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DIURETIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *FRAGARIA ANANASSA* FRUIT IN WISTAR ALBINO RATS

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ABSTRACT: *Fragaria ananassa* fruit are small shrubs belonging to family rosaceae. *Fragaria ananassa* mainly contains anti-oxidants such as tocopherols, ascorbic acid and b-carotene which are effective against cancer, heart disease and cataracts. The focus of the study was to analyze the diuretic activity of hydroalcoholic extract of *Fragaria ananassa* fruit in albino rats. Diuretic activity of hydroalcoholic (70:30) extract of *Fragaria ananassa* fruit (100mg/kg and 200mg/kg body weight orally) was studied in male wistar albino rats (n=5). Group I serve as control receive 1% CMC solution, Group II serve as standard receive Furosemide 10mg/kg, Group III receive 100mg/kg *Fragaria ananassa* and Group IV receive 200mg/kg Urine volume at various interval was measured using metabolic cages (Lipschitz Method). The concentration of Na⁺, K⁺ in the urine at the end of 24 hours was estimated using flame photometer. One-way ANOVA technique was used for statically data analysis by which was followed by Dunnet t test (Graph Pad Prism software). On the basis of analysis, it was found that hydroalcoholic extract of *Fragaria ananassa* fruit showed a significant (P<0.05) dose dependent increase in urine volume. At 100 mg/kg and 200mg/kg hydroalcoholic extract of *Fragaria ananassa* fruit increased the excretion of sodium but decreased the excretion of potassium significantly compared to control.

INTRODUCTION: Traditional medicine is very essential part of day today life but it is often underrate part of health care delivery services worldwide¹. Herbal medicinal treatments have long and reputable history of use in the maintenance of health and various severe illnesses. Primeval plants have been traditionally used as diuretics² from the available categories of diuretics, such as loop and thiazides, they have various adverse effects such as electrolyte abnormalities (hypokalemia, hyperuricemia, and hyponatremia), acid-base imbalance, metabolic abnormalities (hyperglycemia and hyperlipidemia), and acute hypovolemia³.

It is, therefore, vitally important to look forward for the search of a diuretic that is relatively free from such unwanted and sometimes deleterious adverse effects. The agents which increases rate of urine flow and sodium ion secretion are known as Diuretics. Diuresis can be induced by the drugs is very much helpful for treating life threatening conditions such as congestive heart failure (CHF)⁴, hypertension, cirrhosis, kidney damage^{5, 6, 7}. They have been also employed in various cases of overdosing and poisoning for the excretion of unwanted or dangerous substances from the patient's body.

Fragaria ananassa which is commonly known as Strawberry belonged to family Rosaceae⁸. In India, from the total production of strawberry the Panchgani-Mahabaleshwar belt contributes around 85% and rest comes from Himachal Pradesh and Jammu and Kashmir. It is an important small fruit which is grown and distributed throughout the

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world. It is bright red in color and has characteristic fragrance and taste. It has plant height: higher than (32.05 cm) and lower than (17.34 cm), number of leaves: higher than (67.00) and lower than (55.60), number of fruits: the highest number of fruits (43.93) and lowest (21.80), fruit weight: the highest fruit weight (411.40 gm) and lowest (155.33gm) and fruit size: Length; 3.53cm-2.76 cm Diameter; 3.94cm-2.02cm. It have various reported activities such as an antioxidant, anti-lipidemic etc.^{9, 10} Vitamin C, potassium, calcium, phosphorous, phenolic and flavonoids can be obtained from it in a good quantity^{11,12}.

Objective of Research: The objective of the research was to analyze the diuretic activity of hydro alcoholic extract of *Fragaria ananassa* fruit in albino rats.

MATERIALS AND METHODS:

Collection and Authentication of Plant Part: The fresh fruit of *Fragaria ananassa* (strawberry) were procured from the local market of Indore, in the month of July 2018 and authenticated and submitted in department of Modern Institute of Pharmaceutical Sciences with Voucher number HERB/MIPS/2018/0033.



FIG. 1: DRIED *FRAGARIA ANANASSA* FRUITS

Preparation of Fruit Extract: About 500 gm of fresh fruits of strawberry were purchased from the local market of Indore, India. After that the fruit were dried in shade and then made a coarse powder. 50 gm of the coarse powder was subjected for Soxhlet apparatus for extraction¹³.

Phytochemical Screening: The extract was subjected to phytochemical screening of various phytoconstituents according to the procedure described by Trease and Evans. The extract was

tested for the presence or absence of carbohydrate, protein, fats and oils, phenols, phytosterol, saponins, flavonoid, vitamin C and glycoside¹⁴.

Animal Housing: Albino rats were housed in Polycarbonate cages under the maintenances of standard conditions of temperature, humidity and dark/light cycles (12h/12h). The animals were provided pelleted food and drinking water in the sufficient amount. After every 48 h, the bedding of the animal cages was changed.

Approval for Animal Experimentation: The present research protocol was approved by Institutional Animal Ethical Committee (IAEC/ MIPS/01/2018/09). CPCSEA registration No. of Institute 1509/PO/RE/S/11 CPCSEA.

Acute Toxicity Study: Guideline 420 of OECD (Organization for Economic Co-operation and Development) was followed for the acute toxicity study. For determining the dose, fixed dose method was used according to which, the starting dose of 2000 mg/kg body weight was taken. 5 Albino rats were given the starting dose of 2000mg/kg (per oral) and they were retained for observation of behavioral change and death till 72 h¹⁵.

Diuretic Activity: According to the Lipschitz Method following methodology was performed¹⁶. Adult albino rats, weighing 200-220g, were divided into four groups of five animals each. Only the healthy animals were selected for the study. The study was performed at a normal room temperature (25 ± 2 °C). By mild confining of pelvic area and tugging of tails, the bladder of the rats was emptied before the administration of the extract/control. 10 ml/kg of normal saline (1% CMC) was administered to Group I (the control group), 10 mg/kg of Furosemide was administered to Group II (the standard group) and different doses of *Fragaria ananassa* (100 & 200 mg/kg) were administered to the test groups (III and IV)¹⁷. In order to ensure that each animal received the same volume of liquids all the doses were prepared in the same volume of normal saline.

Statistical Analysis: One-way ANOVA technique was used for statically data analysis by which was followed by Dunnet t test results for analyzing the data generated during the study with the level of significance set at $P < 0.05$.

RESULTS: The hydroalcoholic extract of fruit of *Fragaria ananassa* was subjected to qualitative phytochemical tests to identify the phytoconstituents and it revealed the presence of carbohydrates, protein, anthrocyanin, phenolic compounds, tannins, saponins, flavanoids, vitamin C and glycoside **Table 1**.

TABLE 1: PHYTOCHEMICAL SCREENING

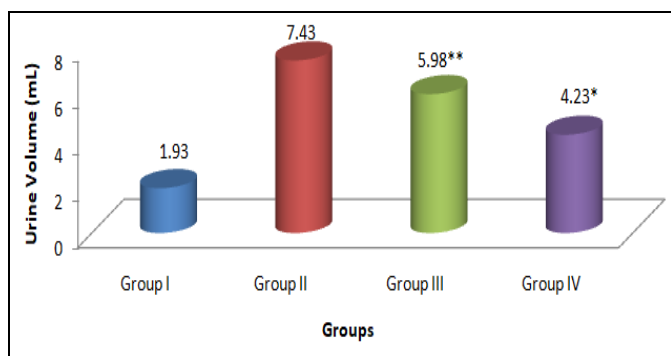
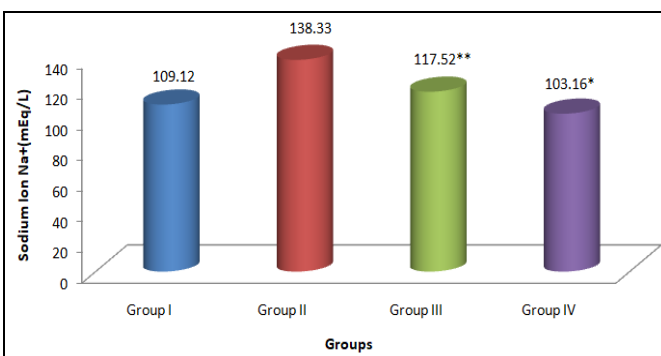
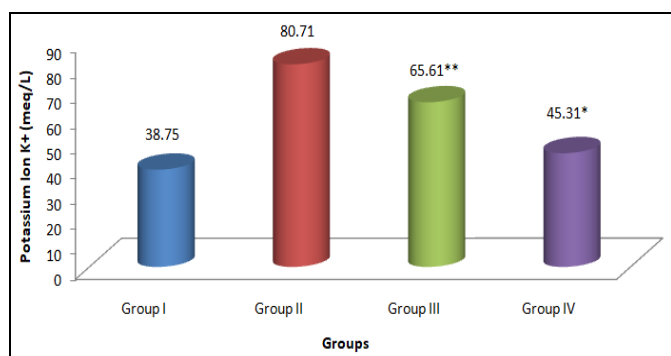
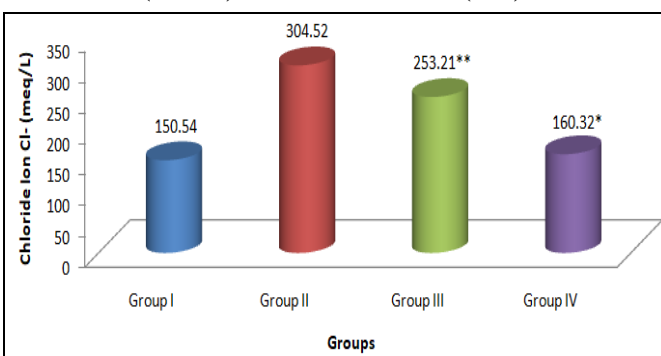
S. no.	Phytochemical Compound	Fruit
1	Carbohydrate	+
2	Protein	+
3	Anthrocyanin	+
4	Phenolic and tannins	+
5	Phytosterols	-
6	Saponins	+
7	Flavonioids	+
8	Vitamin c	+
9	Glycoside	+

The diuretic responses with its electrolyte excretion potency of the hydroalcoholic extract of *Fragaria ananassa* were highly significant in comparison to normal control rats. The hydroalcoholic extract of *Fragaria ananassa* at doses of 100 and 200 mg/kg showed a significant increase in Na⁺, K⁺ and Cl⁻ excretion. The results of urinary electrolyte excretion after treatment of hydroalcoholic extract of *Fragaria ananassa* were comparable to the furosemide group **Table 2**. The crude hydroalcoholic extract of *Fragaria ananassa* effectively increased the urine volume starting at a dose of 100 mg/kg. The highest urine output for the crude extract was observed at the dose level of 100 mg/kg with an appreciable diuretic activity of 80% in comparison with the standard drug Furosemide.

TABLE 2: EFFECTS OF HYDRO-ALCOHOLIC EXTRACT OF FRAGARIA ANANASSA (FRUIT) (HAFA) ON URINE VOLUME AND ELECTROLYTE EXCRETION ON ORAL ADMINISTRATION

Treatment	Dose	Urine Volume (ml)	Na ⁺ (mEq/L)	K ⁺ (mEq/L)	Cl ⁻ (mEq/L)
Group I	(Oral)	1.93±0.20	109.12±2.52	38.75±1.20	150.54±4.32
Group II	10 mg/kg Furosemide	7.43±0.89	138.33±1.86	80.71±1.44	304.52±12.48
Group III	100 mg/kg HAFA	5.98±0.35**	117.52±1.64**	65.61±3.15**	253.21±4.14**
Group IV	200 mg/kg HAFA	4.23±0.40*	103.16±4.95*	45.31±3.41*	160.32±7.89*

All values are expressed as mean ± S.E.M., n=5 in each group. Values are significantly different from Group I control: P values: * <0.05, ** <0.01, *** <0.001 (Student t test analysis). One way ANOVA followed by Dunnett's test.

**FIG. 2: EFFECTS OF HYDRO-ALCOHOLIC EXTRACT OF FRAGARIA ANANASSA (FRUIT) (HAFA) ON URINE VOLUME****FIG. 3: EFFECTS OF HYDRO-ALCOHOLIC EXTRACT OF FRAGARIA ANANASSA (FRUIT) (HAFA) ON SODIUM ION (Na⁺)****FIG. 4: EFFECTS OF HYDRO-ALCOHOLIC EXTRACT OF FRAGARIA ANANASSA (FRUIT) (HAFA) ON POTASSIUM ION (K⁺)****FIG. 5: EFFECTS OF HYDRO-ALCOHOLIC EXTRACT OF FRAGARIA ANANASSA (FRUIT) (HAFA) ON CHLORIDE ION (Cl⁻)**

CONCLUSION: The present study showed that the diuretic activity of the hydro-alcoholic leaves extract of *Fragaria ananassa* is relatively modest and slow in onset as compared to the reference drug, Furosemide. By increasing urinary excretion of Na^+ , K^+ and Cl^- like that of Furosemide the plant extract also increased the urine volume. Therefore the probable diuretic action of hydro-alcoholic leaves extract of *Fragaria ananassa* similar to the mechanism of action of Furosemide could be due to its intervention with the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ co-transport carrier in the luminal membrane of loop of Henle. And, this effect of the extract may be related mainly to the sugar-mannitol. Hydro-alcoholic leaves extract of *Fragaria ananassa* was observed to have diuretic activity in experimentally induced diuresis in Albino rats. The study expands the harmonizing nature of *Fragaria ananassa* with conventional treatment making it comparatively safer, economical, easily available and well tolerated therapy.

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CONFLICT OF INTEREST: The authors confirm that content has no conflicts of interest.

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