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Phytochemial Investigation and Evalution of Diuretic activity of Bambusa Vulgaris

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ABSTRACT:- In a healthy human subject, changes in dietary intake or variations in the extra renal loss of fluid and electrolytes are followed relatively rapidly by adjustments in the rate of renal excretion, thus maintaining the normal volume and composition of extracellular fluid in the body.

Edema is an increase in extracellular fluid volume. In almost every case of edema encountered in clinical medicine, the underlying abnormality involves a decreased rate of renal excretion. One of the factors influencing the normal relationship between the volume of interstitial fluid and the circulating plasma is the pressure within the small blood vessels.

In diseases of hepatic origin (e.g., cirrhosis), the pressure relationships are disturbed primarily within the portal circulation and ascites results. In congestive heart failure, pressure-flow relationships may be disturbed more in the pulmonary or systemic circulation and edema may be localized accordingly.

Keywords:- Diuretics, Saluretics, B. vulgaris, Nephron, Edema, Flavinoids.

I. INTRODUCTION:-

Diuretics (saluretics) elicit increased production of urine (diuresis). In the strict sense, the term is applied to drugs with a direct renal action. the predominant action of such agents is to excrete urine by inhibiting the reabsorption of NaCl and water.

Diuretics are the chemicals that increase the rate of urine formation, by increasing urine flow-rate by increasing the excretion of electrolytes & water from body without affecting protein, vitamin, glucose, or amino acids reabsorption.

II. REVIEW OF LITERATURE

2.1 Zahra Ghanbarinasab et. al. (2021) evaluate the effects of topical Bambusa vulgaris extract on cutaneous leishmaniasis (CL) caused by Leishmania major in BALB/c mice and Bambusa vulgaris extract enhanced wound closure rate through increasing the number of fibroblasts, collagen bundles, and vessels, according to histomorphometric evaluation while it did not affect the parasitic load. Findings of the in vitro study revealed that the extract has substantial mortality for the Leishmania promastigotes.

2.2 Adeyanju O et. al. (2019) extracted Bambusa vulgaris leaves with aqueous and methanolic extracts (1:100 w/v) and used for subsequent investigation. In vitro, the antioxidant capabilities of the extracts were investigated, as well as their inhibitory effect on cisplatin, $FeSO_4$, and nephrotoxicity caused by a single nucleotide polymorphism in rats. The presence of cisplatin and other pro-oxidants in kidney homogenate caused nephrotoxicity in rats by inducing a significant increase in kidney malondialdehyde (MDA) content. Aqueous and methanolic extracts of Bambusa vulgaris leaves were found to reduce this damage, implying that the plant may have antioxidant and therapeutic qualities. The methanolic extract also had the largest free radical scavenging potentials, as well as the highest phenolic and flavonoid content. The extract's free radical scavenging abilities were shown to be proportionate to their phenolic and flavonoid concentration. The findings suggest that methanol may be a good solvent for extracting the antioxidant properties of Bambusa vulgaris leaves and that Fe^{2+}-chelation, DPPH, ABTS*, hydroxyl radical scavenging abilities, and reducing power may be part of the process by which Bambusa vulgaris leaves protect against oxidative stress and also some pro-oxidants induced nephrotoxicity. As a result, Bambusa vulgaris leaves may be utilised to treat cisplatin toxicity and acute renal failure traditionally.

2.3 Ambika K. & Rajagopal B. (2017) revealed that the extract is having broad spectrum of activity against the tested bacteria and fungi but in varying proportion. Because of this wide antimicrobial activity, the extract can be used in the treatment of various infections or inflammations. The mechanism of action should be identified by further works. Our findings demonstrated that acetone extract of B. vulgaris shoot has anti-proliferative effect on human Hela cancer cell line antiproliferative activity using human cancer cell lines may open ways in future in vivo studies on prevention and management of cancer (Zang et al., 2010).

III. RESEARCH OBJECTIVE

The current study was undertaken with the following objectives:

- Phytochemical and physicochemical investigation of selected Vulgaris species
- To prepare polyphenol and flavanoid rich extract from leaves of selected Vulgaris species
- To standardized the extract
- Estimation of diuretic potential of leaf extract using the different in-vitro assay.
- Evaluation of In-vitro diuretic potential of leaf extract
- Evaluation of In-vivo diuretic activity of leaf extract

IV. PLAN OF WORK

- Exhaustive literature survey.
- Identification, collection of Plant.
- Authentication of Plant.
- Extraction of Plant.
- Evalution & Identification of Phytochemicals :-
- ✓ Qualitative Chemical Evalution
- ✓ Determination of Flavonoids content
- ▪ Shinoda Test
- ▪ Zinc Hydrochloride Test
- ▪ Shibita's Reaction Test
- ✓ Determination of content other than flavonoids in extracts

- Estimation of Chemical - Constituents :-
- ✓ TLC,
- ✓ HPLC,
- ✓ HPTLC,

- Pharmacological Studies
- ✓ Biochemical estimation of
- Blood–urea ,
- Creatinine,
- Cholesterol,
- Triglycerides, & Other Parameters.

- Models used

A. In- vivo models

i. GFR Accessment
ii. Urine Analysis
iii. Impaired renal function test

B. **In-vitro models**

i. Lipschitz test
ii. Clearance method

- Compilation of Data
- Summary & Conclusion

V. PLANT PROFILE

5.1 Bambusa Vulgaris

Description of plant :-

Bambusa vulgaris is a rhizomatous plant. The genus of Vulgaris (Arundinaria, Oxytenanthera, Oreobambos and Vulgaris s) is present throughout the continent under rainfall ranging from 700 to 1500 mm. Because of the monocarpic character, episodic flowering, over-exploitation and bush fires, to its very easy adaptation to certain favourable ecological conditions.

Although B. vulgaris is taxonomically a grass, its habit is tree-like. It forms dense stands of cylindrical, jointed woody stems up to 20 m in height and 4-10 cm in diameter; leafy branches at nodes, with narrow lanceolate leaves up to 30 cm long. It is the most widespread member of its genus, and has long been cultivated across the tropics and subtropics.

Chemical analysis of the culms showed 22 % caustic soda and 22.9 % lignin and 21 % pentosans in Vulgaris pulp.

All species of Vulgaris shoots available in the world are not edible. Out of 136 species available in India, the most commonly edible Vulgaris species are Bambus pallida, Bambusa tulda, Bambusa polymorpha, Bambusa balcooa, Dendrocalamus hamiltonii, Dendrocalamus giganteus and Melocanna bambusoides.

Fig. No. :- 3 Leaves of B. Vulgaris

Fig. No. :- 4 Stem (culms) of B. Vulgaris

Fig. No. :- 5 (Whole Plants of B. Vulgaris)

VI. MATERIAL & METHODS

6.1 Plant material

Bambusa vulgaris shoot was collected from Garhi malehra District Chhatarpur, Madhya Pradesh, India for the proposed study. The collected plants were identified by morphological characters.

6.2 Preparation of Plant Extract

The collected fresh leaves are dried in shade for 15-20 days than converted into coarse powder. The shade dried, powdered are extracted separately with Acetone solvents by hot extraction method using soxhlet apparatus. The extracts are used for further experimental use.

The shade dried, powdered are defatted with petroleum Ether then extracted separately by maceration with Chloroform, Ethyl Acetate, Methanol, & Water successively. Extract is collected separately & reduced to small volume under pressure, then further used.

6.3 Chemicals and Reagents

All chemicals & reagents are collected from laboratory of Daksh Institute of Pharmaceutical Sciences, Chhatarpur (M.P.).

6.4 Experimental Animals

Both male and female Sprague Dawley rats aged 8–10 weeks and with a weight range of 260–300 g inbred in the animal house of the Daksh Institute of Pharmaceutical Science, Chhatarpur (MP), were used for the experiment. The animals were housed in polypropylene cages (6 rats per cage) under standard environmental conditions (25 ± 2°C, 55 ± 5% humidity, and 12 h/12 h light/dark cycle). The animals were allowed free access to tap water and laboratory pellet Each rat was placed in an individual metabolic cage 24 h before the commencement of the experiment for adaptation.

6.5 Isolation and characterization of chemical constituent from extract of Bambusa vulgaris leaves by;-

- ✓ TLC,
- ✓ HPLC,
- ✓ HPTLC,

6.6 Phytochemical Screening

Qualitative phytochemical investigation of the aqueous extract was carried out to determine the presence of secondary metabolites like alkaloids, cardiac glycosides, flavonoids, polyphenols, saponins, steroids, tannins, and terpenoids using standard methods.

VII. RESULT & DISCUSSION

7.1 Qualitative Phytochemical Screening

The aqueous extract of Bambusa Vulgaris was tested for the composition of medicinally active compounds and it was found to be positive for alkaloids, flavonoids, polyphenols, and tannins (Table 3).

Table 7.1 Phytochemical Screening of Aqueous Extract of Bambusa Vulgaris Shoot Apex

Phytochemical	Test Method	Observation	Result
Alkaloids	Bouchardat	Brown precipitate was formed	Presence of alkaloids confirmed
	Dragendorff	Red-orange precipitate was formed	Presence of alkaloids confirmed
	Mayer	Yellowish-white precipitate was formed	Presence of alkaloids confirmed
	Wagner	A red-brown precipitate was formed	Presence of alkaloids confirmed
Anthraquinone Glycosides	Borntrager's test	Red color was not occurred	Absence of anthraquinone glycosides
	Ammonium hydroxide test	Pink-red color was not formed	Absence of anthraquinone glycosides
Cardiac Glycosides	Keller-Kiliani test	No formation of brown-ring between layers	Absence of cardiac glycosides
Flavonoids	Alkaline reagent test	Yellow fluorescence was present	Presence of flavonoids
	Shinoda test	Red-pink color was formed	Presence of flavonoids
Polyphenols	Lead acetate test	A bulky white precipitate was formed	Presence of polyphenols
	Ferric chloride test	Blue-green color was formed	Presence of polyphenols
Saponins	Frothing test	A stable persistent froth was not formed	Absence of saponins
Steroids	Libermann-Buchard test	No formation of brown ring at the interface	Absence of steroids
Tannins	Ferric chloride test	Blue or purple or green	Presence of tannins

Phytochemical	Test Method	Observation	Result
		precipitate was formed	
Terpenoids	Salkowski test	A reddish-brown precipitate was not formed	Absence of terpenoids

7.2 Effect on Urine Volume

The effect of aqueous extract of Bambusa Vulgaris shoot apex on urinary output is shown in Table 7.2. The aqueous extract produced diuresis which appeared to be a function of dose and time ($r^2 = 0.855$; $p<0.001$). AQ100 did not produce any detectable difference in urine volume within the first 3 hours after dosing, but it produced a significant increase in urine volume after 4 hours as compared to the negative control ($p<0.001$). AQ200 and AQ400, however, produced a significant increase in urine volume with maximum diuresis of 120% ($p<0.001$) and 136% ($p<0.001$), respectively. AQ200 and AQ400 had a comparable diuretic effect with F10.

Among the three doses of the aqueous extract, AQ400 and AQ200 produced significant diuresis compared to AQ100 at all time-points. AQ200 and AQ400 exhibited a diuretic action of 2.20 and 2.31, respectively. The percent urinary excretion of AQ200 (96%) and AQ400 (100%) was higher as compared to the negative control (44%).

Table 7.2:- Effect of Aqueous Extract of Bambusa Vulgaris Shoot on Urine Volume in Rats

Group	Volume of Urine (mL)					% Urinary Excretion	Diuretic Action	Diuretic Activity
	1 h	2 h	3 h	4 h	5 h			
DW	1.54 ± 0.22	2.04 ± 0.10	2.46 ± 0.03	2.82 ± 0.18	3.01 ± 0.17	44	1.00	
F10	3.02 ± 0.29[a###]	4.07 ± 0.14[a###]	5.65 ± 0.34[a###]	6.67 ± 0.25[a###]	7.03 ± 0.23[a###]	102	2.34	1.00
AQ100	1.48 ± 0.15[b###,]	2.42 ± 0.07[b###, c###,d###]	3.20 ± 0.16 [b###, c###,d###]	3.80 ± 0.19[a#,b###, c###,d###]	4.80 ± 0.29[a###, b###,d###]	69	1.59	0.68
AQ200	2.40 ± 0.20[a#]	3.60 ± 0.19[a###]	5.20 ± 0.16[a###]	6.20 ± 0.18[a###]	6.62 ± 0.16[a###]	96	2.20	0.94
AQ400	2.74 ± 0.13[a##]	3.82 ± 0.16[a###]	5.81 ± 0.40[a###]	6.63 ± 0.32[a###]	6.96 ± 0.30[a###]	100	2.31	0.99

Notes: Each value represents mean ± S.E.M; n=6;
a Against negative control (DW);
b Against standard (F10);
c Against 200 mg/kg;
d Against 400 mg/kg;
#p < 0.05, ##p < 0.01, ###p < 0.001.
Abbreviations: DW, distilled water; F10, furosemide 10 mg/kg; AQ100, aqueous extract 100 mg/kg; AQ200, aqueous extract 200 mg/kg; AQ400, aqueous extract 400 mg/kg.

7.2.1 Effect on Cumulative Urine Output

The cumulative urine output of rats treated with aqueous extract of Bambusa Vulgaris shoot is presented in Figure 2. AQ100 had produced nearly equal urine output as a negative control. Whereas, AQ200 and AQ400 had produced significantly comparable urine output as the standard drug. As it is shown in Figure 7.2.

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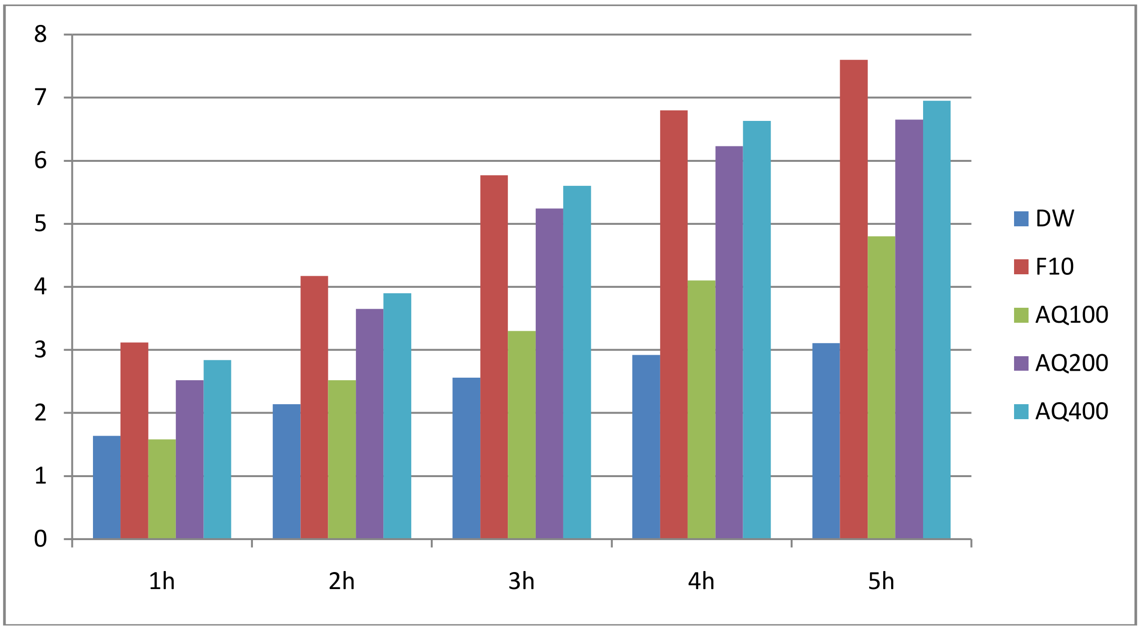

Figure 7.1 The Cumulative Effect of aqueous extract of Bambusa Vulgaris shoot apex on the urinary Output of rats.

Notes: Each value represents mean ± S.E.M; n=6.

VIII. SUMMARY & CONCLUSIONS:-

To sum up, this study provides further evidence that the aqueous extract of the plant Bambusa Vulgaris possessed a comparable diuretic activity with furosemide.

Results obtained from qualitative and quantitative phytochemical tests realized that alkaloids, flavonoids, phenolics, and tannins are responsible for the observed diuretic and natriuretic effects. Secondary metabolites have several mechanisms of diuresis and even they spare the wastage of potassium, which is the main side effect of conventional diuretics, especially loop and thiazide diuretics. Hence, considering the claimed mechanisms of natural diuretics, and the anticipated carbonic anhydrase inhibitory activity, the aqueous extract of Bambusa Vulgaris has multiple mechanisms of action.