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IN VIVO PHARMACOLOGICAL INVESTIGATIONS OF LEAF EXTRACTS OF *CALAMUS TENUIS ROXB.*

F.A.D.MD. OPO, SAJAN DAS, SUMANA KHANDOKAR, RUMANA AKHTER, MOHAMMAD SHAHRIAR

Phytochemistry Research Laboratory, Department of Pharmacy, University of Asia Pacific, Bangladesh

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Abstract: Focus of the present study was to evaluate in vivo neuropharmacological activity, anti-nociceptive activity, gastrointestinal motility, anti-diarrheal and anti-pyretic effect of the leaf extracts of Calamus tenuis. In gastro intestinal motility test the dose of methanol (200 mg/kg), both the doses of ethanol and chloroform extracts (100 & 200 mg/kg) showed maximum charcoal defection time, compared (*p<0.05; **p<0.01; ***p<0.001) with standard drug. In vivo anti-pyretic test methanol 100 mg/kg dose as well as ethanol 100 mg/kg dose showed satisfactory level (*p<0.05) of lowering pyrexia from elevated level. On the other hand methanol 200 mg/kg dose showed a significant level (**p<0.01) of lowering pyrexia from elevated level compared to the standard drug. In forced swimming test the methanol (100 mg/kg), chloroform extract at doses of 100 and 200 mg/kg and ethanol (200 mg/kg) shortened the immobility period in comparison with standard & exhibited a dose dependent antidepressant activity. In anti-diarrheal activity test ethanol 100 mg/kg dose and both the doses of chloroform extracts (100 & 200 mg/kg) showed a significant result (**p<0.01; ***p<0.001) compared to standard drug. In anti-nociceptive activity test, methanol (100 & 200 mg/kg), ethanol (100 mg/kg) and chloroform (100 mg/kg) at 1 minute were showed a significant analgesic activity in tail immersion test as well as acetic acid induced writhing test.

Keywords: *Calamus tenuis*, gastro-intestinal motility, anti-diarrhoeal, anti-nociceptive, anti-pyretic.

Corresponding Author: MR. MOHAMMAD SHAHRIAR

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INTRODUCTION

Calamus is a genus of the palm family Arecaceae. These are among several genera known as rattan palms. *Calamus* species are the spiny, climbing species commonly known as rattans and often described as 'green gold'. Calamus oil extracted from the roots contains palmitic acid, iso-eugenol, calamine, calamol, etc. and is used in perfumery and for flavouring liquors [1]. According to Islam *et al.*, (2015) its fruits are known to have antioxidant and cytotoxic potentials, analgesic and CNS depressant activities [2]. *Calamus tenuis* is an important ayurvedic medicinal herb. Young stem used as medicine for stomach trouble. Young Clum is used as a vegetable [3]. The tribal people of India mostly live in naturally isolated regions like hills and forests, and they use plant for various purposes such as food, fodder, medicines, wild vegetables, fibers and domestic purposes [4]. Tender shoots are used as vegetables and as indigenous medicine [2]. The present study was design to investigate the *in-vivo* gastro-intestinal motility, anti-depressant activity, anti-diarrheal activity, anti-nociceptive activity and anti pyretic activity of the leaf extract of *Calamus tenuis* by using different solvents.

Materials and Methods:

Collection and identification of the plant

The leaves of *Calamus tenuis* was collected from Botanical garden, Curzon Hall at the University of Dhaka in November 2015 and was taxnomically identified with the help of the National Herbarium of Bangladesh, Mirpur-1, Dhaka (DACB Accession Number 42757).

Preparation of the plant extracts

Hot solvent extraction process was used for extraction of the plant material. Soxhlet extractor was used for the extraction procedure. Plant material was extracted by three solvents-methanol, ethanol and chloroform.

Experimental animals

For performing the experiments adult Swiss albino mice weighing between (12-30) gm of either sex were collected from animal sources department of ICDDR, B, Mohakhali, Dhaka. The animals were maintained under normal laboratory condition & kept in standard cages at room temperature of 30°±2° C and 60% to 65% relative humidity and provided with standard diet & water. All protocols for animal experiment were approved by the institutional animal ethical committee.

Group Design

All animals for each and every selective test were divided into 8 well defined groups with 6 mice in each group. Where group 1 served as control and group 2 was standard; from group 3-8 mice were divided as methanol 100, methanol 200, ethanol 100, ethanol 200, chloroform 100 and chloroform 200 mg/kg body weight dosage form respectively. Hence control group always served with 0.9% NaCl in 10 ml/kg body weight dose and standard dose and product will vary with specific test protocol.

***In vivo* Anti-depressant activity**

The Forced swimming test (FST) is currently a popular model, due to the low cost of the experiments and because it is positively the most reliable model available. In a comparative review of drug effects on immobility time in mice a limit of 20% reduction of immobility to consider an antidepressant effective on the test. Immobility observed in the swim test seems not to be related to behavior in the tests used in anxiety models. To perform this test groups were divided as mentioned earlier and diazepam 2 mg/kg body weight dose was considered as standard. All doses were administered per-oral route. As observation total duration of immobility during five minutes swimming test was recorded after 30 minutes administered of drug and extracts. Water should be changed between each data collection to maintain water temperature. Each mice was considered to be immobile when it ceased struggling and remained floting motionless in the water, making only those movement necessary to keep its head above. the duration of immobility during the FST was taken as a measure of anti-depressant activity [5].

***In vivo* Gastro intestinal (GI) motility test**

Except from the traditional test protocol we tried here something different, where the 3Rs (replacement, reduction, and refinement) principle were used to treat animals. They were fasted for 3 hour to control intestinal motility, which reduced stress in the animals. In this new protocol, mice are deprived of food for a short time (3 hour) and are not killed. The mice are observed until evacuation containing charcoal is observed, and the experimental results are based on the charcoal evacuation time. The present study may aid the formulation of recommendations that can be included in revised guidelines relating to the fasting time of mice. All the extracts were administered by oral gavages where butapen 50 mg/kg body weight dose served as standard. 90 min after oral gavages 0.3 ml of charcoal suspension was administered to all the mice. 60 min later, mice were provided with free access to food. The mice were observed at 5 min intervals until feces with charcoal were eliminated (maximum time of observation was 450 min).Charcoal was observed on the feces using normal light when it was easily visible, or using a microscope to help the identification of the black spots. The results were based on the time for the charcoal to be eliminated [6].

***In vivo* Anti-diarrheal activity test**

The present study was performed to evaluate the preventive and curative anti-diarrheal effects of the methanol, ethanol and chloroform extracts. All the animals housed under standard laboratory condition at 25°C and 12 h light such as dark cycle, acclimatized for 10 days before experiment. Standard diet and water were provided constantly. When test started all organized groups served with their respective doses and standard group served with loperamide (5 mg/kg) doses. After 1 hour, all groups received castor oil 2 ml each orally. Then they were placed in cages lined with adsorbent papers and observed for 4 hour for the presence of characteristic diarrheal droppings. 100% was considered as the total number of feces of control group. The activity was expressed as percent inhibition of diarrhea [7].

***In vivo* Anti-pyretic test**

Pyrexia or Fever is defined as an elevation of body temperature. It is a response due to tissue damage, inflammation, malignancy or graft rejection. Cytokines, interleukin, interferon and Tumor Necrosis Factor α (TNF-α) are formed in large amount under this condition, which increase PGE2 which in turn triggers hypothalamus to elevate body temperature [8]. At the starting point of this test mice were divided according to group design and were fasted for 1 hour but had access to water. The body temperature of all the mice was measured by recording rectal temperature. Brewer's yeast suspension was injected subcutaneously in the mice and rectal temperature of the mice was recorded after 18 hours. Only mice showing an increase in temperature of at least)0.5-1(°C was used for experiment. Individual plant extracts were administered orally and temperatures were measured at 1, 2, 3 and 4 hours after administration. Individual plant extracts were administered orally and temperatures were measured at 1, 2, 3 and 4 hours after administration [9].

***In vivo* Anti-nociceptive activity**

Anti-nociceptive activity was evaluated by acetic acid writhing and tail immersion methods.

Acetic acid induced writhing test: Pain is an unpleasant sensation that can be either acute or chronic and that is a consequence of complex neuro-chemical processes in the peripheral and central nervous system. Pain is induced by injection of irritants into the peritoneal cavity of mice. The animals react with a characteristic stretching behavior which is called writhing. The test is suitable to detect analgesic activity although some psychoactive agents also show activity [10]. Here the first group diclofenac sodium 50 mg/kg of body weight as standard. And other groups were treated with extract doses according to group design. Thirty minutes later each mouse was injected intra peritoneal with 0.7% acetic acid at doses of 10 ml/kg of body weight. Full writhing was not always completed by the mice. Accordingly, two half writhing were

considered as one full writhing. The number of writhing responses was recorded for each mouse during a subsequent 5 min period after 15 min intra peritoneal administration of acetic acid and the mean abdominal writhing for the each group was obtained and recorded[11].

Tail immersion test: The tail immersion method was used to evaluate the central mechanism of analgesic activity. Here the painful reactions in animals were produced by thermal stimulus that is by dipping the tip of the tail in hot water. Healthy albino mice of Swiss strain of either sex were used. They were housed in standard conditions of temperature (25±2°C) 12 hours light per day cycle [12]. Diclofenac sodium (50 mg/kg) dose was used as standard drug. Animals were fasted for 16 hours with free access to water. After administration of above drug, the basal reaction time was measured after in a regular interval of 30 minutes, by immersing the tail tips of the mice (last 1-2 cm) in hot water heated at temperature of temperature (55 ± 1)°C. The actual flick responses of mice i.e. time taken in second to withdrawn it's from hot water source was calculated and result were compared with control group. The latent period of the tail-flick response was determined at 0, 30, 60, 90 and 120 minute after the administration of drugs [13].

Results and Discussions:

Gastro intestinal (GI) motility test

Abdominal cramping and pain is a frequent problem in the adult population of western countries, with an estimated prevalence of ≤30%. Pharmacological studies have revealed that hyoscine butyl bromide is an anti-cholinergic drug with high affinity for muscarinic receptors located on the smooth-muscle cells of the GI tract. Its anti-cholinergic action exerts a smooth-muscle relaxing and spasmolytic effect. Blockade of the muscarinic receptors in the GI tract is the basis for its use in the treatment of abdominal pain secondary to cramping. However, because of its high tissue affinity for muscarinic receptors, hyoscine butyl bromide remains available at the site of action in the intestine and exerts a local spasmolytic effect [14]. Results of GI motility showed evidence of lowering GI movement (figure 1) which leads towards delayed charcoal defecation. Both doses (100 & 200 mg/kg body weight) of Chloroform extract showed significant effects (***p<0.001) (figure 1). Both the doses of ethanol (100 & 200 mg/kg) and 200 mg/kg dose of methanol extract showed significant effects compared with standard (*p<0.05, **p<0.01) (figure 1).

Anti-diarrheal activity

Diarrhea is one of the most common causes for thousands of deaths every year. Therefore, identification of new source of anti-diarrheal drugs becomes one of the most prominent focuses in modern research. The present study revealed that the ethanol extract of dose (100 mg/kg b.w) and chloroform extracts of 100 & 200 mg/kg body weight doses showed significant

results (***p<0.001) compared to standard drug loperamide (50 mg/kg b.w) (figure 2). And methanol at 100 mg/kg body weight dose also showed significant result (**p<0.01) in this test (figure 2).

Anti-dipressant activity

Forced swimming test (FST): The result of different leaf extracts of *Calamus tenuis* in mice in forced swimming test is represented by figure 3. Ethanol 200 mg/kg dose and chloroform 100 and 200 mg/kg doses showed significant (*p<0.05; **p<0.01) immobility then standard. It is still one of the best models for this procedure. It is a low cost, fast and reliable model to test potential antidepressant treatments with strong predictive validity. It has a great sensitivity with all antidepressant classes and all mechanisms of action of treatments could be determined, but clinical correlations should be considered very carefully. When mice are forced to swim to in a confined place, they tend to become immobile after vigorous activity (struggling). This stressful inescapable situation can be evaluated by assessing different stress. The development of immobility when the mice are placed in an inescapable container of water reflects the cessation of persistent escape directed behavior [5].

Anti-pyretic activity

Figure 4 demonstrated that in case of every dose of the leaf extracts temperature reduces from brewer's yeast induced temperature and it raises in the 2nd hour eventually. On the other hand from percent reduction of temperature (table 1) found very small amount of percent reduction of temperature of all the doses of the extracts. Lower dose of ethanol (100 mg/kg b.w) and both the doses of methanol extract (100 and 200 mg/kg b.w) showed (*p<0.05; **p<0.01) significant reduction of temperature close to standard group (figure 4). Brewer's Yeast induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermo-regulatory center at a lower temperature. So inhibition of prostaglandin synthesis could be possible mechanism of antipyretic action as that of acetylsalicylic acid. There are several mediators may bring about anti-pyresis. As to how they interfere with prostaglandin synthesis, further studies need to carry out. This study showed some significant effect as anti pyretic agent which may reflect the presence of flavonoid that helps to draw this effect [15].

Anti-nociceptive activity

Tail immersion test: Tail immersion method, the heat itself acts as a source of pain. The different concentrations of methanol, ethanol and chloroform extracts of the leaf (100 and 200 mg/kg) were administered to mice and observed the basal reaction time in different time intervals. The basal reaction time varies with increasing the concentrations along with increasing the time. According to the Kumar and Shankar (2009)[13] after administration of

different extracts, the basal reaction time was measured after in a regular interval of 30 minutes, by immersing the tail tips of the mice (last 1-2 cm) in hot water heated at temperature of temperature (55 ± 1) °C. The actual flick responses of mice i.e. time taken in second to withdrawn it's from hot water source was calculated. In the tail immersion test oral pre-treatment with different extract caused a profound and dose related analgesia. Both the doses of chloroform extracts showed (*p<0.05; **p<0.01) significant activity comparable to standard drug (figure 5).

Acetic acid induced writhing test: Pain is induced by injection of irritants into the peritoneal cavity of mice. The animals react with a characteristic stretching behavior which is called writhing. The test is suitable to detect analgesic activity although some psychoactive agents also show activity. The anti-nociception activity was evaluated by acetic acid induced writhing responses. The intra peritoneal administration of acetic acid produced both peripheral and central nociception action which acted through release of endogenous mediators and blocked by non-steroidal anti-inflammatory drugs. Finally it blocked the sensation of pain. Antinociceptive effect may be due to presence of kaempferol-7-O-α-l-rhamnopyranoside]- 4'-O-4'-[kaempferol-7-O-α-l-rhamnopyranoside (EJ-01) into the plants [10]. Anti-nociceptive activity was evaluated by acetic acid writhing test. Methanol (100 mg/kg and 200 mg/kg), ethanol (100 mg/kg) at 1 minute and chloroform (100 mg/kg) also at 1 minute were showed (***p<0.001) significant analgesic activity. Ethanol (200 mg/kg) as well as methanol (100 and 200 mg/kg mg/kg) doses were shown also (**p<0.01; ***p<0.001) significant analgesic effects at 2 & 3 minutes (figure 6).

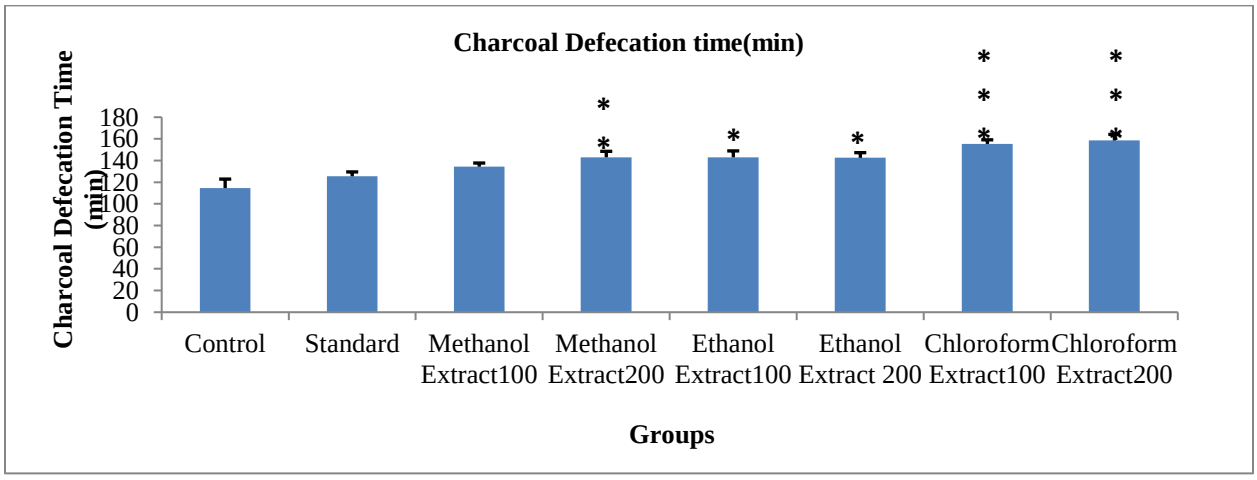
Conclusion

Calamus tenuis is widely used as a medicinal plant but studies strongly support its use as food as well as medicine. There are many scientific and traditional claims documenting the benefits of this plant such as its fruits are known to have antioxidant and cytotoxic potentials, analgesic and CNS depressant activities. The tribal people of our country culms of this rattan species' tender shoots are used as vegetables and as indigenous medicine. Present study found some interesting results from which we could assume that the *C. tenuis* leaves can be used against analgesic, pyretic and diarrhea.

Table 1: Percent (%) reduction of temperature after dosing of leaf extracts of *C. tenuis*

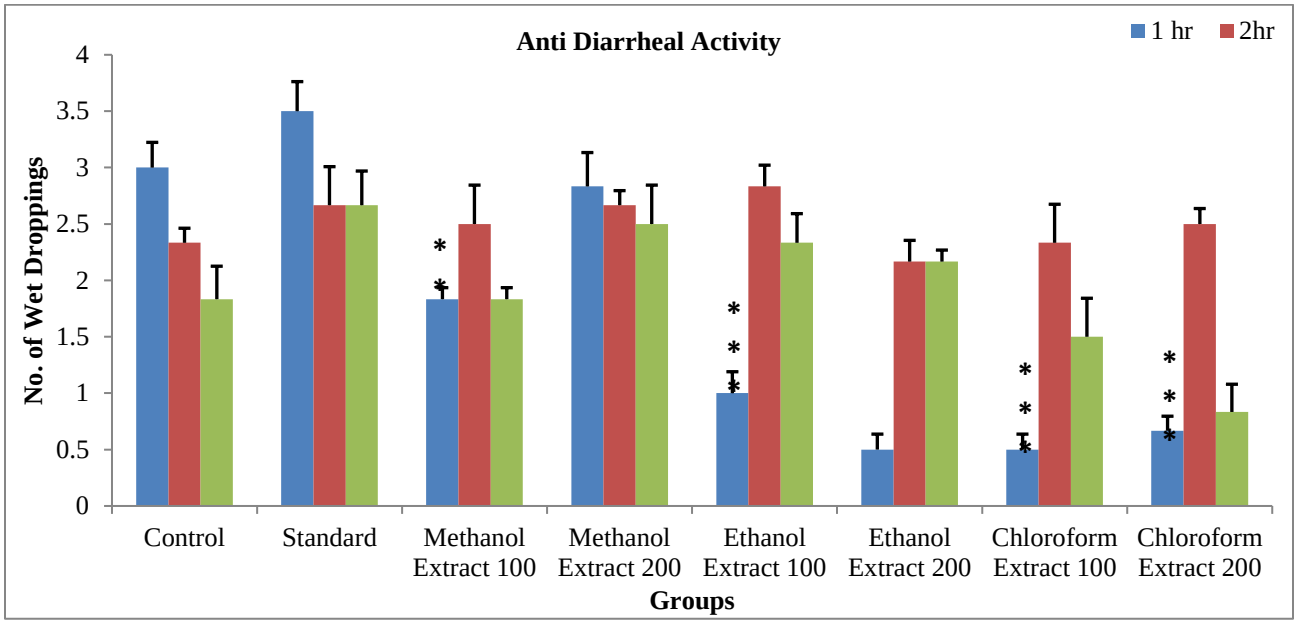
Group	Treatment	Dose (mg/b.w)	% Inhibition of Temperature (^{0}F)		
			1hr	2hr	3hr
1	Control	1ml/100 gm b.w	49.5±1.97	0.53±0.34	0.06±0.38
2	Standard	50	4.55±0.98	4.43±0.88	3.96±1.18
3	Methanol Extract	100	0.24±0.11**	0.27±0.34**	0.11±0.23*
4		200	0.32±0.44**	1.06±0.67**	0.66±0.69*
5	Ethanol Extract	100	0.44±0.11**	0.11±0.08**	0.35±0.12*
6		200	0.5±0.11**	1.44±0.38*	0.97±0.2
7	Chloroform Extract	100	0.46±0.31**	0.83±0.45**	0.65±0.23*
8		200	0.54±0.23**	1.28±0.25**	0.12±0.23*

(Values are expressed as mean ± S.E.M (n=6), *p<0.05; **p<0.01 significant when compared with the corresponding value of standard)



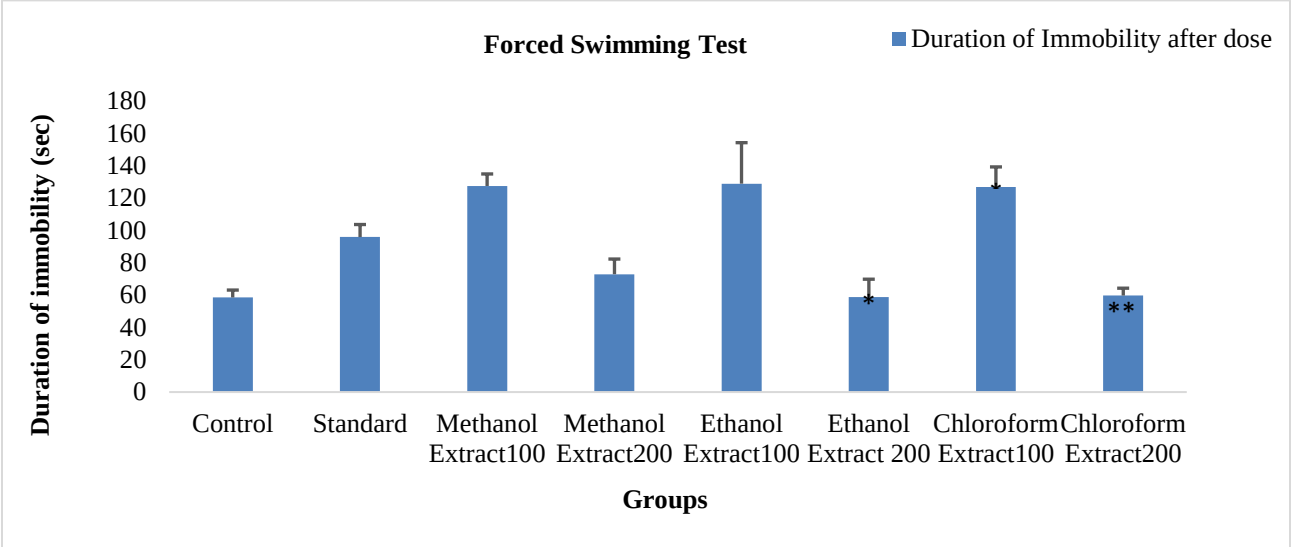
(Values are expressed as mean ± S.E.M. (n=6), *p<0.05; **p<0.01; ***p<0.001 significant when compared with the corresponding value of standard group)

Figure 1. Graphical presentation of gastro intestinal (GI) motility test of the leaf extracts of C. tenuis



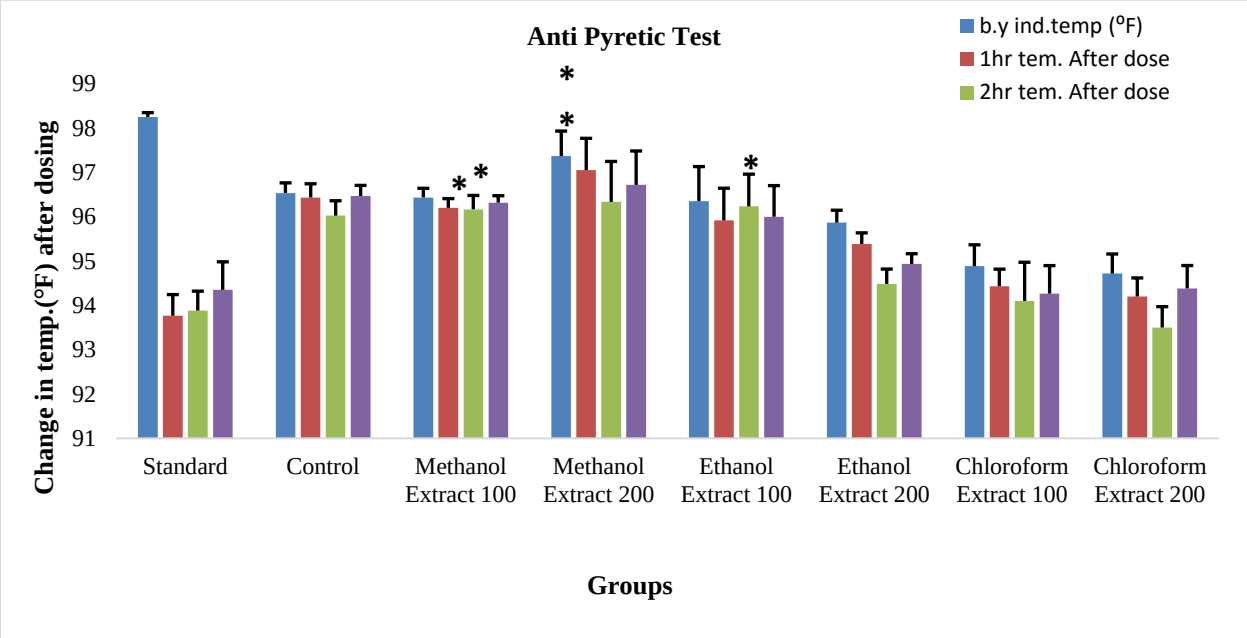
(Values are expressed as mean ± S.E.M (n=6), **p<0.01; ***p<0.001 significant when compared with the corresponding value of standard)

Figure 2: Anti-diarrheal activity of the leaf extracts of C. tenuis



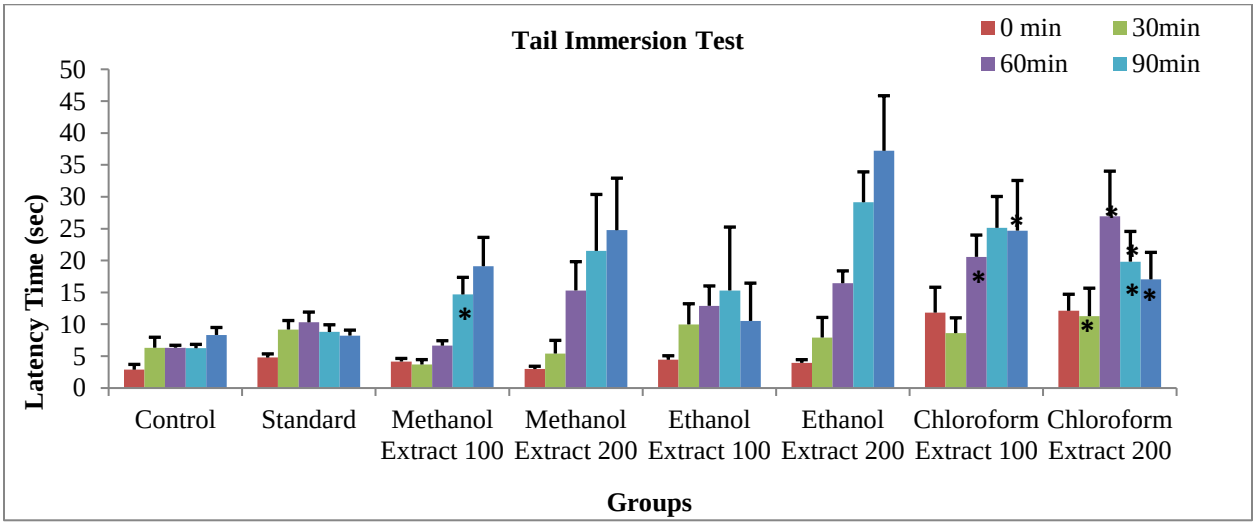
(Values are expressed as mean ± S.E.M (n=6), *p<0.05; **p<0.01 significant when compared with the corresponding value of standard)

Figure 3. Graphical presentation of forced swimming test of the leaf extracts of C. tenuis



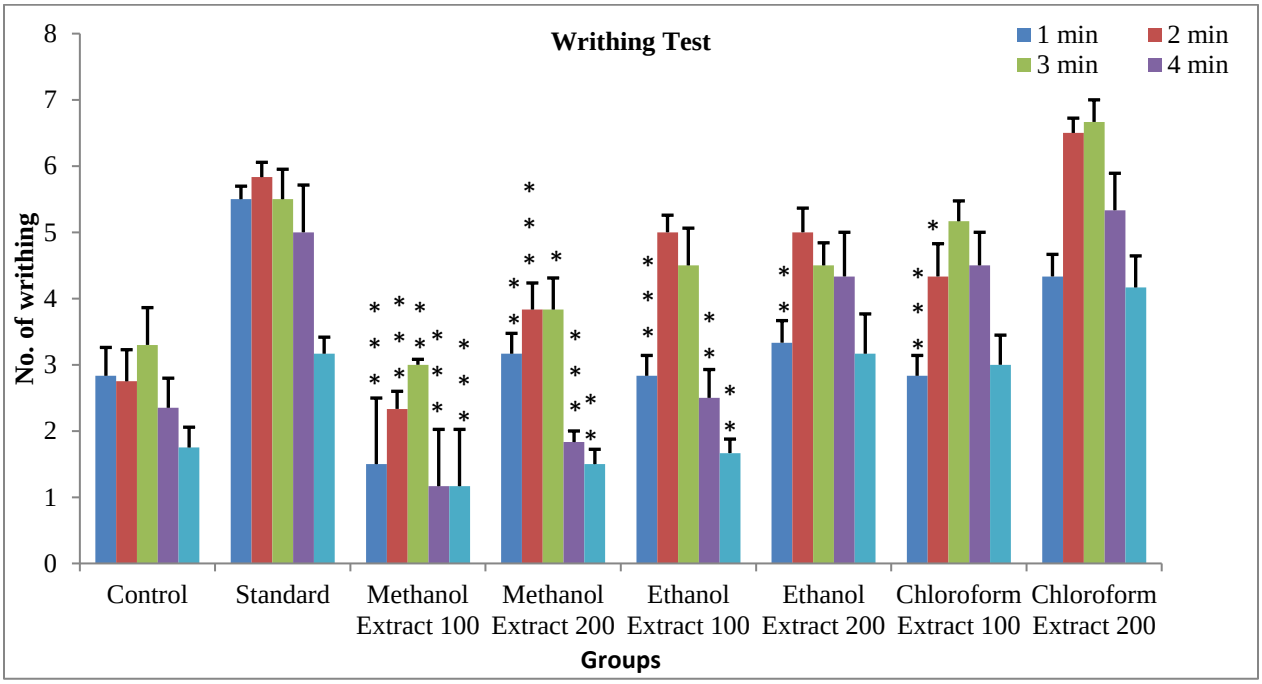
(Values are expressed as mean ± S.E.M (n=6), *p<0.05; **p<0.01 significant when compared with the corresponding value of standard)

Figure 4**. Comparative study of temperature variations after dosing of leaf extracts of *C. tenuis***



(Values are expressed as mean ± S.E.M. (n=6), *p<0.05; **p<0.01 significant when compared with the corresponding value of standard)

Figure 5. Graphical presentation of tail immersion test of leaf extracts of *C. tenuis*



(Values are expressed as mean ± S.E.M. (n=6), *p<0.05; **p<0.01; ***p<0.001 significant when compared with the corresponding value of standard)

Figure 6. Graphical presentation of acetic acid induced writhing test of leaf extracts of *C. tenuis*

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