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EVALUATION OF DIURETIC AND ANTIULCER ACTIVITY OF METHANOLIC EXTRACT OF *LASIA SPINOSE* (RHIZOME) IN RATS

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Abstract In the present study, the plant drug (100g) was subjected to extraction by (maceration) using Hydroalcohol as solvent for about 24 hrs. The yields were found to be (13.5% w/w of crude drug) of Hydroalcoholic extract *Lasia spinosa rhizomes*. The results of phytochemical revels that the all polar and Methanolic and aqueous soluble compound was found to be present in *Lasia spinosa* rhizomes extract.Oral administration of ethanol produced severe ulceration. The Hydroalcoholic extract of *Lasia spinosa* significantly reduced the incidence and severity of ulceration in ethanol-induced ulcer model. The animal treated with Hydroalcoholic extract of *Lasia spinosa* at the dose of 200 and 300 mg/kg per oral, produced significant decrease in ulcer index compared with disease . The extract at the dose of 200 and 300 mg/kg body weight afforded 36.62 and 54.41 % ulcer protection respectively, whereas Omeprazole (20 mg/kg) exhibited 70.41 % ulcer protection.

Key words: *Lasia spinosa,* TPC , TFC, Antiulcer and Diuretic

Introduction

Worldwide trend towards the utilization of natural plant remedies has created an enormous need for information about the properties and uses of the medicinal plant. The Indian Traditional Medicine like Ayurvedic, Siddha and Unani are predominantly based on the use of plant materials. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness [1].

Herb used as a diuretic has been used in India for a long time and has been popularized world over by leading pharmaceuticals. Plant medicine was commonly used for traditional treatment of some renal diseases and a lot of plants have been reported to show significant diuretic activity. Many investigators have demonstrated that studies of herbal plant used in traditional medicine as diuretics have increased recent years and might be a useful tool in the treatment of hypertension. Hypertension is considered one of the main and dangerous complications of diabetes mellitus [2]

Lasia spinosa (L) Thwaites belongs to Araceae. Ethan medicinally, the entire plant used in folklore medicine for many medical uses such as cancer, constipation and rheumatism. The critical issue with herbal drug standardization is lack of adequate plant source recognition. [3]

Collection of plant materials

The rhizomes of *Lasia spinosa* were collected from local area of Bhopal in the period of March 2022, considering the seasonal conditions for obtaining maximum phytoconstituents. Authentication of collected plant materials were confirmed by HOD Department of Botany, Barkatullaha University, Bhopal, Madhya Pradesh, India.

Extraction (By Maceration Method) [4]

Maceration

Collected plant drugs namely *Lasia spinosa* (rhizomes) were cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned and dried plant drugs were converted into moderately coarse powder in hand grinder. Powdered plant drugs were weighed (50 gm) and packed in (1 liter) air tight glass Bottle. The plant drugs were subjected to extraction by Metahnol: water (20:80) as solvent for about 24 hrs. The liquid extracts were collected in a tarred conical flask. The solvent

removed from the extract by evaporation method using hot plate. The extracts obtained with each solvent were weighed to a constant weight and percentage w/w basis was calculated.

Phytochemical Analysis

Preliminary Phytochemical Screening

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the Hydroalcoholic extract of rhizomes of *Lasia spinosa*, were subjected to the phytochemical tests as per standard methods. Phytochemical screening was revealed for the presence of alkaloids, glycosides, carbohydrates, tannins, resins, flavonoids, steroids, proteins and amino acids [5].

Estimation of total phenolic content

Estimation of total phenolic content Total phenolic content of all the extracts was evaluated with Folin-Ciocalteu method. Samples containing polyphenols are reduced by the Folin-Ciocalteu reagent there by producing blue colored complex. The phenolic concentration of extracts was evaluated from a gallic acid calibration curve. To prepare a calibration curve, aliquots of 5, 10, 15, 20 and 25µg/mL methanolic gallic acid solutions were mixed with 2.5 mL Folin– Ciocalteu reagent (diluted ten-fold) and 2.5 mL (75 g/L) sodium carbonate. After incubation at 25°C for 30 min, the quantative phenolic estimation was performed at 765 nm against reagent blank by UV Spectrophotometer 1650 Shimadzu, Japan. The calibration curve was constructed by putting the value of absorbance vs. concentration. A similar procedure was adopted for the extracts as above described in the preparation of calibration curve. All determinations were performed in triplicate. Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per g of extract [6].

5.5.2 Estimation of total flavonoids content

The aluminum chloride colorimetric method was modified from the procedure reported by Marjanovic A et al.,2021 [7]. Quercetin was used to make the calibration curve. Ten milligrams of quercetin was dissolved in 80% ethanol and then diluted to 10 to 50 µg/mL. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of

95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a Shimadzu spectrophotometer. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5 mL of Hydroalcoholic extracts and Flavonoid standard solutions (100 ppm) were reacted with aluminum chloride for determination of Flavonoid content as described.

Material and methods

Pharmacology

Experimental animals

Healthy adult Wistar albino rats of either sex were selected randomly for the study. The rats were obtained from College of Veterinary Sciences and Animal Husbandry, Mhow, Indore. Rats of 12–16 weeks, weighing 160–200 g, were used for the experiment. Each rat was housed in a plastic box cage under standard conditions at 19–25°C and was kept under 12/12 h light/dark cycle. The rats were allowed free access to standard pellet feed and water ad libitum. The study was carried out according to the CPCSEA and Organization of Economic Co-operation and Development (OECD) guidelines. Approval from Institutional animal ethical committee (IAEC) was also obtained.

ANTIULCER ACTIVITY

Alcohol Induced Ulcer [8]

Over-nights fasted rats were divided into five groups of six rats each. All the groups of rats were given treatments as follows:

Group	Drug	Dose	No of animals
Group I (Control)	Normal saline	2ml/kg	6
Group II	HELS	100mg/kg/p.o	6
Group III	HELS	200mg/kg/ p.o	6
Group IV	HELS	300mg/kg/p.o	6
Group V	Omeprazole (STD)	20 mg/kg	6

After fourth day, ulcers were induced by administering 1 ml absolute ethanol (99%) to each rat. All administrations were by per oral route. After 1 hr of absolute ethanol administration, the animals were sacrificed by cervical dislocation with anesthesia. Stomachs were removed, cut along the greater curvature, and then washed with ice-cold saline, scored for macroscopic gross mucosal lesions.

The area of hemorrhagic ulcer in the glandular part of stomach was measured by using transparent graph sheet and after that, the stomach were fixed in 10% normal formalin buffer solution for further histopathological study.

Ulcer inhibition

(UI) was calculated using the formula below.

Ulcer index = 10/X

Where, X is Total mucosal area/ Total ulcerated area.

The percentage protection was calculated by the following formula:

Percentage protection = (Ulcer index of control group - Ulcer index of treated group) / Ulcer index of control group × 100

Diuretic activity [9]

Grouping and dosing of animals

Group	Drug	Dose	No of animals
Group I (Control)	Normal saline	2ml/kg	6
Group II	furosemide (STD)	10 mg/kg	6
Group III	HELS	100mg/kg/p.o	6
Group IV	HELS	200mg/kg/ p.o	6
Group V	HELS	300mg/kg/p.o	6

Animals were randomly assigned into 5 groups each consisting of 6 male mice for diuretic test.

Diuretic activity

Diuretic activity was determined following the methods used by (Lahlou et al) with slight modification. Each male mouse was placed in an individual metabolic cage 24 h prior to commencement of the experiment for adaptation and then fasted overnight with free access to water.

The animals were pretreated with physiological saline (0.9% NaCl) at an oral dose of 0.15 ml/10 g body weight, to impose a uniform water and salt load . Each group was then treated as described in grouping and dosing section orally by gavage.

Immediately after administration, the mice were individually placed in a metabolic cage. Urine was then collected and measured 1, 2, 3, 4, and 5 h after dosing. The urine was then filtered and finally stored at −20°C for electrolyte analyses.

The following parameters were calculated in order to compare the effects of the extracts with vehicle and standard on urine excretion. The urinary excretion independent of the animal weight was calculated as total urinary output divided by total liquid administered.

The ratio of urinary excretion in test group to urinary excretion in the control group was used as a measure of diuretic action of a given dose of a drug. A parameter known as diuretic activity was also calculated. To obtain diuretic activity, the diuretic action of the extract was compared to that of the standard drug in the test group.

$$\text{Urinary Excretion} = \frac{\text{Total urinary output}}{\text{Total liquid administered}} \text{x100}$$

$$\text{Diuretic Action} \frac{\text{Urinary excretion of treatment groups}}{\text{Urinary excretion of control group}}$$

$$\text{Diuretic Activity} = \frac{\text{Diuretic action of test drug}}{\text{Diuretic action of standard drug}}$$

Analytical procedures

Sodium, potassium and chloride levels of urine and the plant extract were analyzed. Sodium and potassium concentrations were determined by making use of flame photometry

A calibration was performed automatically in both cases prior to analysis with different levels of standards. Ratios of electrolytes; Na+/K+ and Cl−/K++Na+ were calculated to evaluate the saluretic activity of the different extracts. In addition, pH was directly determined on fresh urine samples using a pH meter. Moreover, the salt content of the extract was also determined to rule out its contribution on urinary electrolyte concentration.

Statistical analysis

Data are expressed as mean ± S.E.M (standard error of mean). Statistical analysis of the data were performed with one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Significant differences were set at P values lower than 0.05.

Results

The plant drug (100g) was subjected to extraction by (maceration) using Hydroalcohol as solvent for about 24 hrs. The liquid extracts were collected in a tarred conical flask. The solvent removed from the extract by evaporation method using hot plate. The extracts obtained with each solvent were weighed to a constant weight and percentage w/w basis was calculated. The weighed extract of plant drug was stored in desiccators for further use. The yields were found to be (13.5% w/w of crude drug) of Hydroalcoholic extract *Lasia spinosa rhizomes*. Obtained results were recorded in table

Results of Phytochemical test showed the presence of Carbohydrates, Flavonoids, Proteins & Amino acids, Phenols, Diterpenes and Saponins, Alkaloid was found to absent in extract *Lasia spinosa* rhizomes. The results of phytochemical revels that the all polar and Methanolic and aqueous soluble compound was found to be present in *Lasia spinosa* rhizomes extract.

Estimation of total phenolic content

Total phenolic content was estimated by gallic acid and expressed as milligrams of gallic acid equivalent (GAE). The extract contained a considerable amount of phenolic contents of GAE/g of extract. The results were presented in Fig 1.

Table: 1 Absorbance of standard and sample at 760nm

Standard	Concentration (µg/ml)	Mean Absorbance
Gallic acid	5	0.127
	10	0.241
	15	0.338
	20	0.421
	25	0.578

n= 3, values are given in SEM

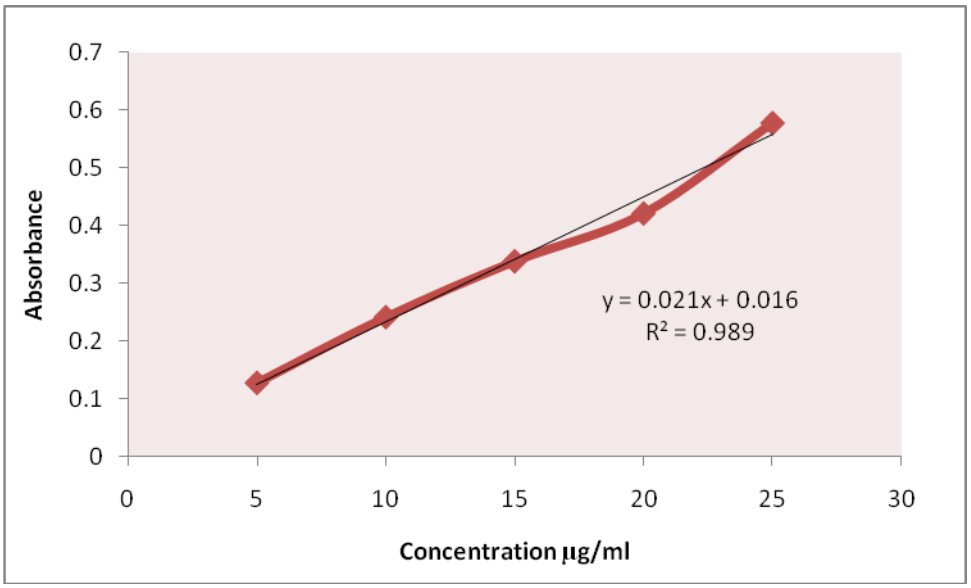

Figure: 1 Standard (Gallic acid) Calibration curves

Table: 2 Total Phenolic Content of Hydroalcoholic extract of *Lasia spinosa*

Sample	Total phenolic content GAE mg/100mg
Hydroalcoholic extract 100µg/ml	0.314

n=3, values are given in SEM

6.3 Estimation of total flavonoids content

Flavonoid content was calculated from the regression equation of the standard plot (y=0.020x, R^2 =0.992) and is expressed as quercetin equivalents (QE) (fig.). Total Flavonoid content was 0.165mg/100mg quercetin equivalent in HELS. Flavonoids are the most common and widely distributed group of plant's phenolic compounds.

Table: 3 Absorbance of standard and Hydroalcoholic extract of *Lasia spinosa*

S. No	Concentration of Quercetin (µg/ml)	Mean absorbance
1	10	0.214
2	20	0.464
3	30	0.624
4	40	0.814
5	50	1.024

n=3, values are given in SEM

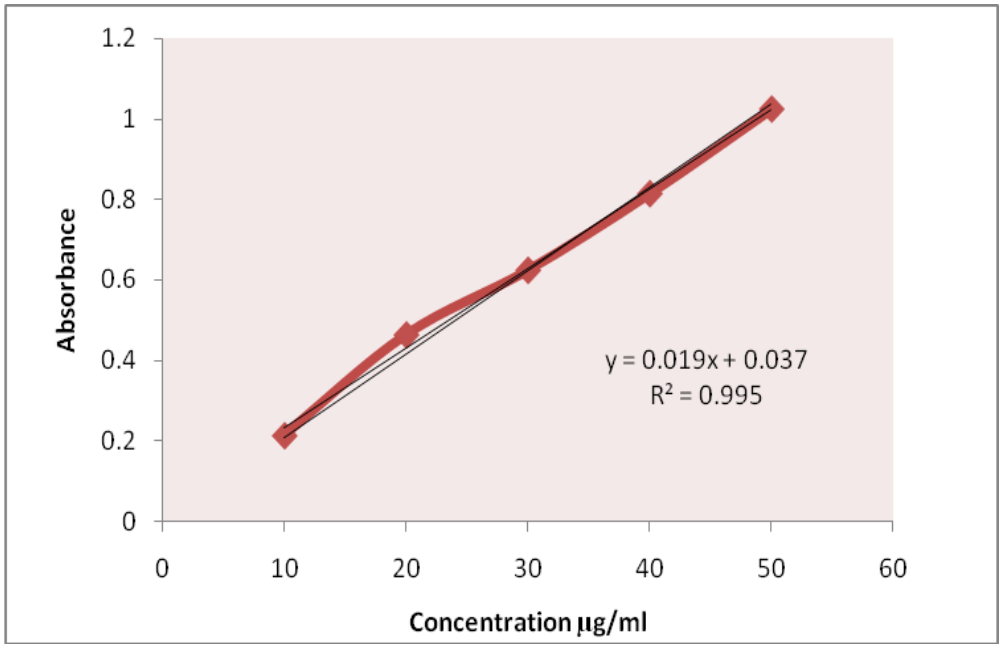

Figure: 2 Standard (Quercetin) Calibration curves

Table: 4 Total Flavonoid content of Hydroalcoholic extract *Lasia spinosa*

S. N.	Extracts 100µg/ml	Flavonoid content Quercetin equivalent mg/100mg
1	Hydroalcoholic extract (100µg/ml)	0.178

n=3, values are given in SEM

Antiulcer activity

Effect of Hydroalcoholic extract of *Lasia spinosa* on alcohol-induced gastric ulceration in rats .The Hydroalcoholic extract of *Lasia spinosa* was evaluated for its anti-ulcer activity against alcohol-induced ulceration in rats. The results are tabulated in Table 1.

Table:5 Effect of Hydroalcoholic extract of *Lasia spinosa* on alcohol-induced gastric ulceration in rats

Group	Drug/dose	Ulcerative Area Mean±SEM (mm^2)	Ulcer index	% Protection
Group I (Control)	Normal saline	107 ± 6.4	1.154±0.541	--
Group II	HELS(100mg/kg/p.o)	95.40 ± 3.6	0.874±0.241	25.31
Group III	HELS(200mg/kg/p.o)	76.21 ± 5.0	0.724±0.197**	36.62
Group IV	HELS(300mg/kg/p.o)	47.24 ± 4.1	0.574±0.152***	54.41
Group V	Omeprazole (STD) 20 mg/kg	32.44 ± 2.1	0.353±0.101***	70.41

(Mean ± SEM; N=6 in each group) **p<0.01, ***p<0.001

Oral administration of ethanol produced severe ulceration. The Hydroalcoholic extract of *Lasia spinosa* significantly reduced the incidence and severity of ulceration in ethanol-induced ulcer model. The animal treated with Hydroalcoholic extract of *Lasia spinosa* at the dose of 200 and 300 mg/kg per oral, produced significant decrease in ulcer index by 0.724 ± 0.197 (p<0.01) and 0.574 ± 0.152 compared with disease control (p<0.001) respectively. The extract at the dose of 200 and 300 mg/kg body weight afforded 36.62 and 54.41 % ulcer protection respectively, whereas Omeprazole (20 mg/kg) exhibited 70.41 % ulcer protection.

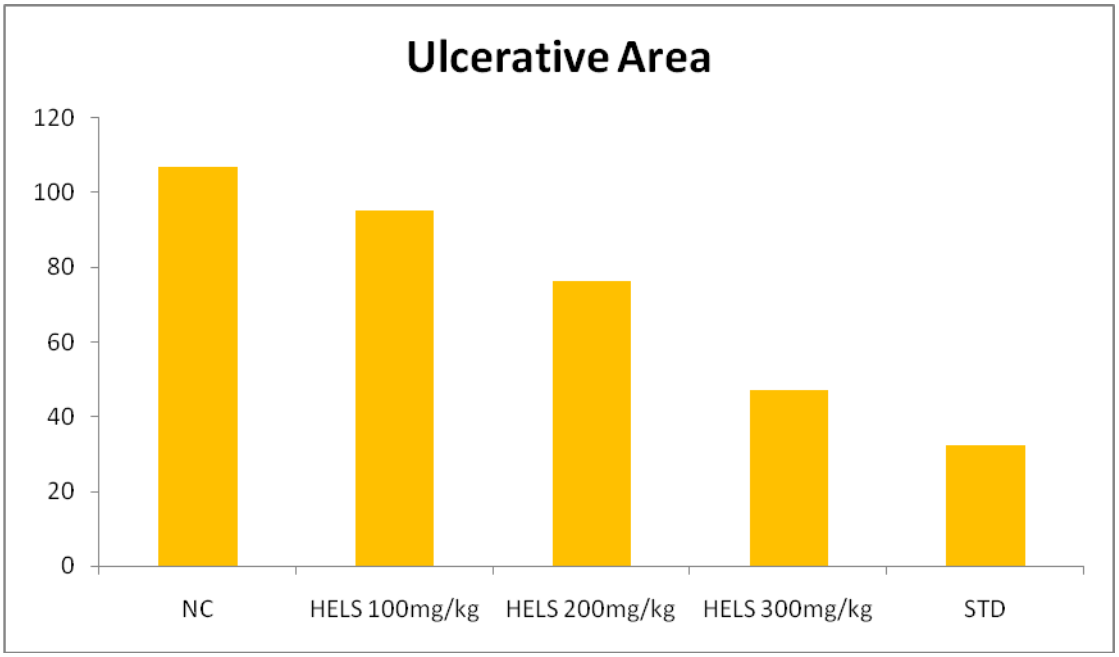

Figure: 3 Effect of Hydroalcoholic extract of *Lasia spinosa* on alcohol-induced gastric ulceration in rats

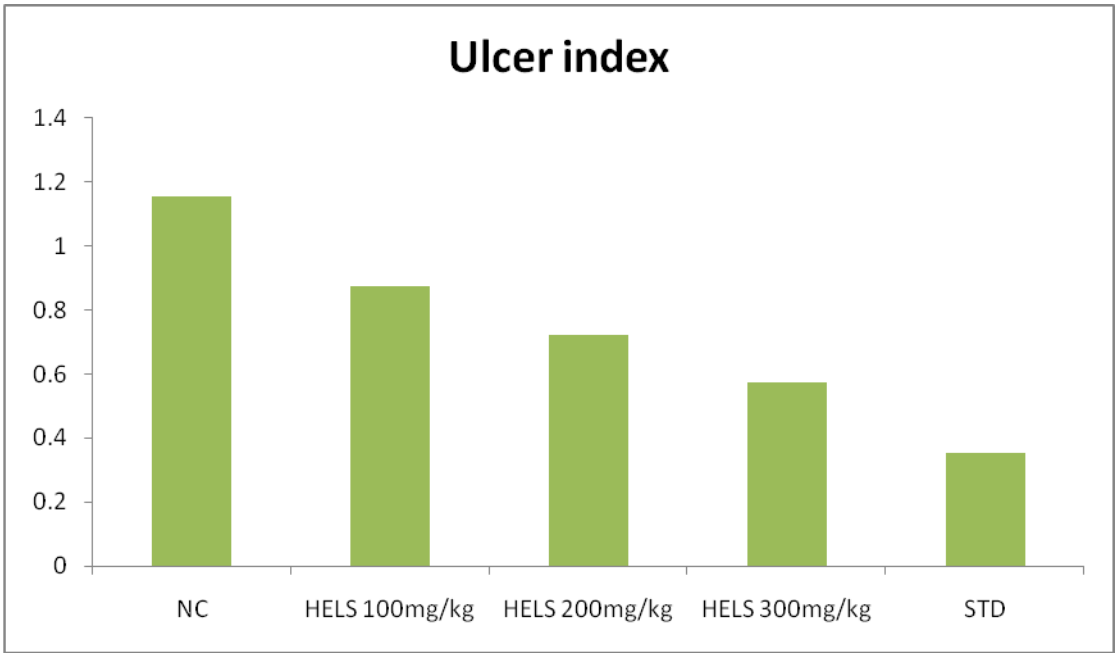

Figure: 4 Effect of Hydroalcoholic extract of *Lasia spinosa* on alcohol-induced gastric ulceration in rats

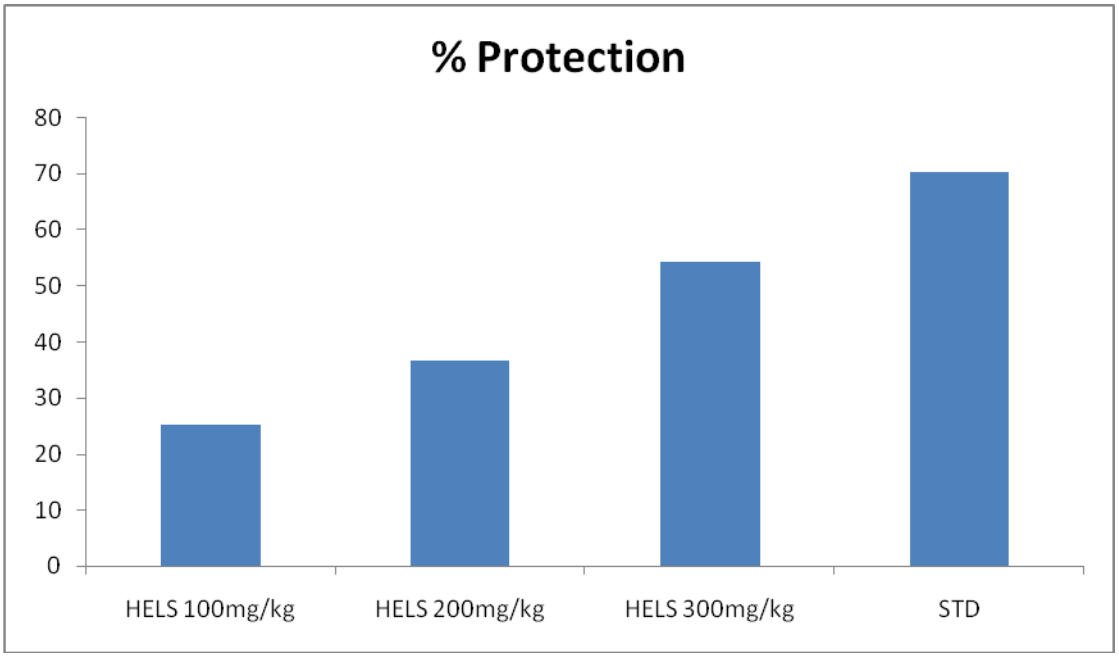

Figure: 5 Effect of Hydroalcoholic extract of Lasia spinosa on (%protection) alcohol-induced gastric ulceration in rats

Diuretic activity

Diuretic activity: effect on urine volume

The Hydroalcoholic extract of *Lasia spinosa* the plant produced diuresis, which appeared to be a function of dose (Table 1). The time course of action of diuresis is also depicted in Figure 2.

HELS at 100mg/kg did not produce any detectable difference in urine volume compared to Control group animals at all time points. HELS at 200mg/kg started to increase urine volume from the second hour, but changes reached statistical significance at the fourth (67%, $p < 0.05$) and fifth (92.1%, $p < 0.01$) hour.

By contrast, the increase in urine volume with the highest dose (HELS at 300mg/kg) was significant (81.1%, $p < 0.01$) starting from the very first hour and a maximum increase was noted at the fifth hour (95%, $p < 0.01$).

Furosemide treated mice exhibited a significantly greater urine volume from the first hour (91%, $p < 0.01$), which continued until the end of the fifth hour (94%, $p < 0.01$).

Furosemide displayed a significantly greater effect compared to HELS at 100mg/kg ($p < 0.001$) throughout the time points, but had no significant difference with that of HELS at 200mg/kg from the second hour onwards. Comparable effects were observed between Furosemide and HELS at 300mg/kg across all time points.

Table:6 Effect of Hydroalcoholic extract of *Lasia spinosa* on 5 h urine volume in mice

Group	Volume of urine (ml)					Diuretic action	Diuretic activity
	1 h	2 h	3 h	4 h	5 h		
Group I	0.63± 0.14	0.82± 0.12	0.98± 0.12	1.0± 0.11	1.04± 0.10	1.0	---------
Group II	1.19± 0.06	1.45± 0.08	1.63± 0.09	1.78± 0.08	2.04± 0.12	1.96	1.0
Group III	0.37± 0.03	0.68± 0.02	0.97± 0.16	1.12± 0.17	1.15± 0.16	1.10	0.57
Group IV	0.57± 0.09	1.08± 0.06	1.42± 0.11	1.67± 0.12	2.02± 0.12	1.92	0.98
Group V	1.14± 0.12	1.25± 0.13	1.53± 0.18	1.72± 0.16	2.04± 0.20	1.95	1.02

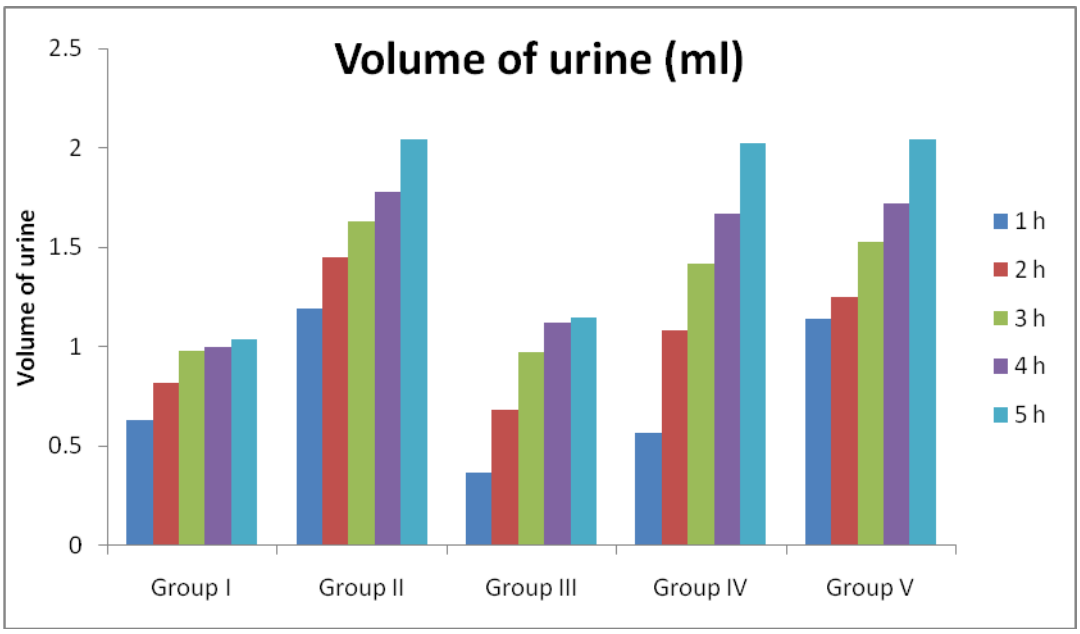

Figure: 6 Effect of Hydroalcoholic extract of *Lasia spinosa* on 5 h urine volume in mice

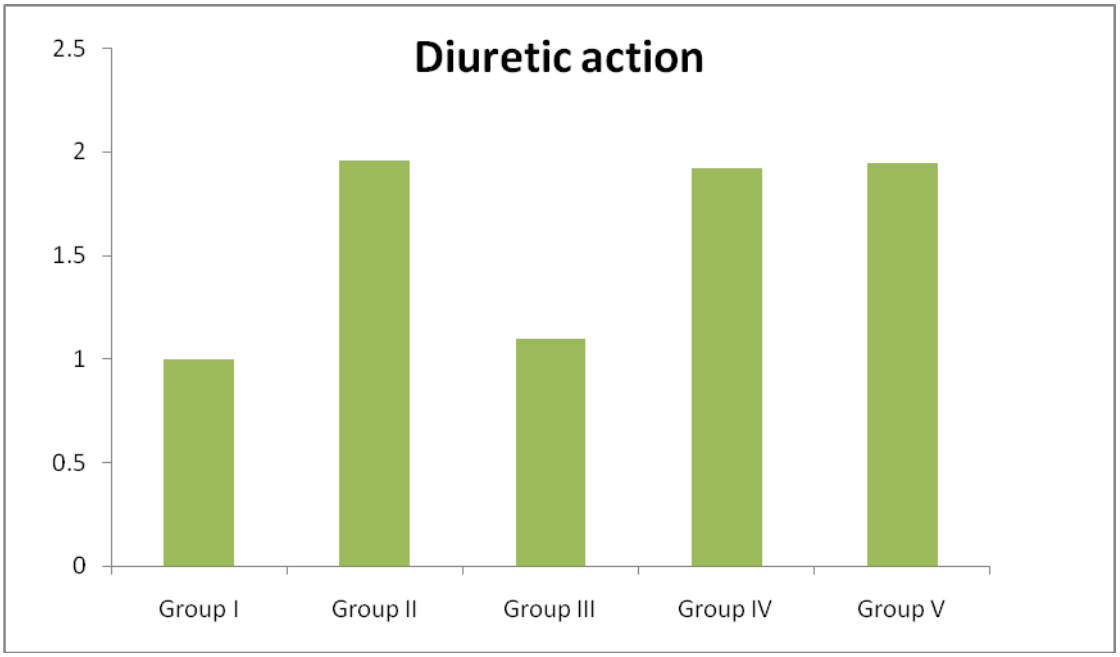

Figure: 7 Effect of Hydroalcoholic extract of *Lasia spinosa* on diuretic actions

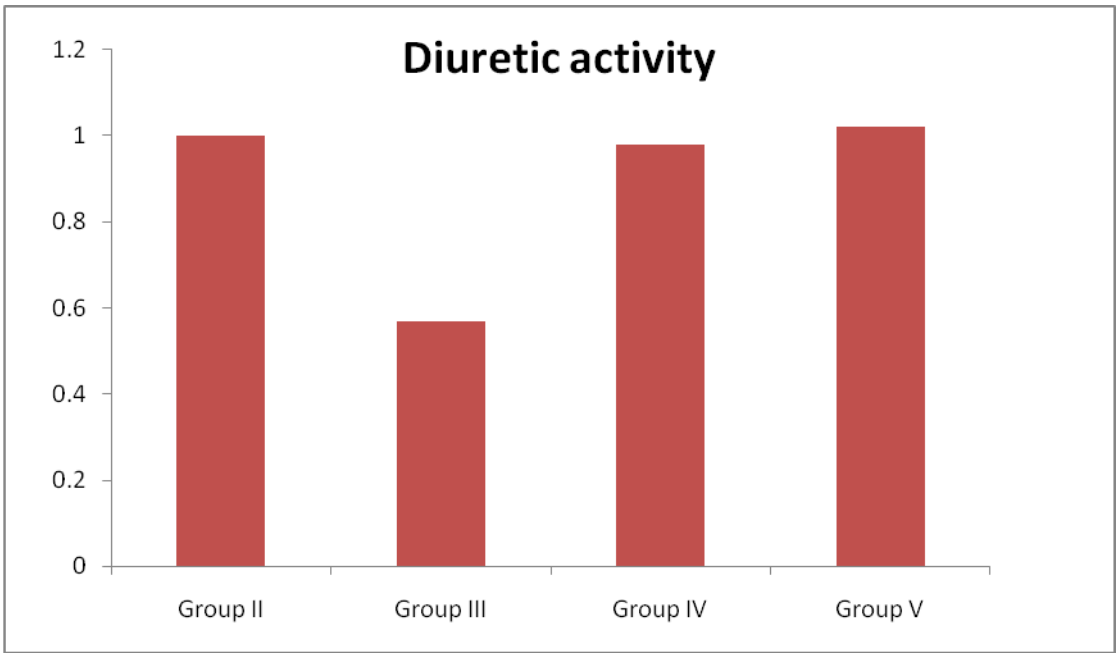

Figure: 8 Effect of Hydroalcoholic extract of *Lasia spinosa* on diuretic activity

Saluretic activity: effect on electrolyte content of the urine

The urine samples collected over the five hours were analyzed for the electrolyte content (Na+, K+, and Cl−).. Whilst HELS at 100mg/kg tended to decrease sodium loss

by 16 %, HELS at 200mg/kg tended to increase by 33% compared to Control group group. By contrast, HELS at 300mg/kg and furosemide significantly increased sodium loss by 68% ($p < 0.001$) and 69% ($p < 0.001$), respectively, compared to Control group of animals.

Urinary K+ excretion was significantly higher with furosemide (107%, $p < 0.001$) compared to Control group. HELS at 200mg/kg and HELS at 300mg/kg tended to increase kaliuresis by 32 % and 67%, respectively, which was not statistically significant compared to control mice. The larger dose of the Hydroalcoholic extract of *Lasia spinosa*, on the other hand, reduced K+ loss by 9% compared to controls, but the decrease was significant (42%, $p < 0.001$) when compared to Furosemide. In the case of Cl− excretion, though treatment tended to increase loss of the anion compared to control mice, the increase reached statistical significance only with HELS at 300mg/kg (89%, $p < 0.001$).

Electrolyte content of the extract

Water soluble salts could be present in the extract and subsequently interfere with the urinary excretion of electrolytes. The content of Na+, K+ and Cl− in extracts was therefore determined to exclude this possibility. The result revealed that Na+ and Cl− were not detectable at all doses in the extracts as tested by the instrument used in the present study.

Table: 7 Effect of Hydroalcoholic extract of *Lasia spinosa* 5 h urinary electrolyte excretion in mice

Group	Urinary electrolyte concentration (m mol/L)			Saluretic index			Na^+/K^+	$Cl^-/Na^+ + K^+$
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-		
Group I	58.31 ± 6.24	43.51 ± 4.87	43.21 ± 3.21				1.36	0.44
Group II	102.5 ± 7.01	91.47 ± 14.01	74.55 ± 12.11	1.67	2.2	1.68	1.10	0.39
Group III	48.60 ± 1.73	58.24 ± 6.33	49.57 ± 11.01	0.82	1.33	1.14	0.87	0.48
Group IV	77.61 ± 5.21	73.44 ± 3.24	59.44 ± 8.32	1.33	1.66	1.32	1.12	0.39
Group V	101.7 ± 7.01	37.44 ± 2.01	83.44 ± 2.71	1.68	0.86	1.86	2.65	0.61

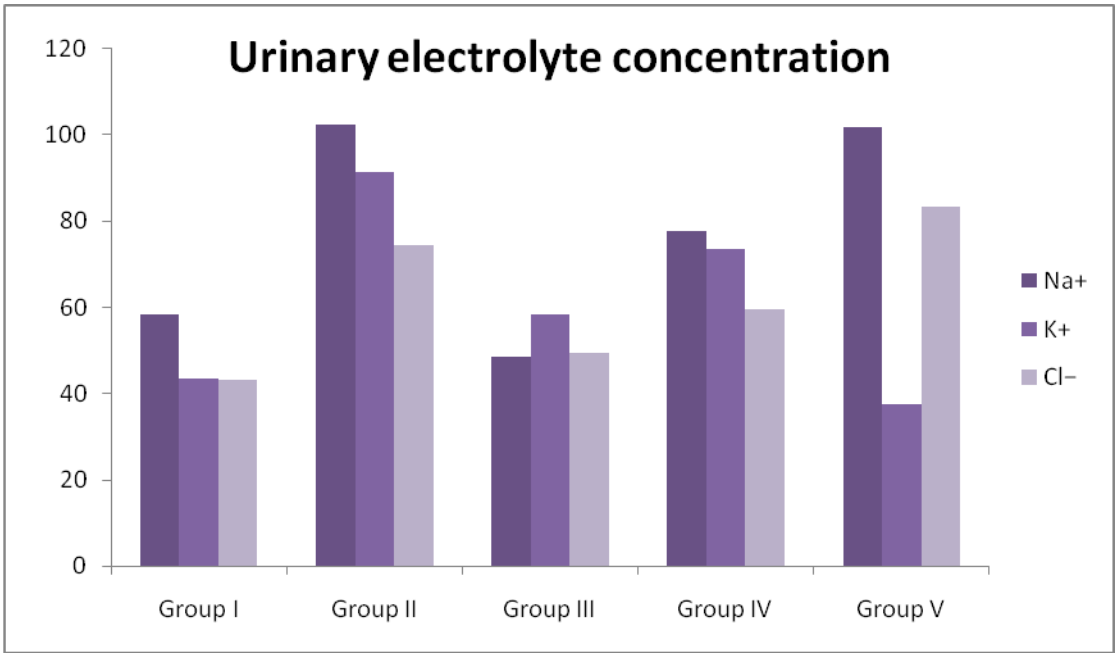

Figure: 9 Effect of Hydroalcoholic extract of *Lasia spinosa* 5 h urinary Urinary electrolyte concentration (Cl^-, Na^+,K) in mice

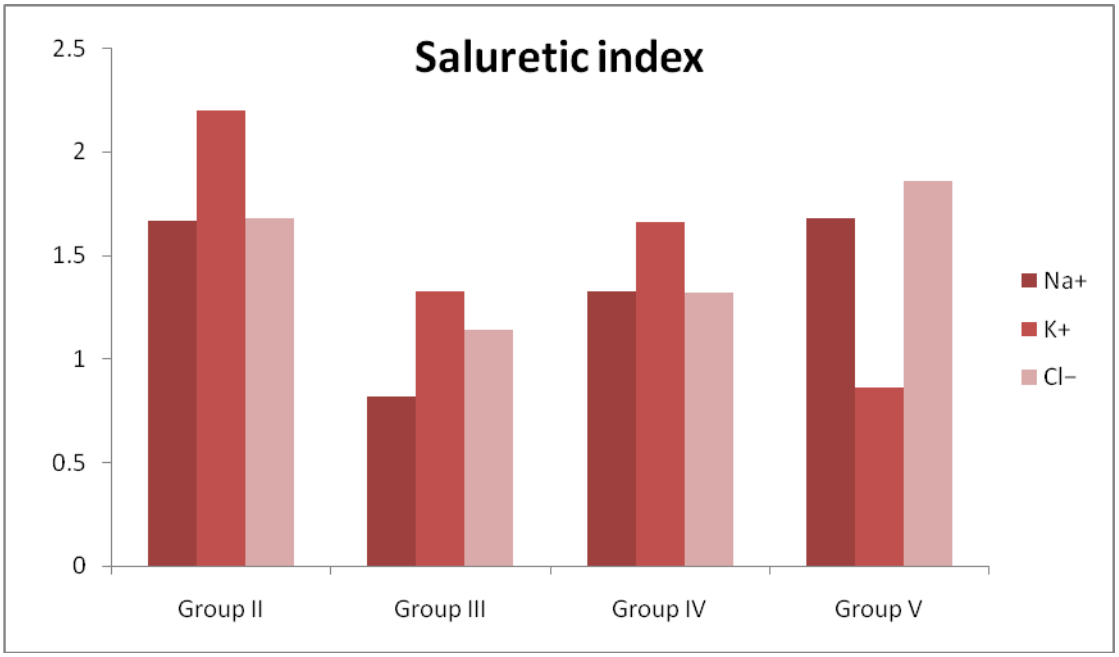

Figure: 10 Effect of Hydroalcoholic extract of *Lasia spinosa* on Saluretic index in mice

Conclusion

Form the above studies it was concluded that the Hydroalcoholic extract of *Lasia spinosa* significantly reduced the incidence and severity of ulceration in ethanol-induced ulcer model, due to the presence of Carbohydrates, Flavonoids, Proteins & Amino acids, Phenols, diterpenes and Saponins. Phenols and flavonoids were present in good quantity. The Hydroalcoholic extract of *Lasia spinosa* also show significantly increase the volume of urine ,so the hydroalcoholic extract of *Lasia spinosa* can be used as diuretics and antiulcer drug. Further isolation and characterization of required to identify the active compounds.

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