

Original research article

Extraction & Pharmacological Evaluations Of Various Parts ‘*Cenchrus Ciliaris*’ And ‘*Cenchrus Setigerus*’**Tanvi Gaur, Dr. Dharmendra Ahuja****Department of Pharmaceutical Sciences, Faculty of Pharmaceutical Science, Jayoti Vidyapeeth Women's University , Vedaant Gyan Valley, Village-Jharna, Mahala Jobner Link Road, Jaipur Ajmer Express Way, NH-8, Jaipur-303122, Rajasthan, India.****Corresponding Author: Tanvi Gaur****Abstract**

Cenchrus ciliaris L. also called as “Dhaman grass”, which is a general starvation food employed during drought and deficiency of food in Rajasthan. Among the collected germplasm accessions, one among the *Cenchrus setigerus* accessions exhibited prostrate activities which would be of enormous use in the greening of degraded lands where with few plants whole area can be covered and used for grazing. Actual aim of this study was intended to find out the pharmacological activities of the extract of various parts (e.g. roots, stems, leaves, seeds) of *Cenchrus Ciliaris* and *Cenchrus Setigerus* and the following evaluations were carried out like-analgesic activity, anti inflammatory and diuretic activity on animal model. The actual goal of this research work was to carry out the pharmacological screening of ethyl acetate (EA) and glacial acetic acid (GAA) extracts of various parts (e.g. roots, stems, leaves, seeds) of *Cenchrus Ciliaris* and *Cenchrus Setigerus*. All the extracts of both the plants were found to be safe up to 2000 mg/kg body weight. Finally, 200 mg /kg b.w. and 400 mg /kg b.w. were selected for this study and acute toxicity study revealed the nontoxic nature for each selected plant extracts. From all the test compounds, GAA extracts of both the plants with a dose size of 400 mg/kg body weight were found to be potent in analgesic activity which is comparable to standard, but EA extracts having lower activity, compared to others. Drugs that reduce the total number of writhing upto in 30 minutes can be considered as an effective analgesic. Moreover, group IX (Treated with mixtures of GAA extracts of *Cenchrus Ciliaris* dissolved in DMSO) is exhibited fairly better activity compared to other extracts. In the same way GAA extracts with higher doses showed significant anti-inflammatory activity. The group of II, IX & X was treated by Diclofenac sodium (10 mg/kg b.w.) and GAA extracts of *Cenchrus Ciliaris* and *Cenchrus Setigerus* (400 mg/kg body weight) showed maximum inhibition of edema after 5hrs, which was 83.72, 81.37 & 67.45% respectively. The furosemide (10 mg/ kg) and GAA extracts of both the selected plants at higher doses (400 mg/kg) showed potent natriuretic and saluretic activity as compared to normal control.

Keywords: *Cenchrus Ciliaris*, *Cenchrus Setigerus*, roots, stems, leaves, seeds, analgesic activity, anti inflammatory and diuretic activity on animal model.

Introduction

Cenchrus ciliaris L. (Poaceae) typically documented as “Dhaman grass”, which is a general famine food employed during drought and insufficiency of food in Rajasthan. This study could isolate numerous natural bioactive compounds from the leaf using solvents of different polarity *i.e.* methanol, ethyl acetate and hexane. Arora S and Kumar G, 2018, investigated to establish the phytochemical composition of leaf of *C. ciliaris* using gas chromatography-mass spectrometry technique [1]. Ambreen Aleem and Khalid Hussain Janbaz, 2017, conducted to

rationalize the traditional uses of *Cenchrus ciliaris* in gastrointestinal disorders using *in vivo* and *ex vivo* assays. The antidiarrheal effect was performed in rats by the castor oil-induced diarrheal model. *C. ciliaris* (100, 300 and 500 mg/kg) reduced the castor oil-induced diarrhea significantly. They also carried out another study in mice to establish the intestinal transit rate showed that *C. ciliaris* (100 and 200 mg/kg) inhibited the transit rate significantly. These results indicated the occurrence of antispasmodic, antidiarrheal and antiemetic activities in *C. ciliaris*, thus providing the scientific basis for its traditional uses [2]. Kalamani. A *et al.*, 2011, were collected 60 germplasm accessions of *Cenchrus ciliaris* and *Cenchrus setigerus* from different habitats in and around Coimbatore and Erode districts and planted in field trials. Among the collected germplasm accessions, one among the *Cenchrus setigerus* accessions exhibited prostrate behavior which would be of vast use in the greening of degraded lands where with few plants whole area can be covered and used for grazing [3]. Wani TA *et al.*, 2012, was evaluated for its analgesic activity by making use of different central and peripheral pain models on ethanolic extract of *S. robusta* resin (SRE). The extract produced significant central and peripheral analgesic effects, as is evident from increase in reaction time in hot plate and tail flick tests, inhibition in writhing counts in acetic acid-induced writhing test, inhibition of licking time in formalin-induced hind paw licking, increased pain threshold in paw withdrawal latency in carrageenan-induced hyperalgesia and increased paw withdrawal threshold in post-surgical pain [4]. Girish Gulab Meshram *et al.*, 2016, evaluated the possible anti-inflammatory activity of the aqueous (AE) and ethanolic (EE) extracts of the leaves of *A. lebbeck* to support the ethno-pharmacological claims. *Albizia lebbeck Benth.* (Mimosaceae) is a medicinal tree used to treat several inflammatory ailments in the Indian traditional Ayurvedic system of medicine. The study was carried out using Wistar rats (100-150 g). The AE and EE were prepared using the Soxhlet extraction process. The anti-inflammatory activity of the AE and EE of the leaves of *A. lebbeck* were studied using carrageenan-induced paw edema and cotton pellet-induced granuloma models [5]. Fahad I. Al-Saikhan & Mohd N. Ansari, 2016, this study was aimed to envisage the true effect and magnitude of diuresis of methanolic extract of *P. harmala* (MEPH) in comparison with a well-known diuretic drug furosemide using Wistar albino rats. MEPH was administered orally in three different doses (150, 300 and 450 mg/kg) to experimentally dehydrated rats. Furosemide (10 mg/kg orally) was used as a reference drug. The diuretic effect of the MEPH was evaluated by measuring urine volume, urine pH, urinary electrolyte levels, natriuretic and saluretic effects. The urine volume (in mL) measured at 5 h and 24 h and electrolyte excretion (Na⁺, K⁺, and Cl⁻) at 24 h duration were measured [6]. Sreelakshmi R *et al.*, 2015, evaluated the diuretic activity of the plant *Mussaenda frondosa* Linn., using Lipschitz method. Wistar albino rats were divided into 4 groups of 6 animals in each. Frusemide (20 mg/Kg p.o) used as a standard diuretics. Two doses of plant extract were used for the study [7].

The present study was intended to find out the pharmacological activities of the extract of various parts (e.g. roots, stems, leaves, seeds) of *Cenchrus Ciliaris* and *Cenchrus Setigerus* and compared the results to each others. The dry and powder portions (roots, stems, leaves and seeds) were extracted with petroleum ether (PE), ethyl acetate (EA) and glacial acetic acid (GAA) using Soxhlet assembly. The actual goal of this research work was to carry out the pharmacological screening of ethyl acetate (EA) and glacial acetic acid (GAA) extracts of various parts (e.g. roots, stems, leaves, seeds) of *Cenchrus Ciliaris* and *Cenchrus Setigerus*. The following evaluations were carried out like-analgesic activity, anti inflammatory and diuretic activity on animal model.

Material & Methods:

Methods for extraction of various parts of plants:

The plant materials were collected and moved promptly into the laboratory, then cleaned with deionized water and chose plant parts were independently conceal dried for multi week. Each shade dried plant parts were powdered and fine powder of each sample was utilized for Soxhlet extraction with various solvents system. The dried plant material was pounded into fine powder utilizing a processor (blender). About 10 gm of powdered material was separated in Soxhlet extraction mechanical assembly progressively with various solvents (250 ml) as per their expanding extremity (oil ether < ethyl acetic acid derivation < cold acidic corrosive) for 18 hours at a temperature not surpassing the breaking point of the individual dissolvable. The acquired concentrates were separated by utilizing Whatman No. 1 channel paper and afterward accumulated at 40°C by utilizing an evaporator. The remaining concentrates were put away in fridge at 4°C in little and sterile glass bottles [2-6].

Pharmacological screening on animal models

Pharmacological evaluation of the ethyl acetate (EA) extracts and glacial acetic acid (GAA) extracts of various parts (e.g. roots, stems, leaves, seeds) of *Cenchrus Ciliaris* and *Cenchrus Setigerus* includes-

- A) Acute toxicity study following OECD guidelines [8] in mice
- B) Analgesic activity in acetic acid induced writhing in mice
- C) Diuretic activity
- D) Anti inflammatory activity in formalin induced rat paw edema method.

Animals and environment condition

All the animals used for the study were healthy and active in their cage. The animals were group housed in polyacrylic cages (38×23×10cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2 °C) and relative humidity 50% ($\pm 10\%$), with a dark and light cycle of 12 ± 1 h. They were allowed free access to standard dry pellet diet (Amrut, India) and water *ad libitum* and kept in quarantine for a week to acclimatize with animal house facility.

All procedures described were reviewed and approved by the institutional committee for ethical use of animals.

Acute toxicity study

Acute toxicities were carried out by using albino mice, following OECD text guideline [8] 423(2001) usage in defined dosage and results allowed the substances to be ranked and classified according to globally harmonized system (GHS) for the classification of chemicals which can cause acute toxicities.

The acute toxicities of ethyl acetate (EA) extracts and glacial acetic acid (GAA) extracts of various parts (e.g. roots, stems, leaves, seeds) of *Cenchrus Ciliaris* and *Cenchrus Setigerus* were determined according to the OECD text guideline 423(2001) [8]. Ethyl acetate (EA) extracts and glacial acetic acid (GAA) extracts of various parts (e.g. roots, stems, leaves, seeds) of *Cenchrus Ciliaris* and *Cenchrus Setigerus* were mixed in a ratio of 1:1:1:1 separately. For each extracts of plants, acute toxicity study was conducted in nine animal groups containing 6 male albino mice in each group, for two weeks. Animals were fasted overnight and treatments were given in the morning.

Table 1:

Group I	Control	Animals received only the vehicle orally.
Group II		Treated with mixtures of ethyl acetate (EA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Ciliaris</i> through gastric intubation suspended in 5% acacia solution.
Group III	Test (1000mg /Kg b.w.)	Treated with mixtures of ethyl acetate (EA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Setigerus</i> through gastric intubation suspended in 5% acacia solution.
Group IV	(volume <2ml)	Treated with mixtures of glacial acetic acid (GAA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Ciliaris</i> through gastric intubation suspended in 5% acacia solution.
Group V		Treated with mixtures of glacial acetic acid (GAA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Setigerus</i> through gastric intubation suspended in 5% acacia solution.
Group VI		Treated with mixtures of ethyl acetate (EA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Ciliaris</i> through gastric intubation suspended in 5% acacia solution.
Group VII	Test	Treated with mixtures of ethyl acetate (EA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Setigerus</i> through gastric intubation suspended in 5% acacia solution.
Group VIII	(2000mg/ Kg b.w.)	Treated with mixtures of glacial acetic acid (GAA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Ciliaris</i> through gastric intubation suspended in 5% acacia solution.
Group IX	(volume <2ml)	Treated with mixtures of glacial acetic acid (GAA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Setigerus</i> through gastric intubation suspended in 5% acacia solution.

Animals were observed for 8 hours on the day of administration and subsequently once daily for the period of 3 days and finally observed up to fourteen days to record possible food-water intake and body weight changes and observed activity [8] Special attention given during first two hours treated animals observed by an observer blind to the treatment protocol. Since no mortality was observed in this dose range, hence this was considered as safer dose and no further toxicity study done at higher doses. Finally, 200 mg kg b.w. 400 mg kg b.w. was selected for this study and acute toxicity study revealed the nontoxic nature for each selected plant extracts, which was tabulated in results part.

Analgesic activity [4, 9-12]

Animals used for the experiment were maintained in the animal house facility of the institution under standard animal house conditions (followed to CPCSEA guideline), and were fed with commercial feed pellets and water *ad libitum*. Animals were acclimatized to the laboratory condition for a week prior to start the experiments.

For analgesic activity:

Adult, healthy, overnight fasted, either sex albino mice, weighing between 35-45 grams.

Methods:

Analgesic activity was evaluated in glacial acetic acid (1% solution) treated albino mice of male sex by writhing method. A compound may be analgesic, if it is effectively reduces the number of writhing produced by acetic acid solution (1% v/v) i.p.

Procedure:

Adult, healthy animal of either sex over night fasted were randomly assigned to Group I to Group VII (n=6).

Earlier reported method was used with slight modification. Mice of either sex were divided into ten groups with each consisting of six animals. After 30 minutes of drug administration, 0.1 ml of 1% acetic acid solution was injected i.p. Mice were placed individually into glass beakers and five minutes were allowed to elapse. These are characterized by specific abdominal contractions and are accompanied by elongation of the body with concave arching of the back and/or twisting or turning of the hip together with the hind paw and/or side rolling of the body and circling. The numbers of writhes observed up to 30 min were recorded and percent inhibition of writhing up to 30 min was also calculated and compared among control and drug-treated groups.

Percentage inhibition was calculated using the following formula:

$$\% \text{ inhibition} = [(Wc - Wt) \times 100] / Wc$$

Where, Wc = No. of writhes in control group, Wt = No. of writhes in test group

Extracts with less than 70% inhibition were considered to have minimal analgesic activity.

Anti-inflammatory activity [5, 13-20]:

For anti-inflammatory activity: Adult, healthy, overnight fasted, male albino rats, weighing between 180-220 grams.

Chemicals used-

- 1) Diclofenac sodium
- 2) 1% glacial acetic acid solution prepared from commercial acetic acid solution.
- 3) 1% formalin was prepared from commercial formaldehyde solution.

All stock solution of either extracts/ standard drugs was prepared fresh on the day of experiment with DMSO (Dimethyl sulfoxide).

The activity was determined by formalin induced rat hind paw oedema method. Diclofenac sodium was used as the standard drug & the % inhibition of oedema was determined. Formalin (0.1 ml of 1%) was used as phlogistic agents and produced inflammation at the injected site of the animals. A compound may be anti-inflammatory agents, if it is effectively suppresses phlogistic agent induced paw edema.

Procedure:

Adult, healthy animal of either sex over night fasted were randomly assigned to Group I to Group X Groups (n=6).

Table 2:

Group I	Control	Animals received only the vehicle orally.
Group II	Standard	Animals received Diclofenac sodium i.p (dose 10 mg/kg) (standard drugs in reference).
Group III	Test (200mg/Kg b.w. administered orally)	Treated with mixtures of ethyl acetate (EA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Ciliaris</i> dissolved in DMSO.
Group IV		Treated with mixtures of ethyl acetate (EA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Setigerus</i> dissolved in DMSO.
Group V		Treated with mixtures of glacial acetic acid (GAA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Ciliaris</i> dissolved in DMSO.
Group VI		Treated with mixtures of glacial acetic acid (GAA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Setigerus</i> dissolved in DMSO.
Group VII	Test (400mg/Kg b.w. administered orally)	Treated with mixtures of ethyl acetate (EA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Ciliaris</i> dissolved in DMSO.
Group VIII		Treated with mixtures of ethyl acetate (EA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Setigerus</i> dissolved in DMSO.
Group IX		Treated with mixtures of glacial acetic acid (GAA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Ciliaris</i> dissolved in DMSO.
Group X		Treated with mixtures of glacial acetic acid (GAA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Setigerus</i> dissolved in DMSO.

Pedal edema was induced by injecting 0.1ml of freshly prepared 1% formalin solution into the planter aponeurosis of right hand paw. Change in paw volume was measured immediately after zero hour, half an hour, one hour, two hours, three hours, four hours and five hours following injection using digital calipers. The treatment was continued for 6 consecutive days and the edema was measured on 1 and 6 days.

The percentage inhibition of paw edema was calculated for all the models using the following formula.

$$\% \text{ inhibition} = [(V_c - V_t) / V_c] \times 100.$$

Where V_t represent paw volume of test or Diclofenac sodium, V_c represent paw volume of treated control group.

Inflammation was indicated by significant ($P < 0.05$) increase in paw thickness. The paw thickness of the groups treated with the test samples and that of the group treated with diclofenac sodium (standard drug) was compared with the negative control group. Significant reduction of the paw thickness (expressed as mean \pm S.E.M) among the treated groups compared to the control group was used to indicate oedema inhibition (anti-inflammatory activity).

Diuretic activity [6, 21-25]

Animals

Adult Wistar albino rats of either sex weighing 180–220 g were used. The animals were kept under standard laboratory conditions, with food and water ad libitum, under a 12 h light/12 h dark cycle.

Chemicals

Methanol was purchased from S. D. Fine Chem Ltd, Biosar, India, Furosemide was obtained from Sigma–Aldrich, USA. All other chemicals used were of reagent grade.

Diuretic activity

Wistar albino rats were randomly divided into ten groups of six rats each, e.g. control group, furosemide treated group, and herbal samples treated group. Control group was treated with 2 ml/100 g of body weight 0.5% acacia solution with water, furosemide treated group was treated with furosemide 10 mg/kg orally. Herbal samples treated group was subdivided into eight sub groups; they were treated with 200 and 400 mg/kg b.w. doses of each plant extracts of different solvent systems, respectively. All administered drugs were given orally.

Each animal was placed in isolation in metabolic cages, 24 h prior to commencement of the experiment for adaptation and then fasted overnight with free access to water. Urine samples were collected after 5 h and 24 h of the last dose. The urine samples were filtered and finally stored at 20 °C for measurement of various other parameters. Immediately after administration rats were placed in metabolic cages, one rat per cage. The metabolic cages were provided with a funnel for urine collection and mesh to separate faeces from urine. The bladder was emptied by pulling the base of tail of each rat. The urine was collected in beaker and covered with aluminium foil to avoid evaporation. The volume of urine collected after 24 hrs was recorded and subjected to analysis for determination of sodium, potassium ions by flame photometry and chloride and bicarbonate ions were determined by titrimetric analysis after 24hrs.

Table 3:

Group I	Control	Treated with vehicle-0.5% acacia orally.
Group II	Standard	Treated with furosemide 5mg/kg P.o
Group III	Test (200mg/Kg b.w. administered orally)	Treated with mixtures of ethyl acetate (EA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Ciliaris</i>
Group IV		Treated with mixtures of ethyl acetate (EA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Setigerus</i>
Group V		Treated with mixtures of glacial acetic acid (GAA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Ciliaris</i>
Group VI		Treated with mixtures of glacial acetic acid (GAA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Setigerus</i>
Group VII		Treated with mixtures of ethyl acetate (EA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Ciliaris</i>
Group VIII		Treated with mixtures of ethyl acetate (EA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Setigerus</i>
Group IX	Test (400mg/Kg b.w. administered orally)	Treated with mixtures of glacial acetic acid (GAA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Ciliaris</i>
Group X		Treated with mixtures of glacial acetic acid (GAA) extracts of various parts (e.g. roots, stems, leaves, seeds in a ratio of 1:1:1:1) of <i>Cenchrus Setigerus</i>

Measurement of urine parameters

Total urine volume was measured after 5 h and 24 h for all rats. Urine pH and conductivity of fresh urine samples were measured with a digital pH meter and conductivity meter, respectively. The total urine output samples (24 h) were then diluted (1:1000 in deionized water) to estimate the total concentrations of electrolytes (sodium, potassium, and chloride ions) in urine.

Calculation of diuretic index, Lipschitz value, saluretic index and Na⁺/K⁺ ratio

The following formulas were used for the calculation of different urinary parameters:

Diuretic index = Mean urine volume of the test group/Mean urine volume of the control group;

Lipschitz value = Mean urine volume of the test group/Mean urine volume of the reference group;

Saluretic index (Na⁺ Index or Cl⁻ index or K⁺ Index etc) = Concentration of electrolyte in urine of the test group/Concentration of electrolyte in urine of the control group;

Na⁺/K⁺ ratio = Concentration of Na⁺ in urine of a group/Concentration of K⁺ in urine of the same group.

Diuretic action and activity

The diuretic action and diuretic activity were derived from the ratio of urine volume in the test group and that in the control and positive control groups, respectively. It was decided prior to the start of the experiment that diuretic activity will be considered “nil”, “little”, “moderate”, and “good”, if the values were <0.72, 0.72–1.00, 1.00–1.5, and >1.5, respectively.

Statistical analysis:

All the results were expressed as mean ± SEM, and were subjected one way ANOVA followed by Dunnett test (post test). Values were considered significant when p<0.05. The potency of test compound was also determined by measuring its percentage inhibition/reduction in compare with standard as well treated groups.

Results & discussion

Pharmacological screening on animal models

Analgesic activity:

Table 4: Analgesic activity in acetic acid induced writhing in mice method

Group	Compound code	Dose	No. of writhing in mice up to 30 min (Mean ± SD)	% inhibition
Group I	Control Vehicle	DMSO	95.23±4.66	-----
Group II	Diclofenac sodium	10 mg/kg body weight	25.08 ± 2.06**	73.66**
Group III	Treated with mixtures of EA extracts of <i>Cenchrus Ciliaris</i> dissolved in DMSO.	200 mg/kg body weight	40.22 ±3.12	57.76
Group IV	Treated with mixtures of EA extracts of <i>Cenchrus Setigerus</i> dissolved in DMSO.	200 mg/kg body weight	49.43 ±3.22	48.09

Group V	Treated with mixtures of GAA extracts of <i>Cenchrus Ciliaris</i> dissolved in DMSO.	200 mg/kg body weight	35.38 ±2.16**	62.84
Group VI	Treated with mixtures of GAA extracts of <i>Cenchrus Setigerus</i> dissolved in DMSO.	200 mg/kg body weight	47.44 ±2.11	50.18
Group VII	Treated with mixtures of EA extracts of <i>Cenchrus Ciliaris</i> dissolved in DMSO.	400 mg/kg body weight	28.17 ± 2.86**	70.41**
Group VIII	Treated with mixtures of EA extracts of <i>Cenchrus Setigerus</i> dissolved in DMSO.	400 mg/kg body weight	29.70 ±2.08 *	68.81*
Group IX	Treated with mixtures of GAA extracts of <i>Cenchrus Ciliaris</i> dissolved in DMSO.	400 mg/kg body weight	25.75 ±3.14 **	72.96**
Group X	Treated with mixtures of GAA extracts of <i>Cenchrus Setigerus</i> dissolved in DMSO.	400 mg/kg body weight	27.07 ± 4.75**	71.57**

All values are expressed as means ± SEM (n =6), * P < 0.05, ** P < 0.01 (Dunnett's test) as compared to control was considered as accepted values and significant.

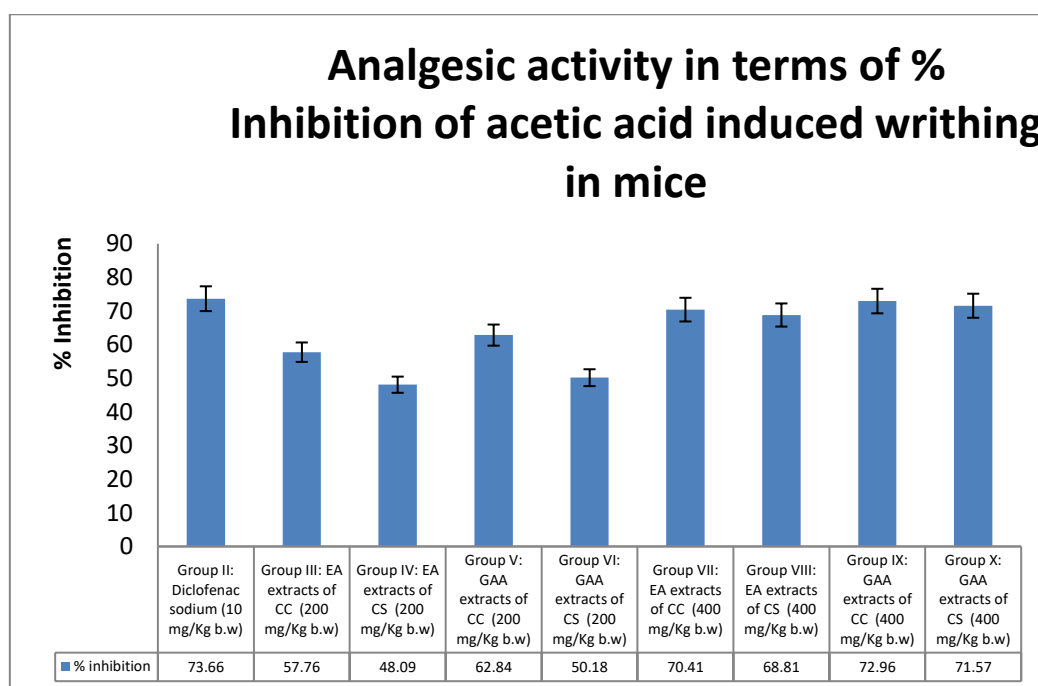


Figure 1: Histogram showing analgesic activity of standard & of the various extract of both the selected plants: % inhibition with respect to control group [writhing in mice up to 30 min (Mean ± SD)].

Anti inflammatory activity in formalin induced rat paw edema method

Table 5: Anti inflammatory activity in induced rat paw edema method: Change in paw edema volume in mm (Mean \pm SEM) at various time intervals.

Group No.	Compound	Dose	Change in paw edema volume in mm (Mean \pm SEM) at various time intervals						
			0 min	30 min	60 min	120 min	180 min	240 min	300 min
Group I	Control (Vehicle)	DMSO	0.75 \pm 0.04	1.78 \pm 0.06	2.81 \pm 0.12	3.89 \pm 0.28	4.85 \pm 0.38	5.02 \pm 0.22	5.10 \pm 0.24
Group II	Diclofenac sodium	10 mg/kg body weight	0.74 \pm 0.02	0.76 \pm 0.04	0.79 \pm 0.04	0.81 \pm 0.06	0.82 \pm 0.08	0.82 \pm 0.08	0.83 \pm 0.08
Group III	Treated with mixtures of EA extracts of <i>Cenchrus Ciliaris</i> dissolved in DMSO.	200 mg/kg body weight	0.76 \pm 0.08	0.98 \pm 0.04	1.83 \pm 0.13	2.86 \pm 0.16	2.92 \pm 0.19	4.00 \pm 0.22	4.39 \pm 0.26
Group IV	Treated with mixtures of EA extracts of <i>Cenchrus Setigerus</i> dissolved in DMSO.	200 mg/kg body weight	0.75 \pm 0.09	1.08 \pm 0.08	1.98 \pm 0.14	2.99 \pm 0.18	3.85 \pm 0.21	4.25 \pm 0.24	4.54 \pm 0.28
Group V	Treated with mixtures of GAA extracts of <i>Cenchrus Ciliaris</i> dissolved in DMSO.	200 mg/kg body weight	0.76 \pm 0.04	0.96 \pm 0.06	1.28 \pm 0.14	2.01 \pm 0.14	2.22 \pm 0.18	3.54 \pm 0.21	4.08 \pm 0.24
Group VI	Treated with mixtures of GAA extracts of <i>Cenchrus Setigerus</i> dissolved in DMSO.	200 mg/kg body weight	0.74 \pm 0.02	0.99 \pm 0.02	2.07 \pm 0.12	2.90 \pm 0.14	3.22 \pm 0.18	3.86 \pm 0.28	4.11 \pm 0.31
Group VII	Treated with mixtures of EA extracts of <i>Cenchrus Ciliaris</i> dissolved in DMSO.	400 mg/kg body weight	0.73 \pm 0.04	0.84 \pm 0.03	0.99 \pm 0.06	1.81 \pm 0.12	2.02 \pm 0.11	2.24 \pm 0.14	2.65 \pm 0.23
Group VIII	Treated with mixtures of EA extracts of <i>Cenchrus Setigerus</i> dissolved in DMSO.	400 mg/kg body weight	0.75 \pm 0.06	0.86 \pm 0.04	1.06 \pm 0.14	1.98 \pm 0.12	2.32 \pm 0.22	2.64 \pm 0.13	2.85 \pm 0.16
Group IX	Treated with mixtures of GAA extracts of <i>Cenchrus Ciliaris</i> dissolved in DMSO.	400 mg/kg body weight	0.73 \pm 0.03	0.77 \pm 0.03	0.78 \pm 0.06	0.82 \pm 0.03	0.83 \pm 0.04	0.85 \pm 0.08	0.95 \pm 0.16
Group X	Treated with mixtures of GAA extracts of <i>Cenchrus Setigerus</i> dissolved in DMSO.	400 mg/kg body weight	0.73 \pm 0.04	0.84 \pm 0.04	0.89 \pm 0.06	1.11 \pm 0.04	1.42 \pm 0.05	1.54 \pm 0.02	1.66 \pm 0.04

All values are expressed as means \pm SD (=6), The observations are expressed as mean \pm S.E.M., * $P < 0.05$, ** $P < 0.01$, (Dunnett's test) as compared to control was considered as accepted values and significant.

Table 6: Anti inflammatory activity of various groups in induced rat paw edema method: % inhibition in paw thickness at various time intervals

Group No.	Compound	Dose	% inhibition in paw thickness at various time intervals					
			30 min	60 min	120 min	180 min	240 min	300 min
Group II	Diclofenac sodium	10 mg/kg body weight	57.30	71.88	79.17	83.09	83.66	83.72
Group III	Treated with mixtures of EA extracts of <i>Cenchrus Ciliaris</i> dissolved in DMSO.	200 mg/kg body weight	44.94	34.87	26.47	39.79	20.31	13.92
Group IV	Treated with mixtures of EA extracts of <i>Cenchrus Setigerus</i> dissolved in DMSO.	200 mg/kg body weight	39.32	29.53	23.13	20.61	15.33	10.98
Group V	Treated with mixtures of GAA extracts of <i>Cenchrus Ciliaris</i> dissolved in DMSO.	200 mg/kg body weight	46.06	54.44	48.32	54.22	29.48	20.00

Group VI	Treated with mixtures of GAA extracts of <i>Cenchrus Setigerus</i> dissolved in DMSO.	200 mg/kg body weight	44.38	26.33	25.44	33.60	23.10	19.41
Group VII	Treated with mixtures of EA extracts of <i>Cenchrus Ciliaris</i> dissolved in DMSO.	400 mg/kg body weight	52.8*	64.76*	53.47*	58.35*	55.37*	48.03*
Group VIII	Treated with mixtures of EA extracts of <i>Cenchrus Setigerus</i> dissolved in DMSO.	400 mg/kg body weight	51.68*	62.27*	49.10*	52.15*	47.41*	44.11*
Group IX	Treated with mixtures of GAA extracts of <i>Cenchrus Ciliaris</i> dissolved in DMSO.	400 mg/kg body weight	56.74**	72.24**	78.92**	82.88**	83.06**	81.37**
Group X	Treated with mixtures of GAA extracts of <i>Cenchrus Setigerus</i> dissolved in DMSO.	400 mg/kg body weight	52.80*	68.32*	71.46*	70.72*	69.93*	67.45*

All values are expressed as means ± SEM (n=6), P* < 0.05, P** < 0.01 compared to control, Student t-test (Unpaired),

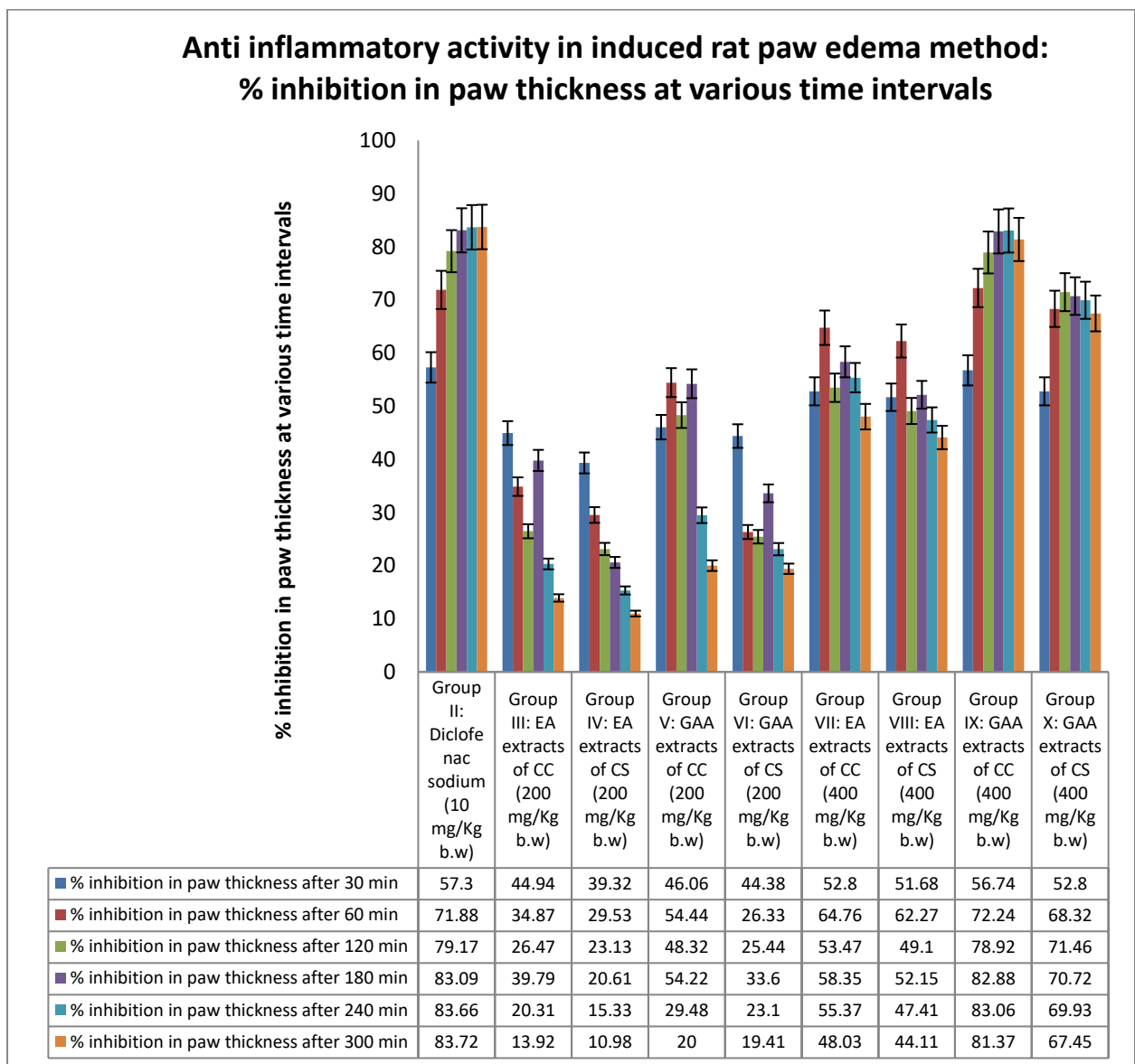


Figure 2: Histogram showing anti inflammatory activity of standard & different extracts of selected plants:: % inhibition in paw thickness at various time intervals

Diuretic activity

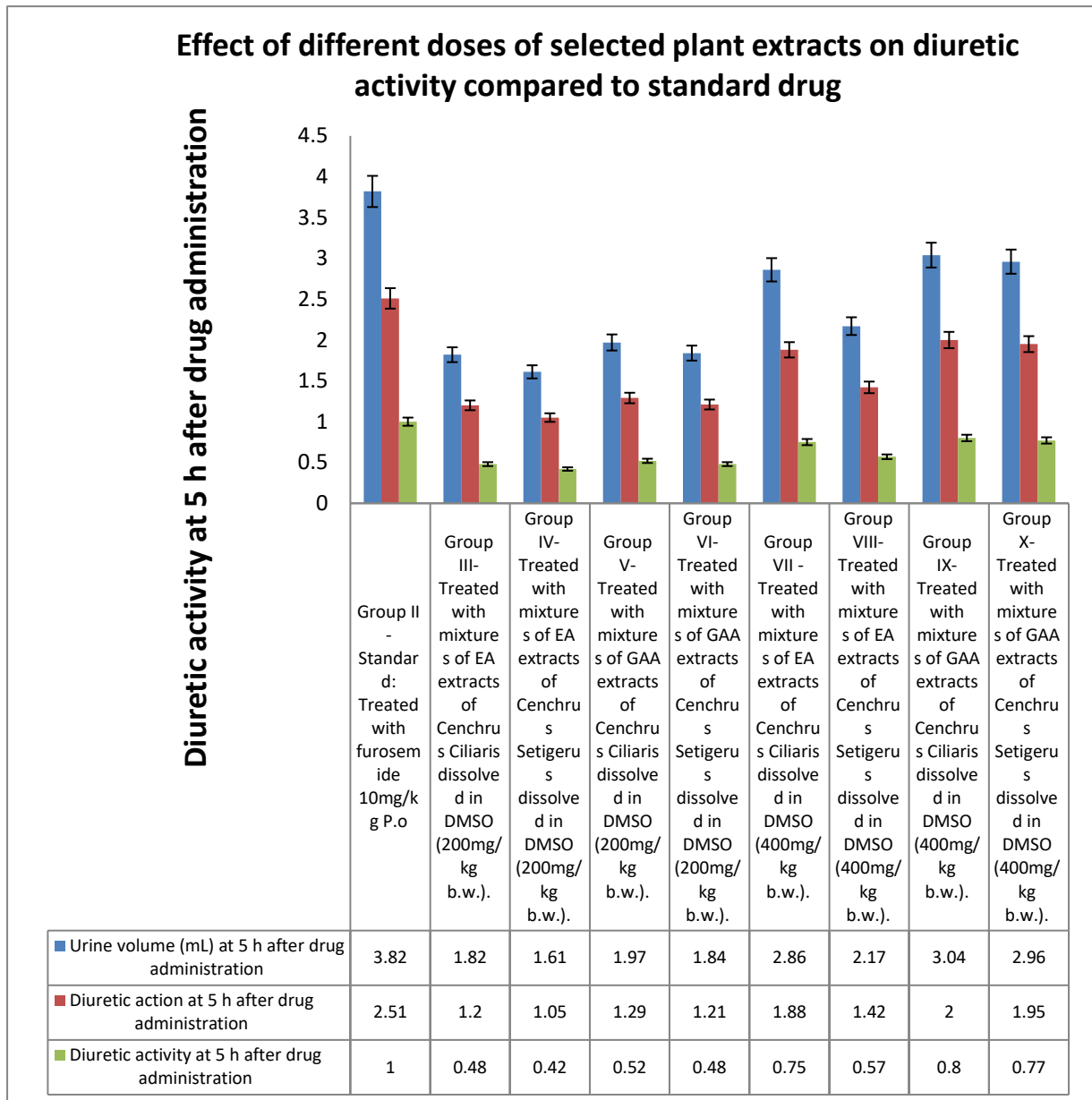


Figure 3: Histogram showing effect of standard & different extracts of selected plants on urine volume at 5 h interval and its diuretic action and activity.

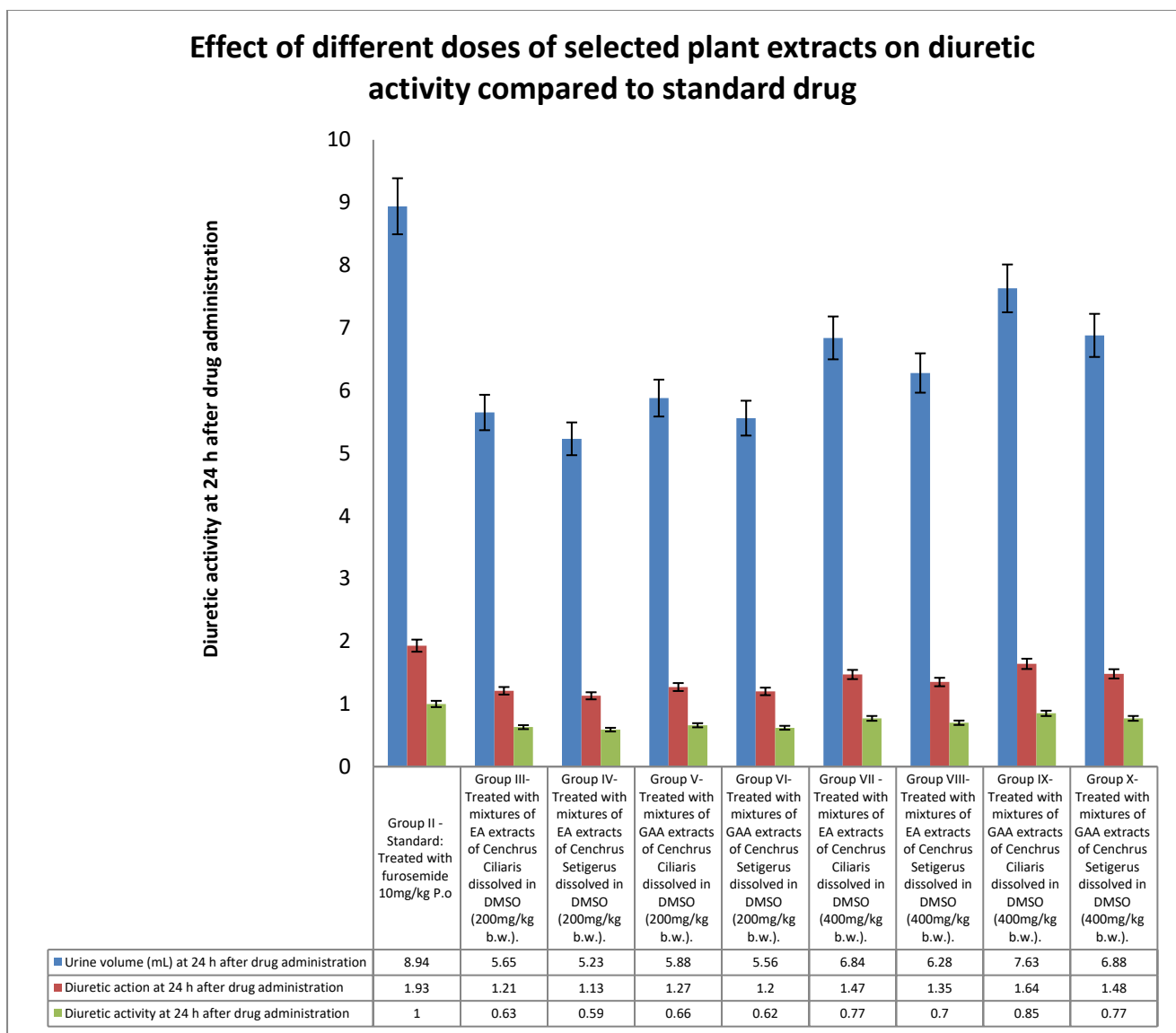


Figure 4: Histogram showing effect of standard & & different extracts of selected plants on urine volume at 24 h interval and its diuretic action and activity.

Table 7: Comparison of saluretic, natriuretic, electrolytes indexes, Lipschitz value and diuretic indexes of various extracts of *Cenchrus Ciliaris* & *Cenchrus Setigerus* to that of control.

Groups		At 24 h after drug administration						Lipschitz value
		Saluretic Index [Na ⁺⁺ Cl ⁻]	Na ⁺ index	K ⁺ Index	Cl ⁻ index	Natriuretic Index [Na ⁺ /K ⁺]	Diuretic Index	
Group I	Control: Treated with vehicle-0.5% acacia orally (2 ml/kg b.w.).	1.00	1.00	1.00	1.00	1.92	1.00	-
Group II	Standard: Treated with furosemide 10mg/kg p.o	1.80	1.77	2.07	1.82	1.63	1.93	1.00
Group III	Treated with mixtures of EA extracts of <i>Cenchrus Ciliaris</i> dissolved in DMSO (200mg/kg b.w.).	1.19	1.20	1.10	1.25	2.07	1.21	0.63
Group IV	Treated with mixtures of EA extracts of <i>Cenchrus Setigerus</i> dissolved in DMSO (200mg/kg b.w.).	1.1	1.10	1.05	1.05	2.01	1.13	0.59
Group V	Treated with mixtures of GAA extracts of <i>Cenchrus Ciliaris</i> dissolved in DMSO (200mg/kg b.w.).	1.26	1.26	1.45	1.24	1.43	1.27	0.66
Group VI	Treated with mixtures of GAA extracts of <i>Cenchrus Setigerus</i> dissolved in DMSO	1.20	1.25	1.28	1.14	1.87	1.20	0.62

Group VII	(200mg/kg b.w.). Treated with mixtures of EA extracts of <i>Cenchrus Ciliaris</i> dissolved in DMSO	1.51	1.58	1.76	1.41	1.72	1.47	0.77
Group VIII	(400mg/kg b.w.). Treated with mixtures of EA extracts of <i>Cenchrus Setigerus</i> dissolved in DMSO	1.38	1.40	1.70	1.37	1.57	1.35	0.70
Group IX	(400mg/kg b.w.). Treated with mixtures of GAA extracts of <i>Cenchrus Ciliaris</i> dissolved in DMSO	1.63	1.64	1.83	1.62	1.71	1.64	0.85
Group X	(400mg/kg b.w.). Treated with mixtures of GAA extracts of <i>Cenchrus Setigerus</i> dissolved in DMSO	1.48	1.55	1.75	1.40	1.69	1.48	0.77

Values are expressed in terms of Mean ± SD; Values given are the mean ± SEM of six observations. All values are compared with control group and considered significant at *p < 0.05.

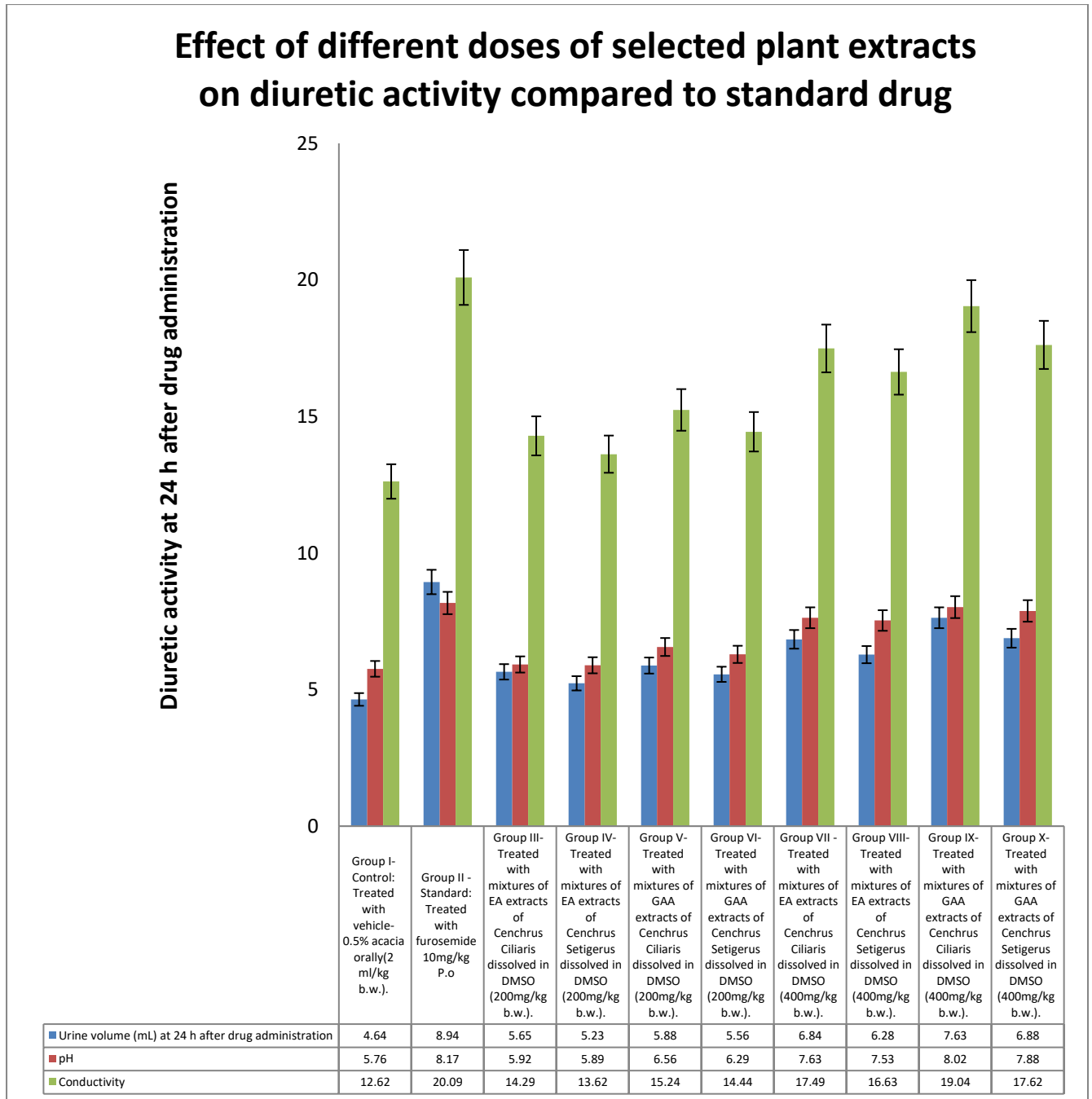


Figure 5: Histogram showing effect of standard & & different extracts of selected plants on urine volume at 24 h interval and its pH and conductivity.

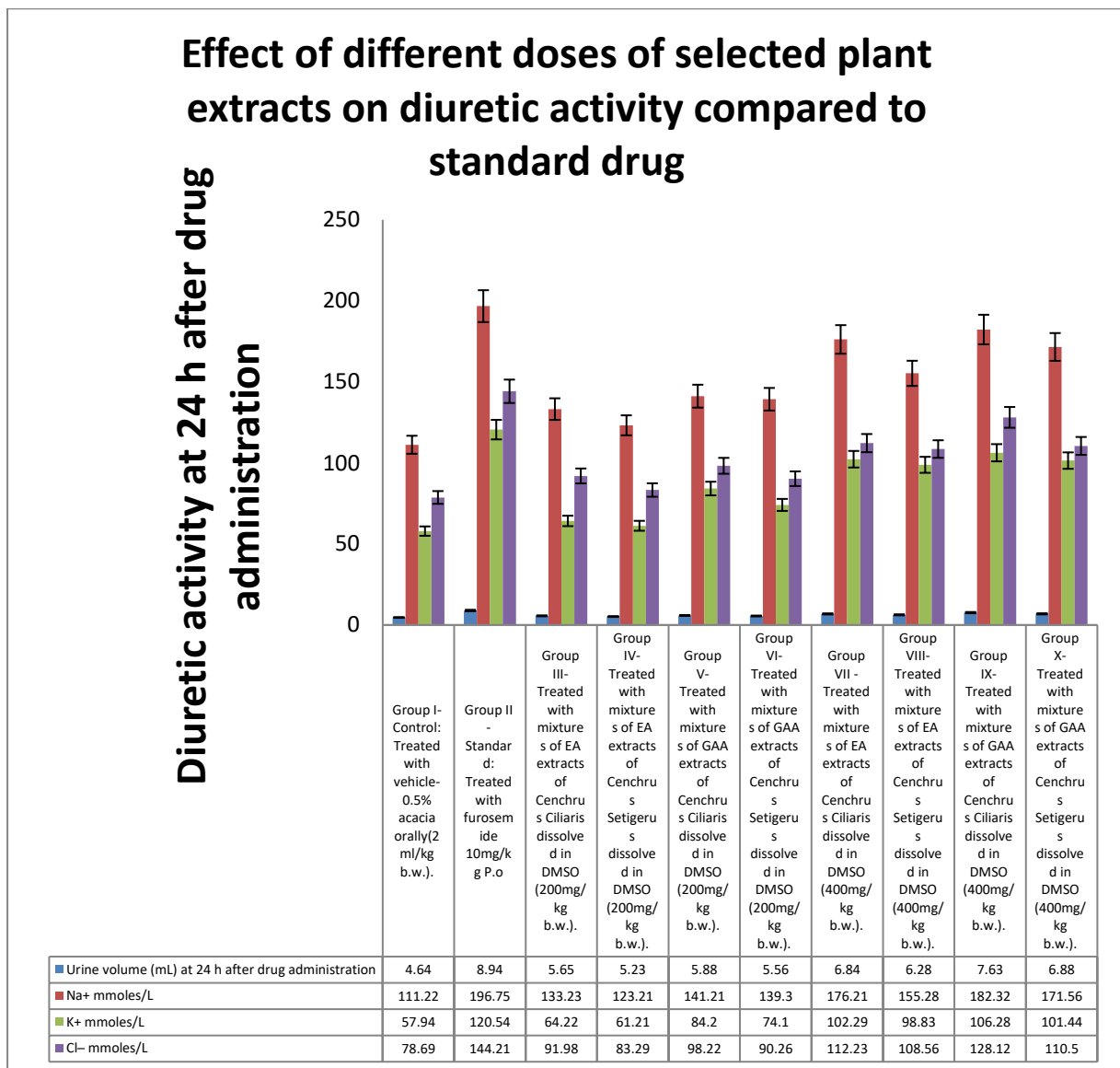


Figure 6: Histogram showing effect of standard & different extracts of selected plants on urine volume at 24 h interval and various concentrations of electrolytes.

Discussion

ACUTE TOXICITY STUDIES

Since no mortality was observed in this dose range of 1000to 2000mg/Kg b.w., hence this was considered as safer dose and no further toxicity study done at higher doses. Acute toxicity studies carried out prior to pharmacological evaluation studies of all the extracts using OECD Test Guideline [8] to find out the safe and toxic dose. All the extracts of both the plants were found to be safe up to 2000 mg/kg body weight. Finally, 200 mg /kg b.w. and 400 mg /kg b.w. were selected for this study and acute toxicity study revealed the nontoxic nature for each selected plant extracts, which was tabulated in **table 3**.

ANALGESIC ACTIVITY

Analgesic activity [4, 9-13] in acetic acid induced writhing up to 30 min and % inhibition in mice are tabulated in **tables 1 & 2 and figure 1**. Histogram of the analgesic activity of

standard & various extracts of selected plants are shown in figure 1. The analgesic activity of the various extracts of selected plants has been evaluated by using acetic acid induced writhing method.

Group I was Control group (DMSO), in Group II, Diclofenac sodium standard drug was administered in a dose of 10 mg/kg body weight and in Group III-X Test: various extracts of selected plants was administered at different dose. The results of the evaluation have been viewed by taking Diclofenac sodium as the standard drug.

Total no. of writhing in mice up to 30 min (Mean \pm SEM) of group I (control vehicle) was 95.23 ± 4.66 and group II of Diclofenac sodium standard drug was 25.08 ± 2.06 and % inhibition compared to control group was 73.66%, which was reduced significantly with $p < 0.01$ level. In the same way % inhibition of test compounds were calculated and it was seen that all the compounds shown a significant effect in a level of $p < 0.05$ except some extracts with lower doses.

From all the test compounds, GAA extracts of both the plants with a dose size of 400 mg/kg body weight were found to be potent in analgesic activity which is comparable to standard, but EA extracts having lower activity, compared to others. Drugs that reduce the total number of writhing upto in 30 minutes can be considered as an effective analgesic. Among all the treated test groups it has been seen that the mixtures with GAA extracts of *Cenchrus Ciliaris* shows highest No. of writhing in mice up to 30 min (25.75 ± 3.14) with 72.96% of inhibition.

ANTI INFLAMMATORY ACTIVITY

In anti inflammatory activity in induced rat paw edema method; change in paw edema (Mean \pm SEM) and % inhibition in paw thickness at various time intervals are shown in **tables 2 and 3**. Histogram showing anti inflammatory activity of standard & various extracts of both the selected plants with change in paw edema volume in mm (Mean \pm SEM) and % inhibition in paw thickness at various time intervals are shown in **figure 2**.

The results of the evaluation have been observed by taking diclofenac sodium as the standard drug. Among all the test groups, group IX: treated with mixtures of GAA extracts of *Cenchrus Ciliaris* dissolved in DMSO with a administered dose of 400 mg/kg body weight was shown potent anti inflammatory activity and which was compared with standard drug with a $P < 0.01$ significant level.

Some earlier reported researchers also studied on carrageenan-induced inflammation are a useful experimental model of acute inflammation for detecting orally active anti-inflammatory agents. Edema formation in the rat paw is a biphasic response. The first phase is mediated through the release of histamine, serotonin, and kinins, whereas the second phase is due to the release of prostaglandin and slow reacting substances. The formalin model was developed >30 years ago to assess pain and evaluate analgesic drugs in laboratory animals. In this test, a dilute (0.5–5%) formalin solution (in which formaldehyde is the active ingredient) is injected into the paw of a rodent, and pain-related behaviors are assessed over two temporally distinct phases. In the present study the analgesic activity of ethanolic and aqueous extracts of *Abutilon Indicum* was evaluated by formalin induced paw licking and the tail flick model. The persistent pain model of formalin induced hind paw licking was used in the study. The first phase of pain is attributed to the direct activation of nociceptors and primary afferent fibers by formalin, causing the release of bradykinin and trachykinins. This phase is inhibited by opioid analgesics. The second phase is due to an inflammatory reaction caused by tissue injury leading to the release of histamine, serotonin, prostaglandin and excitatory amino acids [6, 13-20].

Injection of formalin into the hind paw induced a progressive edema reaching its maximum at 5 hours. In case of Group I animals paw thickness found at $t = 0$ was 0.75 ± 0.04 mm. Group I animals showed an increase in paw thickness of 1.78 ± 0.06 mm ($t = 0.5$ hours), 2.81 ± 0.12

mm (t = 1 hour), 3.89 ± 0.28 mm (t = 2 hours), 4.85 ± 0.38 mm (t = 3 hours), 5.02 ± 0.22 mm (t = 4 hours) and 5.10 ± 0.24 mm (t = 5 hours). Whereas in Group II, animals showed an increase in paw thickness of 0.74 ± 0.02 mm (t = 0.0 hours), 0.76 ± 0.04 mm (t = 0.5 hour), 0.79 ± 0.04 mm (t = 1 hours), 0.81 ± 0.06 mm (t = 2 hours), 0.82 ± 0.08 mm (t = 3 & 4 hours) and 0.83 ± 0.08 mm (t = 5 hours). Moreover, group IX (Treated with mixtures of GAA extracts of *Cenchrus Ciliaris* dissolved in DMSO.) is exhibited fairly better activity compared to other extracts. In the same way GAA extracts with higher doses showed significant anti-inflammatory activity. The group of II, IX & X was treated by Diclofenac sodium (10 mg/kg b.w.) and GAA extracts of *Cenchrus Ciliaris* and *Cenchrus Setigerus* (400 mg/kg body weight) showed maximum inhibition of edema after 5hrs, which was 83.72, 81.37 & 67.45% respectively, as shown in **table 3 and figure 2** compared with other treated groups.

The methanol extract and derived fractions were evaluated for anti-inflammatory activity by using in vitro heat induced albumin denaturation assay and various in vivo assays; carrageenan-induced hind paw edema method, Freund's complete adjuvant induced arthritis, histamine induced paw edema and xylene induced ear edema in Sprague Dawley rat. The extracts/fractions were also evaluated for analgesic effects by using hot plate analgesic test and acetic acid induced writhing test in rat. The results of this study suggested that the presence of polyphenols, sterols, terpenoids and other constituents might contributed towards the anti-inflammatory and analgesic activities of the crude methanol extract of *A. nitida* bark and its derived fractions. This study endorsed the folklore use of *A. nitida* bark for inflammation related disorders [13-20].

DIURETIC ACTIVITY

Effects on urine volume, diuretic action and activity

Effect of various extracts of *Cenchrus Ciliaris* & *Cenchrus Setigerus* on urine volume in Wistar albino rats at 5 h and 24 h interval and its diuretic action and activity were tabulated in **figures 3 & 4**.

It has been seen that the urine volume at 5 h after drug administration of control group and standard reference group was 1.52 ± 0.14 and 3.82 ± 0.24 respectively, whereas at 24 h after drug administration of control group and standard reference group was 4.64 ± 0.56 and 8.94 ± 1.24 respectively. At the lower doses, it has been seen that the diuretic activity was not so prominent and not comparable to the standard reference. But at higher doses of test samples it has been seen that the group IX and X showed significant level of increase of urine volume and diuretic activity compared with standard sample.

Effects on urine pH and conductivity

Urinary pH and conductivity were measured at 24 h of drug administration and it was represented in **Fig. 5**. The urinary pH of control and standard group rats was 5.76 ± 0.12 and 8.17 ± 0.29 respectively. The urine pH after administration of various extracts of both the plants, at doses of 200 and 400 mg/kg body weight at 24 h urine sample were collected. Standard group with furosemide increased the urine pH 8.17 ± 0.35 from acidic pH to the more alkaline urine. In the same way it has been seen that GAA extracts with higher doses have a significant increase of pH compared to the standard sample.

Conductivity of group I animals was reported 12.60 ± 0.87 at 24 h collected urine sample and conductivity of urine of standard group (furosemide treated) was significantly increased to 20.19 ± 2.29 ($P < 0.05$), where as GAA extracts of both the selected plants showed a significant level of higher conductivity, compared to the standard reference.

Effects on concentration of electrolyte excretion

The diuretic responses with its electrolyte excretion potency of various extracts of both the selected plants were highly restrained in comparison to normal control rats except lowest dose (200 mg/kg b.w.). Presences of electrolytes were measured at 24 h of drug administration and it was represented in **Fig. 6**. The concentration of electrolytes (Na⁺, K⁺ and Cl⁻) of control and standard group rats was 111.22±9.66 and 196.75 ±8.28; 57.94±4.75 and 120.54 ±7.76; 78.69±5.34 and 144.21±8.55 respectively. The extract at doses of 400 mg/kg showed a significant increase in Na⁺, K⁺ and Cl⁻ excretion compared to the groups of lower doses. The results of urinary electrolyte excretion after treatment of GAA extracts were comparable to the furosemide group.

Effects on natriuretic, saliuretic and Diuretic Index

Table 4 depicts the results for natriuretic, saliuretic activity, electrolytes indexes, diuretic Index and Lipschitz value. The furosemide (10 mg/ kg) and GAA extracts of both the selected plants at higher doses (400 mg/kg) showed potent natriuretic and saliuretic activity as compared to normal control.

CONCLUSION

All the extracts of both the plants were found to be safe up to 2000 mg/kg body weight. Finally, 200 mg /kg b.w. and 400 mg /kg b.w. were selected for this study and acute toxicity study revealed the nontoxic nature for each selected plant extracts.

From all the test compounds, GAA extracts of both the plants with a dose size of 400 mg/kg body weight were found to be potent in analgesic activity which is comparable to standard, but EA extracts having lower activity, compared to others. Drugs that reduce the total number of writhing upto in 30 minutes can be considered as an effective analgesic.

Moreover, group IX (Treated with mixtures of GAA extracts of *Cenchrus Ciliaris* dissolved in DMSO) is exhibited fairly better activity compared to other extracts. In the same way GAA extracts with higher doses showed significant anti-inflammatory activity. The group of II, IX & X was treated by Diclofenac sodium (10 mg/kg b.w.) and GAA extracts of *Cenchrus Ciliaris* and *Cenchrus Setigerus* (400 mg/kg body weight) showed maximum inhibition of edema after 5hrs, which was 83.72, 81.37 & 67.45% respectively.

The furosemide (10 mg/ kg) and GAA extracts of both the selected plants at higher doses (400 mg/kg) showed potent natriuretic and saliuretic activity as compared to normal control.

REFERENCES:

1. Arora S and Kumar G, Screening of bioactive compounds from leaf of *Cenchrus ciliaris* L. from Thar region of Rajasthan, India. Int J Pharm Sci & Res., 2018, 9(5): 1878-85.doi: 10.13040/IJPSR.0975-8232. 9(5).1878-85.
2. Ambreen Aleem and Khalid Hussain Janbaz, Ethnopharmacological evaluation of *Cenchrus ciliaris* for multiple gastrointestinal disorders, Bangladesh J Pharmacol, 2017, 12: 125-132.
3. Kalamani. A, Ameena Premnath and G. Vijayakumar, Variability among germplasm collections for high biomass traits in *Cenchrus sp.*, Electronic Journal of Plant Breeding, 2011, 2(2):270-274.
4. Wani TA, Kumar D, Prasad R, Verma PK, Sardar KK, Tandan SK, Kumar D. Analgesic activity of the ethanolic extract of *Shorearobusta* resin in experimental animals. Indian J Pharmacol 2012;44:493-499.
5. Girish Gulab Meshram, Anil Kumar, Waseem Rizvi, C.D. Tripathi, R.A. Khan, Evaluation of the anti-inflammatory activity of the aqueous and ethanolic extracts of the

- leaves of *Albizzia lebbek* in rats, *Journal of Traditional and Complementary Medicine* 6 (2016) 172-175.
6. Fahad I. Al-Saikhan & Mohd N. Ansari, Evaluation of the diuretic and urinary electrolyte effects of methanolic extract of *Peganum harmala* L. in Wistar albino rats, *Saudi Journal of Biological Sciences* (2016) 23, 749–753
 7. Sreelakshmi R, Shan P Mohammed, Jyoti Harindran, Sriganesan P, Evaluation of diuretic activity of *mussaenda frondosa*, *Asian J Pharm Clin Res, Vol 8, Issue 2, 2015, 117-118*.
 8. WWW.OECDguidelines.org/2001;3rd may 2006.
 9. Pushpendra Kumar Patel, Jyoti Sahu, Saket Singh Chandel, A detailed review on nociceptive models for the screening of analgesic activity in experimental animals, *International Journal of Neurologic Physical Therapy* 2016; 2(6): 44-50.
 10. Amberkar Mohanbabu Vittalrao, Tara Shanbhag, Meena Kumari K., K. L. Bairy and Smita Shenoy, Evaluation of anti-inflammatory and analgesic activities of alcoholic extract of *Kaempferia galanga* in rats, *Indian J Physiol Pharmacol* 2011; 55 (1) : 13–24.
 11. Muhammad Kifayatullah, Haroon Rahim, Naeem Ullah Jan, Kamran Ahmad Chishti, Irfan Ullah & Sudair Abbas, *In vivo* Analgesic, Antipyretic and Anti-inflammatory Activities of Ethanol Extract of *Pericampylus glaucus* in Experimental Animals, *Sains Malaysiana*, 2019, 48(3), 629–635.
 12. Beena Goyal, Shashi Alok, Sanjay Kumar Jain, and Amita Verma, Evaluation of analgesic activity of ethanolic extract of *tamarindus indica* leaves on experimental animal model, *IJPSR, 2013; Vol. 4(5): 1994-1997*.
 13. Vogel HG. Analgesic, anti-inflammatory and antipyretic activity. Drug discovery and evaluation, pharmacological assays. 2nd ed. Springer-Verlog Berlin Heidelberg 2002: 669-714.
 14. Srinivasa U, Rao VJ, Krupanidhi AM, Shivananda K. Anti-inflammatory activity from the leaves of *Justicia beddomei* L. *Biomed* 2006 Oct; 1 (3): 243-246.
 15. Hassan MM, Khan SA, Shaikat AH, Hossain ME, Hoque MA, Ullah MH and Islam S. Analgesic and anti-inflammatory effects of ethanol extracted leaves of selected medicinal plants in animal model, *Vet World*, 2013, 6(2): 68-71. doi: 10.5455/vetworld.2013.68-71.
 16. Heenu Dhar, More Sonali R, Ghongane Balasaheb B, Anti-Inflammatory activity of Methanolic Fraction of Ethanolic Extract of *crocus Sativus* Stigmas in Rats and Mice, *JMSCR Volume 06 Issue 02 February 2018,573-583*.
 17. Margaret O. Sofidiya, Essien Imeh, Chidebelu Ezeani, Flora R. Aigbe, Abidemi J. Akindele, Antinociceptive and anti-inflammatory activities of ethanolic extract of *Alafia barteri*, *Rev Bras Farmacogn* 24(2014): 348-354.
 18. Mahendra K. Patel, Divyesh R. Mandavia, Tejas K. Patel, Manish J. Barvaliya and C. B. Tripathi, Evaluation of anti-inflammatory, analgesic, and antipyretic effects of ethanolic extract of *pedalium murex* linn. fruits, *Afr J Tradit Complement Altern Med.* (2013) 10(4):94-100.
 19. Vaibhav M. Darvekar, Vijay R. Patil and Amol B. Choudhari, Anti-inflammatory activity of *Murraya koenigii* Spreng on experimental animals, *J. Nat. Prod. Plant Resour.*, 2011, 1 (1): 65-69.
 20. Abdul Hafeez, Dr. Upendra Jain, Pinky Sajwan, Sirish Srivastava, Amit Thakur, Evaluation of Carrageenan induced anti-inflammatory activity of ethanolic extract of bark of *Ficus virens* Linn. in swiss albino mice, *The Journal of Phytopharmacology* 2013; 2(3): 39-43 .
 21. Hailu, W., Engidawork, E., 2014. Evaluation of the diuretic activity of the aqueous and 80% methanol extracts of *Ajuga remota* Benth (Lamiaceae) leaves in mice. *BMC Complement. Altern.Med.* 14, 135.

22. Kalpa Sree P. and Ravi Shankar K., Evaluation of diuretic, anti-urolithiatic activities of ethanolic flower extract of *Michelia champaca* (yellow variety), *Int. J. Biopharm. Res.*, 2014, 3 (05), 219-222.
23. Muhammad Asif, Qaiser Jabeen, Amin Malik Shah Abdul Majid And Muhammad Atif, Diuretic activity of aqueous extract of *nigella sativa* in albino rats, *Acta Poloniae Pharmaceutica ñ Drug Research*, Vol. 72 No. 1 pp. 129-135, 2015.
24. Sreelakshmi R, Shan P Mohammed, Jyoti Harindran, Sriganesan P, Evaluation of diuretic activity of *mussaenda frondosa*, *Asian J Pharm Clin Res*, Vol 8, Issue 2, 2015, 117-118.
25. R. V. Ramanarayana Reddy, K. Uma Maheshwara Rao, Yakaiah Vangoori, J. Mohana Sundharam, Evaluation of diuretic and anti-inflammatory property of ethanolic extract of *solanum surattense* in experimental animal models , *Int J Pharm Pharm Sci*, 2014, Vol 6, Issue 1, 387-389.