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DIURETIC ACTIVITY AND STUDY OF BIOCHEMICAL PARAMETERS IN THE METHANOL EXTRACT OF HIBISCUS ESCULENTUS (OKRA) FRESH FRUITS

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Research Article

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ABSTRACT

Hibiscus esculentus (Family: Malvaceae) is a most common weed in India. The wholeplant is used as medicine. According to Ayurveda,. Hibiscus esculentus is used as in the treatment of constipation, inflammation, anemia, abdominal disorders, urinary tract diseases, general debility. The present study was undertaken to investigate diuretic effect of methanol extract of the Hibiscus esculentus (MEHE) in albino rats. Acute oral toxicity study was performed as per OECD guidelines. In acute oral toxicity study, mortality was not observed up to 2000 mg/kg bodyweight. MEHE were administered at the doses of 250 and 500 mg/kg, p.o.Furosemide 20mg per kg body weight was used as positive control in study. The diuretic effect of the extract was evaluated by measuring urine volume, sodium and potassium content. Urine volume is significantly increased at two doses of MEHE 250 & 500 mg/kg body wt in treated rats. The excretion of sodium,Potassium levels was also increased by the MEHE. The diuretic effect of the extract was similar to furosemide. The MEHE had the additional advantage of chloride conserving effect. This study concludes that MEHE produced notable diuretic effect which appeared to be comparable to that produced by the standard diuretic furosemide. The present study provides a quantitative basis for explaining the folkloricuse of Hibiscus esculentus as a diuretic agent. In addition to this different Biochemical parameters were also evaluated.

KEYWORDS: Hibiscus esculentus , Diuretic activity, urine output, Flame Photometry, diuretic index, lipschitz value.

Introduction

Diuretics are the agents which causes increase in excretion of urine. These drugs generally used in the treatment of oedema, hypertension, and congestive heart failure (CHF), Nephritis, toxemia and other UTI disorders. Diuretics are also used in the treatment of pulmonary congestion and play vital role in pregnance and premenstrual tension[1]. Presently in market synthetic diuretics are available which are having significant side effects. These synthetic diuetics significantly inhibit K^+ secretion and leads to K^+ retention [2]. A natural source serves as an additional source for the development of new diuretic agents because of their biological activity. Several plant sources used as diuetics in different systems of traditional medicine and ayurveda. Hibiscus esculentus (Malvaceae)

is most common weed of INDIA. The whole plant used to treat various disorders[3]. On the basis of the traditional use of the plant as diuetic, but review of the literature reveals that no pharmacological and clinical study was carried out to test the diuretic activity of this plant. The main purpose of the present study was to evaluate the diuretic activity of Hibiscus esculentus. We focused on evaluation of the biochemical parameters like creatinine, urea and bicarbonates concentrations in both urine and serum.

Materials and Methods

Plant material: The whole plant of Hibiscus esculentus was collected from N.G. Ranga Agriculture universiy, Hyderabad, Andhra Pradesh, India. The whole plant were dried under shade, powdered and stored in an air tight container.

Drugs and Chemicals:

All the drugs, chemicals and reagents were procured from SD Fine chemical ltd Mumbai, INDIA. All the chemicals were of an analytical grade.

Preparation of extract: The collected whole plant was dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh. About 200g of powdered materials were extracted with Methanol (60°-80°C) using soxhlet apparatus. The extraction was carried out until the extractive becomes colourless. The extracts is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is disolved in normal saline and used for the experiment. The percentage yield of prepared extract was around 8.5%w/w. Preliminary Phytochemical analysis The methanol extract of Hibiscus esculentus . was subjected to qualitative analysis for the various phyto-constituents. Standard methods were used forpreliminary qualitative phytochemical analysis of extract .[4]

Preliminary phyto chemical screening:

The phytochemical examination of the MEHEwas performed by standard methods.

Tests for proteins-xanthoprotein test: To 1 mL of extract, few drops of nitric acid was added by the sides of the test tube and observed for formation of yellow color.

Tests for resins: Five milliliter of distill water was added to the extract a observed for turbidity.

Tests for steroids: Two milliliter of ace anhydride was added to 0.5 g of extract a 2 mL of sulphuric acid was added by t sides of the test tube and observed t colour change from violet or blue-green.

Tests for tannins: About 0.5 g of the each extract was taken in a boiling tube and boiled with 20 mL distilled water and then filtered added few drops of 0.1% ferric chloride was added mixed well and allowed to stand some time. Observed for brownish green or a blue-black coloration.

Tests for glycosides-Keller-killani test: About 0.5 mL of alcoholic extracts was taken and subjected to the following test, 1 mL of glacial acetic acid containing traces of ferric chloride and 1 mL of conc. Sulphuric acid was added to extract and observed for the formation of reddish brown color at the junction of two layers and the upper layer turned bluish green in the presence of glycosides.

Tests for reducing sugar-fehling's reagent: Few drops of Fehling's solution A and B in equal volume were added in dilute extracts and heated for 30 min and observed for the formation of brick red

colored precipitate.

Tests for carbohydrates-molisch test: Small quantities of alcoholic and aqueous extracts was dissolved in 5 mL of distilled water and filtered. To this solution 2-3 drops of α-naphthol was added and 1 mL of concentrated sulphuric acid was added along the sides of inclined test tube so as to form two layers and observed for formation of violet coloured ring at the interface to detect the presence carbohydrates.

Tests for saponins: To 0.5 g of extracts was added to 5 mL of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Tests for sterols-Liebermann-buchard test: The insoluble residue was dissolved in chloroform and few drops of acetic anhydride were added along with a few drops of conc. Sulphuric acid from the sides of the test tube and observed for the formation of blue to blood red color.

Tests for terpenoids-salkowski test: To 0.5 g of the extract, 2 mL of chloroform was added; Conc. H_2So_4 (3 mL) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Tests for phenols: The extracts were taken in water and warmed. To this 2 mL of ferric chloride solution was added and observed for formation of green or blue colour.

Test for cardiac glycosides (Keller Killiani's): Among 100 mg of extract was dissolved in 1 mL of glacial acetic acid containing 1 drop of ferric chloride solution. This was then underlayer with 1 mL of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a de-oxy sugar charactersitcs of cardenolides.

Test for catachol: To 2 mL of test solution alcohol is added and erlich's reagent and few drops of conc.hydrochloric acid was added. The result was obtained.

Test for flavonoids: The qualitative analysis of the flavonoids was performed by thyn layer chromatography (TLC) in the following experimental conditions:
Stationary Phase: Silica gel G (Merck), ready-made plates 10x20 cm
Mobile phase: ethyl-acetate:water:formic acid: acetic acid (72:14:7:7)
The sample Aliquots: 10 µg from the samples spoted.
Migration distance: 7,5 cm
Identification: Neu-PEG Reagent

Phytochemical components of qualitative analysis	MEAI
Proteins	+
Resins	-
Carbohydrates	-
Terpenoids	-
Steroids	+
Tannins	+
Glycosides	-
Reducing Sugars	-
Saponins	+
Sterols	+
Phenols	+
Catachol	+
Flavonoids	+

Mean ± SE (n= 8) values of different biochemical parameters in the furosemide and Hibiscus esculentus textract treated rats :

Parameters	Furosemide	MEHE extract
Diuretics (ml/min.kg)	0.04± 0.03	0.05± 0.01
pH (blood)	7.77 ± 0.04	8.05 ± 0.03
pH (urine)	8.34 ± 0.05	8.63± 0.04
Creatinine (µg/ml)		
Serum	0.09 ± 0.005	0.08 ± 0.06
Urine	0.45 ± 0.008	0.34 ± 0.003
Urea (µg/ml)		
Serum	6.80 ± 0.10	6.72 ± 0.12
Urine	1.3 ± 0.15	1.4 ± 0
Sodium (mEq/L)		
Serum	298.01 ± 1.12	300 ± 1.95
Urine	355.5 ± 0.765	344 ± 1.142
Potassium(mEq/L)		
Serum	80.2 ± 1.25	75.16 ± 0.10
Urine	275 ± 0.75	263.5± 1.020
Chloride (mEq/L)		
Serum	174.10 ± 0.41	187.69 ± 0.96
Urine	290.00 ± 0.426	273.5 ± 0.610
Bicarbonate		
Serum	108.42 ± 2.90	41.38 ± 1.20
Urine	179.70 ± 15.08	98.52 ± 1.72
Renal Clearance (ml/min/kg)		
Creatinine	1.86 ± 0.33	3.36± 0.28
Urea	0.43 ± 0.07	1.66 ± 0.15
Sodium	0.06 ± 0.01	0.06± 0.01
Potassium	1.53 ± 0.14	0.11 ± 0.01
Chloride	0.13 ± 0.01	0.06 ± 0.01
Bicarbonate	0.06 ± 0.01	0.12 ± 0.03

Procurement and selection of animal:

Wister albino rats of either sex weighing between 100-150 gms were obtained from National Institute of Nutrition, Hyderabad. The animals were used for the acute toxicity and diuretic activity. The animals were stabilized for one week, maintained under standard conditions at room temperature 60 ± 5% relative humidity, and 12 hrs light- dark cycle. They had been given standard pellet diet and water throughout the course of the study. The animals were handled gently to avoid giving them too much stress which could result in an increased adrenal output. The study was approved by Institutional animal ethics committee (470/01/a/CPCSEA 24th Aug'01).

Toxicology studies:

The acute toxicity study was carried out in adult male albino rats by the fixed dose method. The animals were fasted and the next day the product containing extract of the plant Hibiscus esculentus was administered orally at 250mg per kg body weight. Then the animals were observed continuously for three hours for general behavioral, neurological and autonomic profiles and then every 30 minutes for next three hours and finally for mortality after 24 hrs till 14 day. In acute oral toxicity study mortality was not observed up to 2000mg/kg body weight [5].

Diuretic activity:

Wister albino rats of either sex weighing 100-150 gms were divided into four groups of 6 animals each. The animal were fasted for 15 hrs, deprived of food and water. All the animals received priming dose of a 0.9% NaCl solution 25ml per kg body weight. The first group served as control and the second group received the standard drug of furosemide 20 mg per kg body weight in a 0.9% NaCl solution. The other two groups received product containing extract of Hibiscus esculentus at doses of 250mg per kg body weight suspended in 0.9% NaCl solution. Immediately after the respective treatment the animals placed in metabolic cages and urine was collected in a measuring cylinder upto 5 hrs. During this period no food and water was made available to animals. Then the volume of urine and Na^+, K^+ and Cl^- were estimated for assessing diuretic activity. Sodium and Potassium concentrations were determined by Flame Photometer and Cl^- conc was estimated by titrated with $AgNO_3$ solution (0.17 N) using 2 ml of Ferric alum solution as indicator[6,7,8]

Statistical analysis

The data were expressed as Mean ± S.E.M. and statistically analyzed using one way ANOVA followed by Tukey- Kramer's Multiple comparison test, $p<0.05$ was considered significant.

Results

Preliminary Phytochemical analysis The Methanol extract of Hibiscus esculentus . revealed the presence of steroids, proteins, Tannins, Alkaloids, flavonoids, saponins and phenols.

Table No : 1

Effect of Hibiscus esculentus . on urine volume and electrolyte concentration

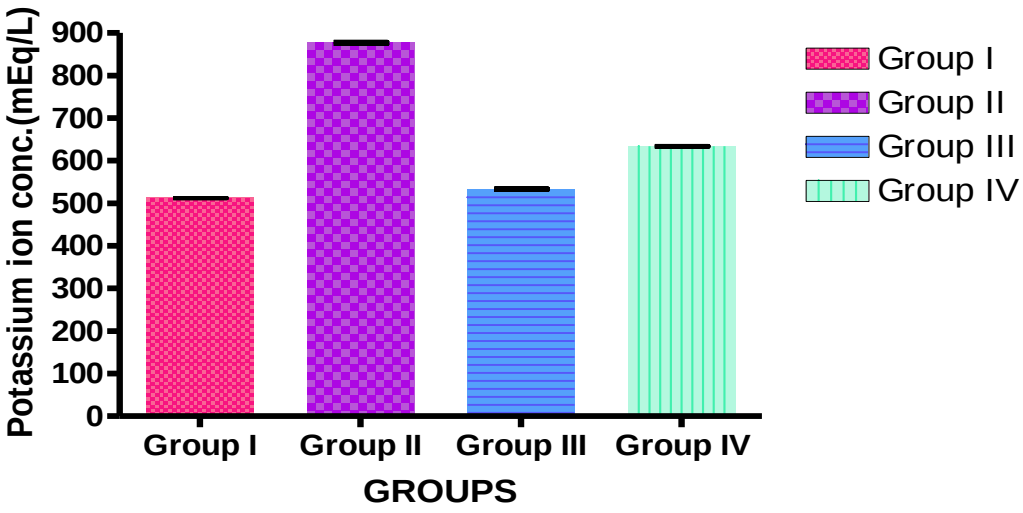
Group	Treatment	Mean urine volume (ml)	Electrolyte Na^+	Concentration (m eq/l) K^+	Na^+ / K^+ ratio	Diuretic index	Lipschitz value
Group-I	Normal Saline 5 ml/kg, p.o	4.655± 0.07591	71.6 ± 0.1683	511.25 ± 0.4530	14.19	---	---
Group-II	Furosemide 20mg/kg, p.o	9.98±0.08396**	174.366± 0.2696**	875.8 ± 2.918**	19.75	2.12	---
Group-III	MEHE-I 250mg/kg, p.o	6.65 ± 0.0855*	81.856±0.1553*	532.17 ± 1.597	15.37	1.41	0.65
Group-IV	MEHE-II 500 mg/kg, p.a	8.683± 0.0875**	103.28±0.2552**	632.85 ±1.004**	16.44	1.85	0.86

Values expressed as Mean S.E.M. One way ANOVA: p<0.01 (urine volume, electrolyte concentration) considered extremely significant. Tukey-Kramer's multiple comparison test

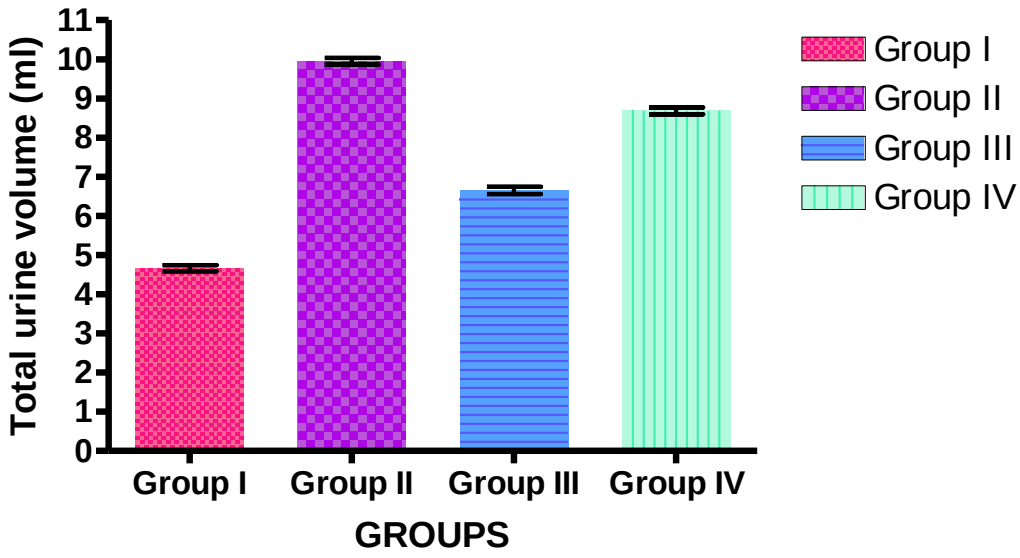
***p<0.05, **p<0.01; when compared with the control group.**

- **Diuretic Index=Mean urine volume of test/Mean urine volume of control.**
- **Lipschitz value = Mean urine volume of test/Mean urine volume of standard.**

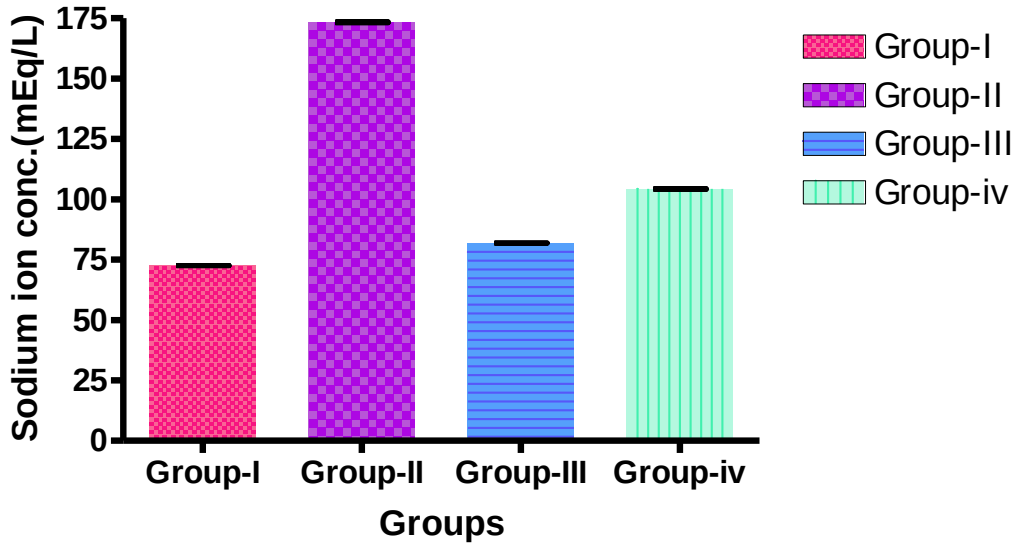
Effect of MEHE on Potassium Ion Concentration in Rats Urine



Effect of MEHE on Urine volume in Rats



Effect of MEHE on Sodium Ion concentration in Rats Urine



Discussion and conclusion

The diuretic activities of the extracts were significant ($P < 0.05$) when as compared to control. The graded doses of the MEHE in normal saline showed a very significant increase in diuresis, natriuresis, kaliuresis, GFR (Table 1). All the extracts cause increase urine elimination and increase in Na+, K+ and Cl+ excretion as compared to normal saline. The extracts possibly act by the synergistic action mechanism of the [HCO3 −/Cl−], [HCO3 +/H+] [9] exchangers and the [N+/H+] antiporter, to cause diuresis. There was an increase in the ratio of concentration of excreted sodium and potassium ions after MEHE treatment.

The the biochemical parameters like creatinine and urea levels shows the significant values compared to standard.

This indicates that the extract increases sodium excretion to larger extent than potassium, which is a very quality of diuretic with lesser hyperkalaemic side effect. The Hibiscus esculentus . extract exerted its diuretic activity possibly by inhibiting tubular reabsorption of water and accompanying anions, as such action has been hypothesized for some other plant species [10]

Therefore Hibiscus esculentus extract significantly increased the GFR due to (a) A detergent like interaction with structural components of glomeruluar membranes. (b) A decrease in renal perfusion pressure, attributable to decrease in the resistance of the afferent arteriole and/or an increase in the resistance of the efferent arteriole and/or. (c) The direct effect on the arteriole wall affecting glomerular blood flow [11].

As emphasized, diuretic properties of MEHE could be due to other active principles such as flavonoids,saponins, and phenols [12]. It is also possible that diuretic effect of the water MEHE could be due to other secondary active(s) metabolites(s) [13]. The other possibility for the observed diuretic effect of MEHE water could be due to indirect changes of some physiological parameters before blood filtration step [14] and/or the consequence of the observed glycosuria [15].

The observed decrease of urine osmolality could be explained by a marked increase in urinary flow, which seemed to be more important than the possible urinary electrolytes excretion. Administration of the MEHE caused a diuretic response, which was accompanied with a slight increase in GFR. This finding suggests different mechanisms of action, like a direct effect on arterial pressure which could affect GFR or glomerular blood flow by decreasing renal perfusion pressure .

MEHE caused diuresis by a mechanism quantitatively similar to that of furosemide and more than one mechanism seems to be involved. The MEHE did not affect plasma urea levels, urine pH, plasma osmolarity and hematocrite indicating that the rapid physiological regulation of these important parameters was not altered after RR infusion.

On basis of the above results, we can conclude that MEHE treatment produced a marked diuresis when rats were acutely treated. In our study, no lethality was observed at least for the dose and duration used. However, advanced toxicological studies remain to be performed in mice and rats. It remains necessary to study eventual adverse effect(s) of this plant such as alteration of some neural, metabolic and hormonal parameters, which are undetermined in this study, before its recommendation to clinical use. The precise site(s) and the molecular and cellular mechanism(s) of MEHE action remain to be elucidated in further studies[16]

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