is native to tropical Asia and Africa. In India it is commonly known as Kokilaksha or Gokulakanta. In Chhattisgarh it is known as Maukhala or Talmakhana. Whole plant, its roots as well as seeds are known for medicinal value in Ayurveda. It is used for treatment of rheumatism, jaundice, inflammation, pain, hepatic obstruction, gout, bacterial infection etc. (Sharma et al., 2002; Chopra et al., 1986; Nandkarni, 1978; Mazumdar et al., 1997; Boily and Vanpuyvelde, 1986). In India cow is called as 'Goumata' or 'Kamdhenu' (that fulfils all wishes). It has high socio-cultural values and plays significant role in rural economy. The cow urine, one of the ingredients of 'Panchagavya' is capable of treating many curable as well as incurable diseases. Cow urine distillate is more effective as a bioenhancer than cow urine and increases the effectiveness of antimicrobial, antifungal and anticancer drugs (Chawla, 2010). Cow urine has shown to have anti pyretic and anti- inflammatory property.

II. MATERIAL AND METHODS

Experimental animals

Thirty adult healthy Wistar albino rats (150-200g) in 5 Groups (n= 6) of any sex at Lab Animal House, College of Veterinary Science and Animal Husbandry, Anjora, Durg were used for these experiments. They were maintained under standard laboratory conditions (27± 2℃ temperature and 12/12 hr light/dark cycle) and offered standard commercial feed and clean drinking water. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC), College of Veterinary Science & A.H., Anjora, Durg (C.G.), India and followed the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of` Forests and Environment, Government of India.

Collection of cow urine and preparation of distillate:

Natural voiding, morning, mid stream urine from Kosli cows of 2-3 year age was collected in a sterile container. Distillate was prepared by condensing it using a glass distillation apparatus. The preparation was stored at 4℃ until use.

Preparation of Hygrophila spinosa extract

Leaves of Hygrophila spinosa were collected from paddy fields of district Rajnandgaon, C.G., shade dried, powdered and hydro-alcoholic (30:70) extract was prepared using Soxhlet apparatus**.**

Anti- inflammatory activity

The rat paw oedema is an acute inflammatory model that can be induced by phlogistic agents like carrageenan, formalin or cotton pellet granuloma. In this study anti - inflammatory activity was evaluated by using carrageenan induced rat paw oedema model according to the method described by Winter et al. (1962). Various pretreatment as per experimental design were given 1hr before carrageenan administration. 1% suspension of carrageenan in 0.9% sterile normal saline was injected into plantar aponeurosis of right hind paw of the rat and results were compared with standard drug - Phenylbutazone. Plethysmometer, which works on water displacement method, was used to measure the paw volume at 0 and after 3 hours of carrageenan injection. The paw of rats was marked below the tibiotarsal joint to assure the equal length of paw dipped in plathysmometer each time. The experimental design was as follows:

Group No.	No. of Animals	Treatment
I	6	Control: Normal saline orally and Carrageenan
II	6	Reference Drug: Phenylbutazone @ 100mg/kg, p.o. and Carrageenan
III	6	Cow urine distillate of kosli cow @ 0.5ml orally and Carrageenan
IV	6	HSE @ 400 mg/kg orally and Carrageenan
V	6	Cow urine distillate of kosli cow @ 0.5ml orally + HSE @400 mg/kg orally and Carrageenan

The percent of inhibition of the edema was determined using following formula:

Percent (%) inhibition = 1 – Vt/Vc X 100,

where Vt- edema volume in test group, Vc-edema volume in control.

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Anti- pyretic activity

Selection of animals for evaluation of antipyretic activity was done by recording of approximate constant rectal temperature for 7 days. Brewer's yeast derived from species Saccharomyces cerevisiae is commonly used for pharmacological screening of antipyretics in rats. Pyrexia was induced by subcutaneous injection of 20 % (w/v) brewer's yeast suspension at the dose rate of 10 ml/kg into the animal's dorsal region below the nape of the neck (Smith and Hambourger, 1935). The rectal temperature of each animal was recorded before injection of yeast (-18h) and at 18 hour after injection of yeast (0h). At 18 hr following treatments were given to the rats showing 1 degree higher temperature. Then rectal temperature was then recorded at 1h, 3h, 6h and18h.

Group No.	No. of Animals	Treatment
I	6	Control: Normal saline orally
II	6	Reference Drug: Aspirin @ 100mg/kg, p.o.
III	6	Cow urine distillate of Kosli cow @ 0.5ml orally
IV	6	HSE @ 400 mg/kg orally
V	6	Cow urine distillate of Kosli cow @ 0.5ml orally + HSE @ 400 mg/kg orally

III. RESULT AND DISCUSSION

Anti- inflammatory activity

In this study, combination of Cow urine distillate of Kosli cow @ 0.5ml orally and HSE @ 400 mg/kg orally resulted in significant inhibition of paw volume (59.29 ± 2.41%), which was comparable to the inhibition of paw volume by standard drug (61.79 ± 2.90 %). Individual treatment with HSE @ 400 mg/kg orally and Cow urine distillate of Kosli cow @ 0.5ml orally also indicated significant inhibition of paw volume, 53.57 ± 1.43 and 33.93 ± 1.02, respectively. But their activity was lower than activity of standard drug Phenylbutazone and the combination of HSE and CUD in the given dose. Percent inhibition of paw volume in CUD treated animals was lowest among the treatment groups.

Table 1: Effect of cow urine distillate, Hygrophila spinosa extract and their combination on carrageenan induced rat paw edema.

	(3h-0h)V Volume of paw (0h)	Volume of paw (3h)	Difference in paw volume (3h-0h)	% inhibition of paw volume
Group I	1.27 ± 0.09	1.74 ± 0.03	0.47 ± 0.10 [a]	-
Group II	1.49 ± 0.03	1.66 ± 0.03	0.18 ± 0.01 [b]	61.79 ± 2.90 [a]
Group III	1.14 ± 0.12	1.44 ± 0.12	0.31 ± 0.00 [b]	33.93 ± 1.02 [c]
Group IV	1.40 ± 0.05	1.61 ± 0.05	0.22 ± 0.01 [b]	53.57 ± 1.43 [b]
Group V	1.43 ± 0.04	1.62 ± 0.04	0.19 ± 0.01 [b]	59.29 ± 2.41 [a]

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Note: 1% suspension of carrageenan was injected into plantar aponeurosis of right hind paw of the rat.
Values indicate Mean ± SE.
Values with different superscript differs significantly ($P \leq 0.05$) within a column.

Acute inflammatory response is mediated primarily by blood leukocytes such as neutrophils and macrophages (Knight and Tait, 1996). Neutrophils stimulation also causes increased vascular permeability and produce oedema (Fantone and Ward, 1982). Development of oedema after carragennan injection follows three phases: Early phase (approx 90 minute) - involves release of histamine and serotonin, Second phase (next 60 minutes) involves kinins and third phase (after 180 minutes)- involves prostaglandins. The significant inhibitory effect is observed after 180 minutes of oedema development. Various studies have demonstrated that plants possess inhibitory activity against COX enzymes, and it is believed that such plants could play a role in the treatment of pain and inflammatory diseases (Jeppesen et al., 2012; Noreen et al., 1998). It can be contemplated that HSE and its combination with cow urine distillate has influence on arachidonic acid pathway and inflammatory mediators like prostaglandins mainly by inhibiting COX enzyme as like Phenylbutazone. In accordance with this study Tekulu et al., 2020 showed anti inflammatory activity of 70 % ethanolic leaf extract of H. schulli by significant inhibition of carrageenan induced hind paw oedema in mice model. In contrast to present study Jagadeesh et al., 2011 investigated effect of cow urine on formalin induced (edema-acute and chronic) inflammatory activity in male and female Wistar albino rats and found that there was no significant decrease in paw thickness in cow urine treated groups when compared to standard control. Literature on anti-inflammatory activity of herbs in combination of cow urine or its distillate has not been published, so no comparison could be made.

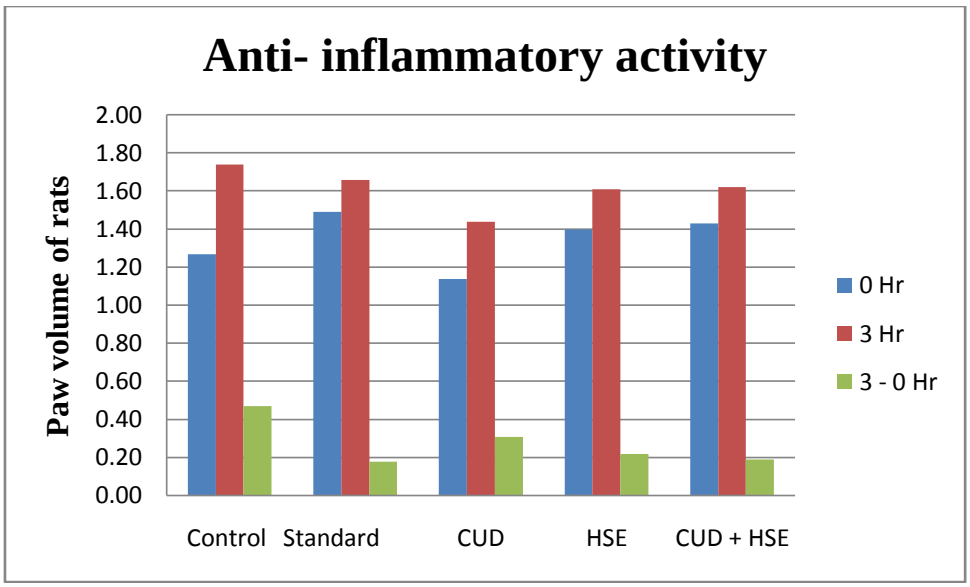

Figure 1: Effect of cow urine distillate, Hygrophila spinosa extract and their combination on carrageenan induced rat paw edema.

Anti- pyretic activity

Fever is the most commonly observed symptom caused by infection or inflammation in many diseases. Measurement of temperature permits the easy identification of a pathological condition and changes in the disease (Conti and Bartfai, 2014). Among animal models of fever, yeast-induced pyrexia in rats is the most widely used model. Evaluation of antipyretic activity showed that animals treated with HSE significantly relieved pyrexia in 1 hour, which was comparable to the standard drug Aspirin. CUD treatment had not shown significant reduction in body temperature of Albino rats. Combination of HSE and CUD also reduced the increased temperature, which was significant at 6 hour and onwards after induction of fever. Similarly, Patra et al., 2009 investigated antipyretic activity of H. spinosa alcoholic and chloroform extract and found that Chloroform extract significantly decreased the

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elevated rectal temperature 3 h after the administration of a dose of 400 mg/kg only, while the alcoholic extract reduced the hyperthermia at both 200 and 400 mg/kg doses 1 h after administration.

Table 2: Effect of cow urine distillate, Hygrophila spinosa extract and their combination on Brewer's yeast induced pyrexia in Albino rats

	-18 hr	0 hr	1hr	3hr	6hr	18hr
Group I	98.12 ± 0.28 [b]	99.28 ± 0.18 [a]	99.28 ± 0.21 [a]	99.22 ± 0.17 [a]	99.07 ± 0.18 [a]	98.97 ± 0.13 [a]
Group II	98.10 ± 0.08 [c]	99.25 ± 0.21 [a]	98.87 ± 0.17 [b]	98.45 ± 0.09 [c]	98.15 ± 0.09 [c]	98.13 ± 0.09 [c]
Group III	97.77 ± 0.20 [c]	99.18 ±0.22 [a]	98.90 ±0.19 [ab]	98.60 ±0.25 [ab]	98.43 ±0.24 [bc]	98.32 ±0.22 [bc]
Group IV	98.02 ± 0.25 [b]	99.23 ± 0.26 [a]	98.40 ±0.30 [b]	98.25 ±0.31 [b]	98.13 ±0.30 [b]	98.12 ±0.28 [b]
Group V	97.90 ± 0.17 [b]	98.90 ±0.22 [a]	98.58 ±0.28 [ab]	98.30 ±0.27 [ab]	98.12 ±0.21 [b]	98.03 ±0.18 [b]

Note: Pyrexia was induced using 20 % (w/v) Brewer's yeast suspension at the dose rate of 10 ml/kg into the animal's dorsal region below the nape of the neck of rats.

Values indicate Mean ± SE.

Values with different superscript differs significantly ($P \leq 0.05$) within a row.

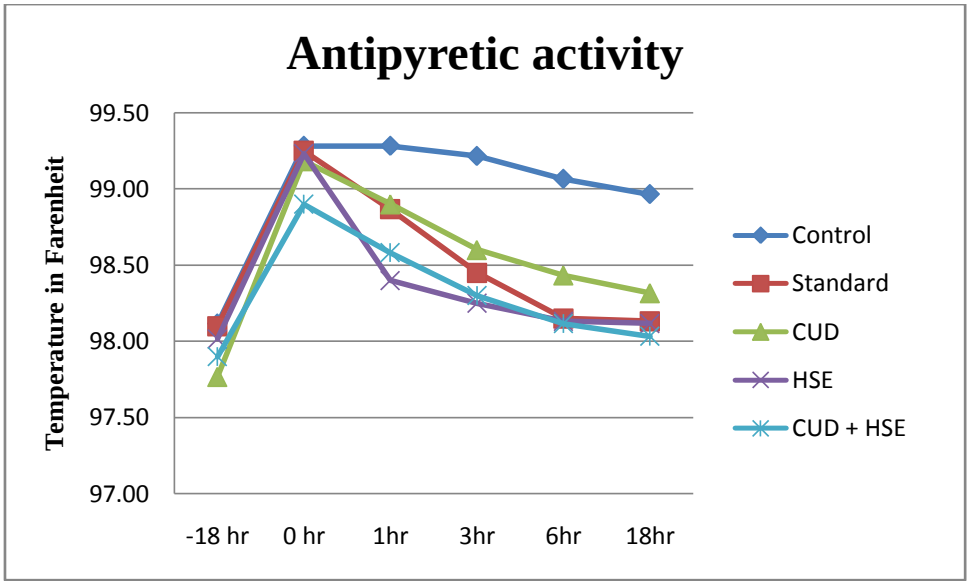

Figure 2: Effect of cow urine distillate, Hygrophila spinosa extract and their combination on Brewer's yeast induced pyrexia in Albino rats

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IV. STATISTICAL ANALYSIS

Results of the experiment were subjected to a one-way analysis of variance (ANOVA). Differences among the groups were obtained using Duncan's test. Statistical significance was accepted as $p<0.05$. All the data was expressed as mean ± SE in each group.

V. CONCLUSION

Among various extracts of herbal plants, hydro-alcoholic extract is known for maximum extraction of the phytoconstituents. The current study exhibited significant anti-inflammatory and antipyretic activities of HSE alone and in combination with Kosli cow urine distillate in Albino rats. Various bioactive molecules present in the leaves of plants are responsible for their activity. The leaves of H. spinosa revealed presence of alkaloids, carbohydrates, proteins, steroids, glycosides, flavonoids, tannins, phenolic compounds, fats, and oils (Patra et al., 2009). Isolation of these phytochemicals is required to determine the active principle responsible for pharmacological activities. The result of this experiment also depicts that cow urine distillate enhanced anti- inflammatory potential of H. spinosa but not its antipyretic action. The study validates the use of H. spinosa hydroalcoholic extract @ 400mg/kg body weight orally alone and in combination with cow urine distillate @ 0.5 ml orally for treatment of painful inflammatory conditions and pyrexia. It can be concluded that herbs and Indian cow urine distillate are novel drugs to combat inflammation and fever without side effect.

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