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Evaluation of Diuretic Activity of *Desmodium gangeticum Linn*

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Abstract: ***Purpose: The present study was undertaken to investigate diuretic effect of methanol extracts of the roots of Desmodium gangeticum L., in normal rats. Method: methanol extract of Desmodium gangeticum L. were administered to experimental rats orally at various doses (100mg/kg, 200mg/kg, 400mg/kg) p.o. Furosemide (10 mg/kg) was used as positive control in the study. The diuretic effect of the extracts was evaluated by measuring urine volume, diuretic index and Lipschitz value, sodium and potassium content, and chloride content. Saluretic index, Natriuretic index and Ion quotient were calculated. Result: Urine volume was significantly increased by the two doses of methanol extracts from root of Desmodium gangeticum L., in comparison to control group. While the excretion of sodium was also increased by both extracts, The highest dose of extract significantly excreted sodium, chlorides in excess and had good saluretic, natriuretic activity which was comparable to that of standard drug. There was no potassium sparing effect found. There was no significant change in the pH of urine but they were mildly alkaline after administration of the Desmodium gangeticum L., extracts. The diuretic effect of the extracts was comparable to that of the reference standard (Furosemide). Conclusion: We can conclude that methanol extracts of Desmodium gangeticum L., produced diuretic effect. Present study provides a quantitative basis for explaining the diuretic use of Desmodium gangeticum L., as a diuretic agent.***

Keywords: Diuretic, urine volume, electrolytes, saluretic, natriuretic, diuretic index

1. Introduction

Water constitutes about 60% of the average adult body weight and is responsible for many physiological processes in the human body. Thus, fluid and electrolyte homeostasis is critical for human survival, as exemplified by the potentially devastating consequences of fluid imbalance. Renal excretion of urine also ensures the elimination of products of metabolic activity and excess electrolytes in addition to water, thus maintaining fluid homeostasis. Diuretic therapy is generally used to treat edematous states in the cases of renal insufficiency, nephrotic syndrome, liver cirrhosis, and heart failure Diuretics are medications that are designed to increase the flow of urine, promoting the removal of excess of water, salts, metabolic products, and toxins from the body. Excessive diuretic use can result in compromised physical performance and health consequences. These synthetic diuretics typically inhibit potassium secretion and leads to potassium retention. (1, 2, 3). To overcome these side effects there is a need to study about plant based drugs with less or no toxicity and also to avoid abuse of diuretics.

It is a common misconception that all weeds are useless or a nuisance to the public; however, some of these weeds have good ethno medicinal values around the world and are good sources for new drug discovery, and they grow naturally in large quantities without the need for specialised good agricultural practises and are available all year. It is our job to safeguard these incredible natural resources. (4)

One such weed is *Desmodium gangeticum L.*, It is found in tropical Africa, India, China, Myanmar, Japan, Cambodia Malaysia, Indonesia. Shalparni is a shrub with woody stem, 2-4 feet in height. Branches are covered with soft hairs. Leaves are unifolate, ovate, oblong, obtuse and pubescent beneath and upto 15cm in length. Lower surface of leaves is light green in colour. Flowers are purple/white in colour. Fruits compressed, slightly falcate, moniliform, six to eight jointed glabrescent lomentum, slightly indented above, joints separating when ripe, indehiscent, one seeded, more or less straight or lightly curved above and rounded on the lower side. Seeds compressed reniform without a strophiole. The tap root is poorly developed and the lateral roots are very strong, nearly uniformly cylindrical, light yellow and smooth.

This herb has anthelmintic, anticatarrhal, carminative, diuretic, expectorant, febrifuge, nervine tonic, anti diarrheal and stomachic properties. Moreover use of this herb is quite good to resolve the complications like enteric fever, respiratory complications and piles. (5, 6, 7, 8)

As there are no scientific evidences on its diuretic potential an attempt has been made to study its diuretic properties.

2. Research Methodology

The plant *Desmodium gangeticum (L).DC was* collected from around Telangana region in the month of January, 2020 and was authenticated by Dr. K Madhava Shetty, Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh India. Voucher number for the authentication of plant is 0449. Roots are thoroughly washed under tap water dried under shade and powdered by using a mechanical grinder.

Approximately 200 g of root powder was placed in the soxhlet apparatus and extracted with methanol. The extraction procedure was carried out for 18 to 20 hours until a colourless solvent appeared in the side tube. The extract collected was dried by evaporating the solvent on a water bath maintained at <50°C. (9)

The extract was examined for their colour and consistency and their percentage yield was calculated with reference to the quantity used for extraction. The extract was stored in airtight containers in a refrigerator below 10°C until use. The preliminary phytochemical investigations were carried out with *Desmodium gangeticum L.,* for qualitative identification of phytochemical constituents present in extract by following standard methods. (10)

2.1 Experimental Animals

Male and female albino rats (*Rattus norvegicus*) weighing 150–200 g were used for the acute toxicology studies. The rats were obtained from the animal house, LNCT University, Bhopal, India. The animals were acclimatized to laboratory conditions for seven days prior to the experiments. The rats were maintained at a room temperature of 22–24 °C, with a 12 h light/dark cycle and humidity around (50 ± 5)%. During acclimatization, the rats were randomized into experimental and control groups and housed individually in sanitized polypropylene cages housed with sterile paddy husk as bedding. Animals were given free access to standard pellet diet and water ad libitum. All experimental procedures were in compliance with the Animal Ethical Committee, Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and were approved by Institute Ethical Committee.

2.2 Acute Oral Toxicity Study (11, 12)

An acute oral toxicity study was performed according to the Organization of Economic Co-Operation and Development (OECD) guideline 425 for testing of extracts. The animals were observed constantly for 3 h after oral dose administration of the sample for behavioral, autonomic, and neurological profiles and then every 30 min for the consecutive 4 h and lastly for mortality after 24 h, 48 h, 7 days, and 14 days (2 weeks) for any change in behavior or mortality. The mice were analyzed for signs of toxicity. on their skin, hair, pupils, mucous membrane, salivation, lethargy, sleep, coma, convulsions, tremors, diarrhea, oral activity, abdominal, and external genitalia. The mice were separated from their cages during the study to assess the survival, morbidity, and general health. The LD_{50} value was determined.

2.3 Diuretic activity (13, 14)

2.3.1 Lipschitz method

A method for testing diuretic activity in rats has been described by Lipschitz et al. (1943). The test is based on water and sodium excretion in test animals and compared to rats treated with a high dose of urea. The "Lipschitz-value" is the quotient between excretion by test animals and excretion by the urea control.

Procedure

Male Wistar rats weighing 150–200 g are used. Three animals per group are placed in metabolic cages provided with a wire mesh bottom and a funnel to collect the urine. Stainless-steel sieves are placed in the funnel to retain feces and to allow the urine to pass. The rats are fed with standard diet (Altromin® pellets) and water ad libitum. Fifteen hours prior to the experiment food and water are withdrawn. Three animals are placed in one metabolic cage. For screening procedures two groups of three animals are used for one dose of the test compound.

2.3.2 Evaluation of Diuretic Activity of *Desmodium gangeticum (L.)*:

2.3.2.1 Lipschitz Model/Hydrated rat model:

Albino rats weighing between 150-200 g and each group containing 6 animals were divided into 5 groups.

Group I - Normal control (Vehicle 2% CMC in normal saline 10 ml/kg b.wt)

Group II - Standard Furosemide (10 mg/kg, p.o) in vehicle

Group III- Low dose of *Desmodium gangeticum (L.)* (100 mg/kg) in vehicle

Group IV- Medium dose of *Desmodium gangeticum (L.)* (200 mg/kg) in vehicle

Group V- High dose of *Desmodium gangeticum (L.)* (400 mg/kg) in vehicle

Urine excretion is recorded after 5h and 24h. Various parameters like total urine volume and concentration of Sodium, Potassium and Chloride in the urine were measured and estimated respectively. Routine urinalysis including determination of pH and specific gravity along with presence of occult blood, bilirubin, urobilinogen, ketone bodies, proteins, nitrite, glucose, and leucocytes in urine was carried out using urocolor test strips (Standard Diagnostics Inc., South Korea) for urine samples of control and extract treated rats

Urine volume excreted per 100 g body weight is calculated for each group. Results are expressed as the "Lipschitz-value", i.e., the ratio *T*/*F*, in which *T* is the response of the test compound, and *F*, that of Furosemide treatment. Indices of 1.0 and more are regarded as a positive effect.. Calculating this index for the 24 h excretion period as well as for 5 h indicates the duration of the diuretic effect. Similar to urine volume, quotients can be calculated for sodium excretion. Dose response curves can be established using various doses. Loop diuretics are characterized by a steep dose-response curve. Saluretic drugs, like hydrochlorothiazide, show Diuretic index around 1.8, whereas loop diuretics (or high ceiling diuretics) like furosemide, bumetanide or piretanide reach values of 4.0 and more. The Lipschitz test has been proven to be a standard method and a very useful tool for screening of potential diuretics.

2.3.2.2 Estimation of Urinary Electrolytes: Electrolytes in urine like Sodium, Potassium and Chloride were determined by Ion Selective Electrode method as described by the user instruction manual of the biochemical kits (Roche Diagnostics Pvt. Ltd, Gurgaon, Haryana.)

2.3.2.3 Evaluation of Salurietic activity and Natriuretic activity

The sum of Na^+ and Cl^- excretion is calculated as parameter for saluretic activity.

The ratio Na^+/K^+ is calculated for natriuretic activity. Values greater than 2.0 indicate a favorable natriuretic effect. Ratios greater than 10.0 indicate a potassium-sparing effect.

The ratio $CL^-/Na^+ + K^+$is calculated to estimate carbonic anhydrase inhibition• Carbonic anhydrase inhibition can be excluded at ratios between 1.0 and 0.8. With decreasing ratios slight to strong carbonic anhydrase inhibition can be assumed.

All values were expressed as mean ± SEM (standard error of mean) of six rats (n = 6). The statistical analysis was done by analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. The value of $p < 0.05$ was considered as significant. (15)

3. Results and Discussion

The *Desmodium gangeticum L.,* roots were examined for their colour and consistency and their percentage yield was calculated with reference to the quantity used for extraction and the extracts were dark brown colour with a yield of 13.79%. (Table 1) Preliminary phytochemical screening showed the presence of alkaloids steroids, triterpenes, phenols, saponins, flavanoids, proteins, aminoacids and Glycosides in *Desmodium gangeticum L.* (Table 2)

In the present study *Desmodium gangeticum L.,* methanolic root extract (DGMRE) was subjected for acute oral toxicity studies. For the LD50 dose determination DGMRE *was* administered upto the dose level of 2000 mg/Kg body weight orally and the extract did not produce any mortality. Hence from the maximum dose tested (2000 mg/Kg) with each extract, three different doses were selected as $1/20^{th}$ low (100 mg/kg), $1/10^{th}$ medium (200 mg/kg), $1/5^{th}$ high (400 mg/kg) doses respectively. (Table 3)

Routine urine analysis including determination of pH and specific gravity along with presence of occult blood, bilirubin, urobilinogen, ketone bodies, proteins, nitrite, glucose, and leucocytes in urine showed that they were absent and urinary pH was 5.86. The urine pH after administration of DGMRE at doses 100, 200, 400mg/kg bw were 7.01, 7.34, 7.63 respectively at 24h urine sample. Furosemide increased the urine pH to 7.89 thus making the urine slightly alkaline. The specific gravity was normal and no abrupt change in any extract treated animals (Table 4). The effect of methanolic extract of roots of *Desmodium gangeticum (L.)* was found to be dose dependent, i.e., among the three doses studied, higher dose produced more effect. A comparison was made with the standard diuretic drug furosemide, the diuretic effect observed after treatment with methanolic extract of roots of *Desmodium gangeticum (L.)* was found to be significant in terms of urinary output and Diuretic activity. The Diuretic Index and Lipschitz value of 100mg/kg DGMRE (1.64, 0.66, 1.71, 0.66 at end of 5hr and 24hr respectively) ($p<0.001$) and the Diuretic Index and Lipschitz value of 200mg/kg DGMRE (1.84, 0.74, 2.06, 0.79 at end of 5hr and 24hr respectively) ($p<0.001$) The 400mg/kg DGMRE (2.11, 2.48 at end of 5hr and 24hr respectively) ($p<0.001$) showed high diuretic activity. The Lipschitz values were 0.85 and 0.95.The diuretic activity of extracts were significantly comparable to standard (2.50, 1, 2.61, 1, at end of 5hr and 24hr respectively) (Table 5, 6, Fig 1)

Although, the dose dependent rise in urinary excretion of water was observed the increase in urinary electrolyte excretion was found to be independent of the dose administered The sodium and chloride electrolyte excretion was found to be high and significant in urine sample of animals which received 400mg/kg DGMRE (251.22±15.04, 224.32±14.50) and was comparable to standard drug Furosemide received urine samples. (279.20±21.67, 265.76±7.02) All the extracts 100.mg/kg, 200mg/kg and 400 mg/kg of DGMRE showed CAI below 0.8 and proved to be having good CAI activity. The values are 0.53, 0.31, 0.59 respectively)The 400mg DGMRE showed appreciable diuretic, saluretic and natriuretic activity as compared to Furosemide. (Table 7, 8; Fig 2)

Remarkably, the diuretic activity of the plant extract was dose and time-dependent indicating that this effect is intrinsic, genuine, and possibly receptor-mediated. Renal excretion of electrolytes is as salient as the excretion of water for treatment of hypertension, peripheral edema, ascites, and congestive heart failure. The increase in diuresis caused by the extracts reflected correspondingly in the excretion of electrolytes. It significantly increased the excretion of urinary electrolytes (Na+, K+, and Cl−) in a dose-dependent manner. Although the methanol extracts (400mg/kg) increased the excretion of K+ as compared to the negative control, it was significantly lower than that induced by the standard drug (Table 7, Fig 2). The natriuretic activity (aldosterone secretory index) of the plant extract can be determined by taking the ratio of Na+/K+ and values greater than 2.0 indicate a favorable natriuretic effect, whereas ratios greater than 10.0 indicate a potassium-sparing effect.137 Since the DGMRE and HCMRE did not increase the Na+/K+ ratio, it is not acting as a potassium-sparing diuretic. Because, potassium-sparing diuretics are usually very weak, have a slow onset of action, 138 and increase the urinary Na+/K+ ratio.137 The ratio of Cl−/[Na+ + K+] is used to estimate the carbonic anhydrase inhibitory activity of the extract. The values between 1.0 and 0.8 can exclude carbonic anhydrase inhibition. With a decreasing ratio, enzyme inhibitory activity can be assumed.137 The DGMRE and HCMRE had carbonic anhydrase inhibitory indices of 0.53, 0.31, 0.59 and 0.53, 0.62, 0.41 at the doses of 100, 200.400 mg/kg respectively (Table 7, 8)

4. Conclusion

Thus, this study indicates that extracts might have an inhibitory action on carbonic anhydrase enzyme in the renal tubules. The active principle (s) responsible detected in *Desmodium gangeticum L.,* after qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, polyphenols, and tannins (Table 5.2). It is reasonable to suggest that these secondary metabolites may act

individually or synergistically to produce the observed diuretic and natriuretic activities of *Desmodium gangeticum L.* Flavonoids are one of the natural antagonist ligands for A1 adenosine receptors, while antagonistic activity to the receptor is known to associate with diuretic activity. The enzyme carbonic anhydrase has a role in the regulation of pH and reabsorption of sodium in the PCT. Interestingly; flavonoids have both diuretic and potassium-sparing activities. The plant extract has less effect on the excretion of potassium (So, this might be another evidence for the potassium-sparing activity of the plant extract as it was found to contain flavonoids Additionally, tannins are implicated in decreasing blood pressure by promoting the excretion of water and electrolytes. Collectively, shreds of evidence suggested that the plants *Desmodium gangeticum L* has diuretic activity via several mechanisms due to the phytochemicals it contained.

The findings from present study support the folklore use of *Desmodium gangeticum L., (*roots) for their diuretic actions. Methanolic extracts of the plant do not seem to have renal toxicity in rats at doses selected in the present study. Based on the pattern of excretion of water and electrolytes, it appears that that there are active principles present in these extracts having a frusemide-like activity. Moreover, efforts should also be geared toward identifying the specific phytochemicals at molecular level responsible for the observed activity and also quantitative estimation should be done for constituents present in extracts.

Table 1: Nature and Percentage Yield of the extracts

S.No.	Name of the extract	Nature	Colour	%Yielding (w/w)
1.	Desmodium gangeticum L.,	Sticky	Dark brown	13.79

Table 2: Phytoconstituents reported after preliminary chemical tests

Phytoconstituents	*Desmodium gangeticum L.,*
Alkaloids	Present
Carbohydrates	Absent
Steroids, Triterpenes, Phenols	Present
Saponins	Present
Tannins	Absent
Flavonoids	Present
Proteins and Aminoacids	Present
Glycosides	Present
Fixed oils and Fats	Absent

Table 3: Determination of acute toxicity (LD_{50}) value of methanolic root extract of *Desmodium gangeticum L.*

Groups	Dose (mg/kg)	D/T	Sign of toxicity/Behavioral changes
A	0.25 ml (H_20)	0/6	No toxic effects
I	500	0/6	No toxic effects
II	1000	0/6	No toxic effects
III	2000	0/6	Calm, but agile after 2 h
IV	5000	0/6	Calm, agile after 2 h but could not eat enough food.

D/T = Number of rat deaths/Total number of rats used.

Table 4: Effect of *Desmodium gangeticum (L.)* root extracts on miscellaneous urinary parameters in control and experimental rats

S. No.	Groups (n=6)	pH	Specific gravity	Glucose	Protein
1	Control (15 ml/Kg b. wt)Saline	5.86	1.023	Absent	Absent
2	Standard (Frusemide 10 mg/kg b.wt)	7.89	1.05	Absent	Absent
3	methanolic root extract of *Desmodium gangeticumL., (*100 mg/kg b.wt)	7.01	1.024	Absent	Absent
4	methanolic root extract of *Desmodium gangeticum L., (*200 mg/kg b.wt)	7.34	1.019	Absent	Traces
5	methanolic root extract of *Desmodium gangeticum L., (*400 mg/kg b.wt)	7.63	1.017	Absent	Absent

Table 5: Effect of *Desmodium gangeticum (L.)*root extracts on urine excretion volume (at 5hr), diuretic action and Diuretic Index.

S. No.	Groups (n=6)	Total Urine Vol (ml/kg b.wt/5 h)	Urinary excretion	Diuretic Index	Lipschitz value
1	Control (15 ml/Kg b. wt)Saline	2.25±0.08	15	---	------
2	Standard (Frusemide 10 mg/kg b.wt)	5.63±0.02	37.53	2.5	1
3	methanolic root extract of *Desmodium gangeticum L., (*100 mg/kg b.wt)	3.70±0.05	24.66	1.64	0.66
4	methanolic root extract of *Desmodium gangeticum L., (*200 mg/kg b.wt)	4.15±0.04	27.66	1.84	0.74
5	methanolic root extract of *Desmodium gangeticum L., (400 mg/kg b.wt)*	4.75±0.06	31.66	2.11	0.85

Values expressed as mean ± S.E.M., n=6, Significance at p<0.05*, p<0.01**, p<0.001***, Compared with control group (One Way ANOVA followed by Dunnetts 't' test).

Table 6: Effect of *Desmodium gangeticum (L.)*root extracts on urine excretion volume (at 24hr), diuretic action and Diuretic Index

S. No.	Groups (n=6)	Total Urine Vol (ml/kg b.wt/24 h)	Urinary excretion	Diuretic Index	Lipschitz value
1	Control (15 ml/Kg b. wt)Saline	4.5±0.13	30	----	-------
2	Standard (Frusemide 10 mg/kg b.wt)	11.75±0.08	78.33	2.61	1
3	methanolic root extract of *Desmodium gangeticum L.,* (100 mg/kg b.wt)	7.72±0.06	51.46	1.71	0.66
4	methanolic root extract of *Desmodium gangeticum L.,* (200 mg/kg b.wt)	9.28±0.08	61.86	2.06	0.79
5	methanolic root extract of *Desmodium gangeticum L.,* (400 mg/kg b.wt)	11.20±0.07	74.66	2.48	0.95

Values expressed as mean ± S.E.M., n=6, Significance at p<0.05*, p<0.01**, p<0.001***, Compared with control group (One Way ANOVA followed by Dunnetts 't' test).

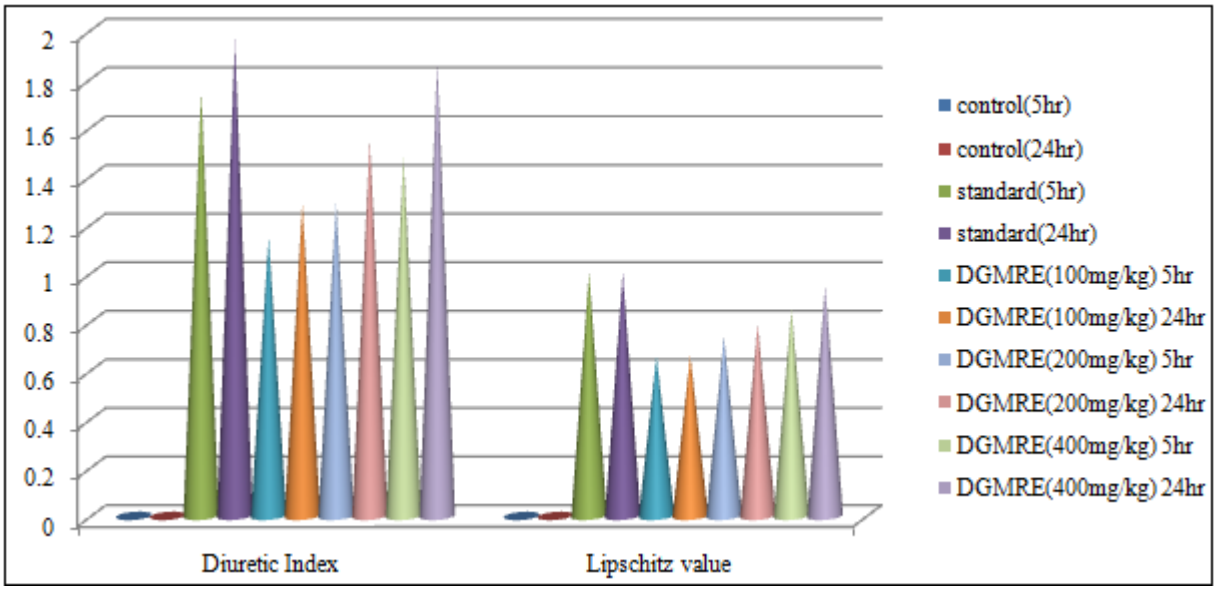

Figure 1: Effect of *Desmodium gangeticum (L.)*root extracts on Diuretic index and Lipschitz value (at 5hr and 24hr)

Table 7: Effect of *Desmodium gangeticum (L.)* root extracts on electrolytes concentration in urine

S. No.	Groups (n=6)	Na^+ (mmol/L)	K^+ (mmol/L)	CL^- (mmol/L)
1	Control (15 ml/Kg b. wt)Saline	85.50±11.03	105.60±18.90	129.55±6.28
2	Standard (Frusemide 10 mg/kg b.wt)	279.20±21.67***	130.31±8.96	265.76±7.02***
3	Methanolic root extract of *Desmodium gangeticumL.,* (100 mg/kg b.wt)	125.36±17.90***	218.28±32.20**	185.11±23.01***
4	Methanolic root extract of *Desmodium gangeticum L.,* (200 mg/kg b.wt)	138.64±10.23	205.68±17.17**	108.05±12.18**
5	Methanolic root extract of *Desmodium gangeticum L.,* (400 mg/kg b.wt)	251.22±15.04***	127.02±14.78	224.32±14.50***

Values expressed as mean ± S.E.M., n=6, Significance at p<0.05*, p<0.01**, p<0.001***, Compared with control group (One Way ANOVA followed by Dunnetts 't' test).

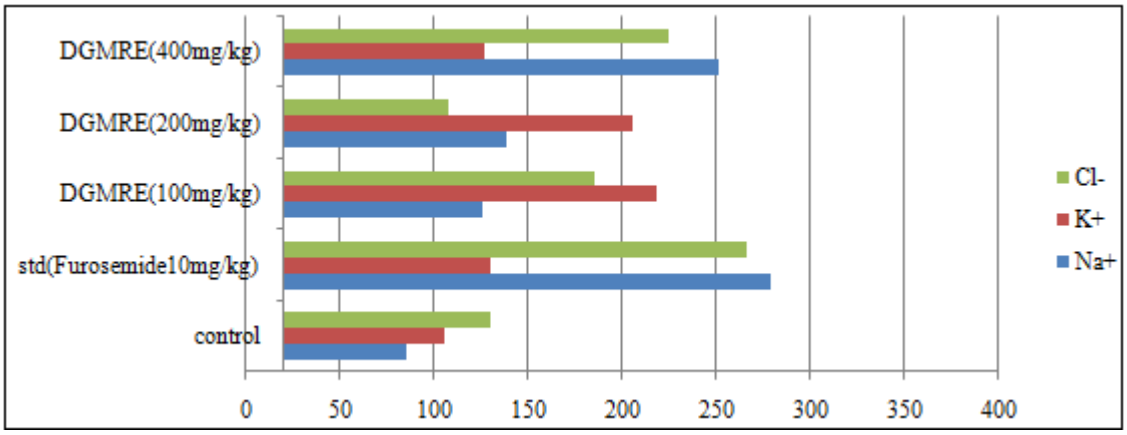

Figure 2: Effect of *Desmodium gangeticum (L.)* root extracts on electrolytes concentration in urine

Table 8: Effect of *Desmodium gangeticum (L.)*root extracts on Saluretic , Natriuretic index and Ion Quotient in Urine

S. No.	Groups (n=6)	Saluretic Index			NatriureticIndex	Ion Quotient
		Na^+	K^+	Cl^-	(Na^+/K^+)	$CL^-/ Na^+ + K^+)$
1	Control (15 ml/Kg b. wt)Saline	1	1	1	0.81	-
2	Standard (Furosemide 10 mg/kgb.wt)	3.26	1.23	2.06	2.14	0.64
3	Methanolic root extract of *Desmodium gangeticum L., (*100 mg/kg b.wt)	1.47	2.07	1.49	0.57	0.53
4	Methanolic root extract of *Desmodium gangeticum L., (*200 mg/kg b.wt)	1.62	1.95	0.83	0.67	0.31
5	Methanolic root extract of *Desmodium gangeticum L., (*400 mg/kg b.wt)	2.94	1.2	1.73	1.97	0.59

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