Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/9661
DOI URL: http://dx.doi.org/10.21474/IJAR01/9661

RESEARCH ARTICLE

TO DETERMINE DIURETIC ACTIVITY OF BOERHAAVIA DIFFUSA ROOT EXTRACT IN RATS.

Vidhi Sharma, Nehal Sharma, Deepak kr. Gupta, Raju choukse and Rakesh patel.
Dr. Apj abdul kalam university, bypass road, arandia village, post jhalaria, indore (m.p).

Manuscript Info

Manuscript History
Received: 04 July 2019
Final Accepted: 06 August 2019
Published: September 2019

Key words:-
Boerhaavia diffusa, Diuretic, phytochemical ,extract,ethanol.

Abstract

The present study was undertaken to determine diuretic activity of boerhaavia diffusa root extract in rats. In this study the extraction process was carried out for 18 - 20 h till the appearance of colourless solvent in the side tube. The extract collected was dried by evaporating the solvents on a water bath maintained at <500C and percentage yield of alcoholic extract was recorded with respect to the total quantity of powder used for the extraction. Phyto-chemical evaluation for the extract was performed using standard procedures Results showed that single dose administration of alcoholic extract of roots of Boerhaavia diffusa as 100,200 and 300 mg/Kg and standard Furosemide (10 mg/kg) have increased the urinary output along with an increase in concentration of Sodium, Potassium and Chloride ions in urine.Alcoholic extract of roots of Boerhaavia diffusa mg/Kg produced a greater diuretic activity which is comparable to that of standard Furosemide (10 mg/kg). In traditional medicine the plant is used for its diuretic activity. This study come up with identification of so many phyto constituents reported earlier for this diuretic effect in alcoholic extract of roots of Boerhaavia diffusa. Thus the study supports and justifies the rationale behind the use of roots of Boerhaavia diffusa its diuretic activity.

Introduction:-

The amount of fluid (water) retained by the body is controlled primarily by the kidneys. This occurs due to the kidney's ability to control the retention and elimination of sodium and chloride, because the amounts of sodium, chloride, and water in the body are carefully balanced. Thus, if sodium and chloride are eliminated from the body, water also is eliminated. Conversely, if sodium and chloride are retained by the body, so is water. The elimination of sodium, chloride, and water from the body is somewhat complex. In the kidneys, sodium, chloride, and other small molecules are filtered out of the blood and into the tubules of the kidney where urine is formed. Most of the sodium, chloride, and water are reabsorbed into the blood before the filtered fluid leaves the kidney in the form of urine. To make matters even more complex, there are different mechanisms that are active in different parts of the tubules that affect the reabsorption of sodium and chloride. [1]

Boerhaavia diffusa (Nyctaginaceae family) is a herbaceous perennial plant, native of India and Brazil, where it was used for centuries as a medicinal plant by indigenous populations. The root of B. diffusa is used for the treatment of many diseases, such as liver disorders (jaundice, hepatitis, etc.), gastrointestinal disorders (as laxative), renal disorders (for calculations, cystitis and nephritis), and for the treatment of anaemia and of menstrual syndrome. The

Corresponding Author:-Vidhi Sharma.
Address:-Dr. Apj abdul kalam university, bypass road, arandia village, post jhalaria, indore (m.p).

drug has recently been used as an adjuvant in an anticancer therapy.[2] Spironolactone is a synthetic 17-lactone drug which is a renal competitive aldosterone antagonist in a class of pharmaceuticals called potassium-sparing diuretics, used primarily to treat heart failure, ascites in patients with liver disease, low-renin hypertension, hypokalemia, and Conn's syndrome.

This Ayurveda herb is found throughout India. It is a creeping and spreading perennial herb, with a stout root-stock and many erect or spreading branches. It grows up to 2 meters in length. The leaves of the plant are simple, broad, somewhat rough, thick and brittle. The flowers are pink or red in colour. The fruits are oval in shape, dull green or brownish in colour and about the size of caraway bean.

White Punarnava

Red Punarnava

Ancient ayurvedic texts describe Punarnava as of two types, white and red. Both of them have identical medicinal value. Punarnava is slightly bitter in taste and is considered hot, light and dry in effect. It balances all Doshas (Vata, Pitta and Kapha) in the body. Besides potassium nitrate, it contains an alkaloid, which is known as punarnavine. Experimental studies have confirmed the diuretic properties of Punarnava. It is useful in obesity, anemia cases, loss of appetite, jaundice and chronic but non-specific febrile conditions. It is also anti-inflammatory, mildly laxative and also a heart tonic. Punarnava is also known to possess properties to cure skin and soft tissue infections.

Scientific Classification

1. Botanical Name: Boerhaaviadiffusa
2. Family: Nyctaginaceae
3. Division: Magnoliophyta
4. Class: Magnoliopsida
5. Order: Caryophyllales
6. Genus: Boerhaavia
7. Species: Diffusa , hirsute

Vernacular names of punarnava in various languages

1. Linn (Latin)Boerhaviadiffusa , Boerhaaviarepens
2. Sanskrit Punarnava
3. Hindi LalPunarnava, Beshakapore, San
4. English Spreading Hogweed, Shothagni, Red Hogweed, Raktapunarnava
5. Bangali Punarnnava
6. Punjabi Khattan
7. Kannada Kommeberu, Komma

Pharmacological Activity

Antioxidant Activity:

The evaluation of the antioxidant potential of ethanolic extract of Andrographis echioides and Boerhaavia diffusa was carried out by determining the levels of enzymatic and non-enzymatic antioxidants. The results showed that both the plant extracts possessed significant levels of enzymatic and non-enzymatic antioxidants. The results of the enzymatic and non-enzymatic antioxidants in Andrographis echioides and Boerhaavia diffusa exhibits that they possess preventive and productive role to maintain the cell survival, cellular interaction and maintenance of cell membrane architecture.

Antidiabetic Activity:
The study indicates that Boerhaavia diffusa and ethanolic extracts exhibit significant anti- hyperglycemic activities in alloxan induced as well as streptazotocin induced hyperglycemic rats. They can also improve the condition of diabetes as indicated by parameters like body weight along with serum cholesterol and triglyceride levels. The number of functionally intact β-cells in the islet organ is of decisive importance for the development course and outcome of diabetes.

Antibacterial Activity:
A Potent antibacterial activity against gram positive and gram negative bacteria shown by the leaves of B. diffusa might be due to the phytochemicals present in the leaves. Ethanol extract showed inhibitory an effect on gram positive bacteria like S. aureus, B. subtilis, S. faecalis, M. luteus and all gram-negative bacteria selected for the present study. Methanol extract showed inhibitory effect against all gram-positive bacteria selected for the present study except M. luteus and gram-negative bacteria like K. pneumoniae, P. vulgaris, S. marcescens and S. flexneri21.

Antistress / Adaptogenic / Immunomodulatory Activity:
Hydroethanolic extract (80%) of Boerhaavia diffusa (HEBD) and a polyherbal formulation (Punarnava mandur) PHF-09 containing Boerhaavia diffusa were compared for their antistress activity using cold restraint stress model. Stress was induced by subjecting animals to cold restraint. Due to cold restraint stress there was an imbalance in the levels of biochemical parameters like glucose, triglycerides, cholesterol, SGOT, SGPT which were near normalized following the administration of HEBD and PHF-09. HEBD and PHF-09 were found to have comparable anti-stress activity.

The ethanol extracts of roots of B. diffusa was evaluated for antistress, adoptogenic activity in albino mice, by swim endurance test and cold restrains stress and the extract showed improved stress tolerance in immunomodulatory activity was shown by increased carbon clearance, indicating stimulation of the reticuloendothelial system. There was an increase in DTH response to SRBC in mice, corresponding to cell mediated immunity and indicating stimulatory effects on lymphocytes and accessory cell types.

Adaptogens is useful in both adrenal hyperstress as well as adrenal hypofatigue. By definition, an adaptogen implies the capability for bi directional or normalizing effects. The most important adaptogens for the adrenals include Panax Ginseng, Siberian Ginseng, Ashwagandha, Rhodiolia, Boerhaavia diffusa, and Holybasil Leaf Extract. Boerhaavia diffusa (Punarnava) has the ability to support both adrenal over and under activation. In stressful conditions it has demonstrated the ability to buffer the elevations of serum cortisol and prevent the suppression of the immune system that takes place with elevated cortisol. On the other hand, Boerhaavia diffusa has also demonstrated the ability to improve cortisol levels with end stage adrenal exhaustion [28].

Hepatoprotective Activity:
The hepatoprotective activity of roots of different diameters were collected in three seasons, rainy, summer and winter, and examined in thioacetamide intoxicated rats. The results showed that an aqueous extract (2 ml/kg) of roots of diameter 1-3 cm, collected in the month of May (summer), exhibited marked protection of a majority of serum parameters, i.e., GOT, GPT, ACP and ALP, but not GLDH and bilirubin, thereby suggesting the proper size and time of collection of B. diffusa L. roots for the most desirable results.

Analgesic/Anti-Inflammatory Activity:
The Decoction (DE) or Juice (JE) of the leaves of Boerhaavia diffusa were used to study the antinociceptive effect in chemical (acetic acid) and thermal (hot Plate) models of hyperalgesia in Mice. The DE, raised the pain thresholds during the first period (30 min) of observation. In the acetic acid-induced abdominal writhing in mice, pre-treatment of the animals with naloxone (5 g/kg, i.p.) significantly reversed the analgesic effect of morphine and JE but not that of DE. The study proves that the active antinociceptive principle of B. diffusa is present mainly in the juice of fresh leaves and has a significant antinociceptive effect when assessed in these pain models28. [18]

Antitumor Activity:
Cancer chemo preventive property of B. diffusa was evaluated on 7,12-dimethyl benz(a)anthracene (DMBA) induced skin papillomagenesis in male Swiss albino mice (6-7 weeks old). The cancer chemopreventive efficacy

was assessed by its ability to modulate the activities of enzymes associated with drug metabolism and bifunctional modulators reduced the availability of ultimate carcinogen metabolites in the epithelial stage.

Anti-Convulsant Activity:
The study was carrired out to investigate the methanolic root extract of B. diffusa and its different fractions including liriodendrin-rich fraction for exploring the possible role of liriodendrin in its anti-convulsant activity. Air-dried roots of B. diffusa were extracted with methanol by cold maceration. The methanol soluble fraction of extract thus obtained was successively extracted to obtain liriodendrin rich fraction and two side fractions, that is, chloroform fraction and phenolic compound fraction. Anti-convulsant activity of methanolic extract and its different fractions, that is, liriodendrin-rich fraction and phenolic compound fraction were studied in pentylenetetrazol (PTZ)-induced seizures.

Antiproliferative and Antiestrogenic Activity:
Antiproliferative and antiestrogenic properties of methanol extract of Boerhaavia diffusa (BME) in MCF-7 breast cancer cell lines. Boerhaavia diffusa extracts exhibited a strong inhibitory effect on the proliferation of human breast cancer cells in vitro and the antiestrogenic effects are mediated by ER. Phytochemical studies have revealed the presence of alkaloids, flavonoids, phenols and saponins in BME. The antiestrogenic activity shown by the extract may be attributed to these diverse compounds. [6,7]

Cytological Activity:
The extract of B. diffusa exhibited a strong depressive effect on the mitosis of C. jagus roots. The study was conducted using B. diffusa extract, the mitotic index of the control experiment was found to be 5.27. There was a negative correlation between the concentrations of the treatment extracts and the mitotic indices obtained from their action. This points to an inhibition of mitosis by this extract. Inhibition of the mitotic index increased significantly with an increase in the concentration of treatment solution of B. diffusa. This again shows a very negative correlation between the concentration of the extract and the mitotic indices produced by the observed action.

Bronchial Asthma:
Dried leaves of Punerneva can be used in dhoomapana in treatment of bronchial asthma. The leaf decoction is said to be an excellent expectorant when decocted with punarnava (Boerhaavia diffusa) and then combined with ginger juice and black pepper. [46]

Anti Fibrinolytic Activity:
A study evaluated the effect of anti-fibrinolytic agents; α aminocaproic acid (α-ACA), tranexamic acid (AMCA); anti-inflammatory drugs (indomethacin, ibuprofen, naproxen); and plant extract (root extract of Boerhaavia diffusa) on endometrial histology of IUD-fitted menstruating monkeys. It is effective in reducing stromal edema, inflammation, and atortuosity of glands, and in increasing the degree of deposition of fibrin and platelets in the vessel lumen. [17]

Anti-Arthritic activity:
The extract of the root of the plant Boerhaavia diffusa was studied for their Anti rheumatoid activity in Freunds Adjuvant induced Arthritis rats with the dose of 500 and 1000 mg/kg p.o. The administration of extract reported significant reduction in paw swelling on 4th,8th,14th and 21st day after sub-plenter administration of complete Freunds adjuvant.[15] The paw swelling was measured as a volume displacement using digital plethysmometer.

Antiurolithic activity:
Evaluated the antiurolithic activity of Boerhaavia diffusa root aqueous extract (BDE) as prophylaxis for renal stones. In vitro calcium oxalate (caOx) crystallization inhibitory effect of BDE was determined by measuring chande in turbidity at 620nm on addition of sodium oxalate in the synthetic urine.

Phytochemicals Present In Punarnava
Generally whole plant consists the following phytochemical constitiutents; those are punarnavine (Alkaloids), B-sitosterol (Phytosterols), Liriodendrin (lignans), Punarnavoside (Rotenoids), Academic Sciences Mayank et al. Int J Curr Pharm Res, Vol 4, Issue 2, 4-8 5 Boerhavine (Xanthones) and Potassium nitrate (Salts).

The roots contain the rotenoidsboeravinones AI, BI, C2, D, E and F besides the new dihydroisofurenoxanthin, Alanine, Arachidic Acid, Aspartic Acid, Behenic Acid, Beta-Sitosterol,BoeravinoneA - F, Boerhaavic Acid, Borhavine, Borhavone, Campesterol, Daucosterol, BetaEcdysone, Flavone, 5-7-dihydroxy-3'-4'-dimetho, Xy-6-8-dimethyl, Galactose, Glutamic Acid, Glutamine, Glycerol, Glycine, Hentriacontane N, Heptadecyclic Acid, Histidine, Hypoxanthine-9-larabinofuranoside, Leucine, Liriodendrin, Methionine, Oleaic Acid, Oxalic Acid, Palmitic Acid, Proline, Proline, hydroxy, Serine, SitosterolOleate, SitosterolPalmitate, Stearic Acid, Stigmasterol, Syringaresinol-mono-beta-d-glucoside, Threonine, Triacontan-1-OL, Tyrosine, Ursolic Acid, Valine, Xylose, triacontanolhentriacontane, ß- sitosterol, ursolic acid, 5, 7-dihydroxy-3, 4-dimethyoxy-6, 8-dimethyl flavone, and an unidentified ketone (mp 86°).

The roots contain the rotenoidboeravinones AI, BI, C2, D, E and F besides the new dihydroisofurenoxanthin and an antifibrinolytic agent, two lignans, liriodendrin and syringaresinol mono-ß-D-glucoside, have also been reported in the root.

Four new compounds were isolated from Boerhaavia diffusa namely (i) eupalitin 3-O-β-D-galactopyranosyl-(1”>2”)-O-β-D-galactopyranoside, (ii) 3,3’,5-trihydroxy-7-methoxyflavone (iii) 4’,7-dihydroxy-3’-methylflavone and (iv) 3,4-dimethoxyphenyl-1-O-β-D-apiofuranosyl-(1”>3’)-O-β-D-glucopyranoside (Maurya et al., 2007).

Material and methods:-

Plant material

The roots of boerhaavia diffusa were collected in the month of September from area Udaipur market, Udaipur (Rajasthan) India. The roots were cut into small pieces and dried. Root was identified by their vernacular names and latter validated at B.N. College of Pharmacy, Udaipur (Rajasthan).

Extraction and Phytochemical screening

Roots were thoroughly washed under fresh tap water and shade dried and powdered by using a mechanical grinder. The preparation of alcoholic extract of roots of Boerhaavia diffusa was done by using soxhletation in the Department of Pharmacology, B.N.College of Pharmacy, Udaipur. About 200 g of root powder was taken into the soxhlet apparatus and extracted using ethanol (95%). The extraction process was carried out for 18 - 20 h till the appearance of colourless solvent in the side tube. The extract collected was dried by evaporating the solvents on a water bath maintained at <500C and percentage yield of alcoholic extract was recorded with respect to the total quantity of powder used for the extraction. Phytochemical evaluation for the extract was performed using standard procedures.

Experimental work:

Physiochemical analysis

Physiochemical analysis of powdered root was done by the standard reported method.

Loss on drying

Five gm of powder was dried in oven at 105ºC and weighed.

Determination of Ash value

2 gm of the crude drug was incinerated at about 450°C. It was cooled, weighed and total ash was calculated.

Determination of acid insoluble ash

The total ash obtained above was boiled with 25ml of 2M HCl for 5 min. It was collected on a tared filter paper, dried and then weighed (Table 2).

Determination of water soluble ash

The total ash was boiled for 5 min. with 25ml of water, collected on a tared filter paper, dried
And weighed. The weight of the insoluble matter was subtracted from the weight of the ash to get water soluble ash.

Determination of solvent extractive value

Percentage of water soluble extract or alcohol soluble extract was determined with reference to air dried drug.

Determination of alcohol soluble extractive value

Five gm of drug powder was macerated with 100ml of ethanol for 24 hr. It was shaken
Frequently for first six hrs. It was then filtered. 25ml of the alcoholic extract was evaporated to dryness in a petridish. It was further dried at 105ºC and weighed. Percentage of ethanol soluble extractive value was calculated with reference to air dried drug taken

Determination of water soluble extractive value

Five gm of drug powder was macerated with 100ml of chloroform water for 24 hr with shaking.After filtration solvent was evaporated to dryness (105ºC), weighed, & percentage of water soluble extractive value was calculated.

Preparation of extract

Material and method:-

1. Soxhelet apparatus
2. Heating mentle
3. Cotton
4. Glass rod
5. Solvents
6. Dried roots
7. Pastal & mortar
8. Pipes

Fig 1:-Soxhlet extraction equipment.

1. Plant material can be fresh or dried. It needs to be crushed, using a pestle and mortar, to Provide a greater surface area.
2. The plant material should be sufficient to fill the porous cellulose thimble.
3. Following this, the solvent (250 ml of ethanol) is added to a round bottom flask, which is attached to a Soxhlet extractor and condenser (Fig. 1) on an isomantle.
4. The crushed plant material is loaded into the thimble, which is placed inside the Soxhlet extractor.
5. The side arm is lagged with glass wool. The solvent is heated using the isomantle and will begin to evaporate, moving through the apparatus to the condenser. 52
6. The condensate then drips into the reservoir containing the thimble.
7. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again. The process should run for a total of 48 hours. The equipment can be turned On and off when over and the dried extract is collected in the petridish for further testing.

In-Vivo Pharmacological Study

Acute toxicity study

Determination of LD50: The acute toxicity [14, 15] of alcoholic extract of roots of Boerhaavia diffusa was determined by using albino rats, maintained under standard husbandry conditions. The animals were fasted for 3 h prior to the experiment and the extract was administered as single dose and observed for the mortality up to 48 h study period (short term toxicity). Based on the short term toxicity profile, the next dose of the extract was determined as per OECD guidelines No.420. The maximum dose tested (2000 mg/kg) for LD50. From the LD50, doses like 1/20th, 1/10th and 1/5th were selected and considered as low, medium and high dose i.e., 100 mg/kg, 200 mg/kg, 400 mg/kg respectively.[10]

Grouping and dosing criteria
Total 20 animals (N=20) will be divided into 5 groups of 4 (n=4) animals each and will be put in metabolic cages.
Group 1 (Control): Normal saline 5 ml/kg body weight, orally
Group 2 (Standard): Frusemide 20mg/kg body weight, orally
Group 3 (Test A): Boerhaavia diffusa linn (Punarnava) (100mg/kg) body weight, orally
Group 4 (Test B): Boerhaavia diffusa linn (Punarnava)(200mg/kg) body weight, orally
Group 5 (Test C): Boerhaavia diffusa linn (Punarnava)(300mg/kg) body weight, orally

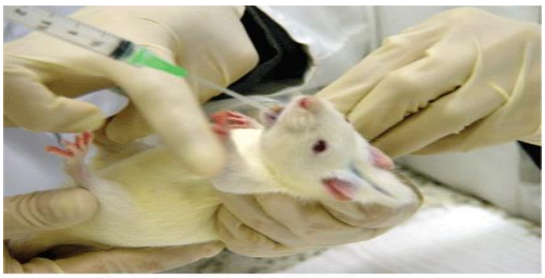


Figure 1 - Administration of DMBA by gavage.

Inclusion criteria:
Adult male wistar rats weighing 150-200grams.
Healthy animals with normal behavior.

Exclusion criteria:
Animals weighing more than 200 grams and less than 150 grams.
Animals previously used for any other experiment procedure.

Sample Collection (Urine)
Male wistar rats were divided into 5 groups of 4 rats in each. The group I serves as normal control received vehicle (CMC 2% in normal saline 10 ml/kg b.wt), the group II received Furosemide (10 mg/kg, p.o) in vehicle; other groups III, IV, V were treated with low, medium, and high doses of alcoholic extract of roots of Boerhaavaia diffusa in vehicle and immediately after the extract treatment all the rats were hydrated with saline (15 ml/kg) and placed in the metabolic cages (2 per cage), specially designed to separate urine and faeces and kept at 21°C±0.5°C. The total volume of urine collected for 5 hr was measured at the end. During this period no food and water was made available to animals. Various parameters like total urine volume and concentration of sodium, potassium and chloride in the urine were measured and estimated respectively.

Urine collection and separation

Result & Observation:-

Study of morphological characteristics roots of Boerhaavia diffusa

S.NO	CHARACTER	OBSERVATION
1	Color	Yellowish to dark Brown
2	Odour	Characteristic odour
3	Taste	Bitter
4	Size	20cm in length
5	Shape	Oval

6	Texture	Rough

Physicochemical parameters and solvent extractive values

S. No	PARAMETER VALUE	(% W/W)	IP LIMIT
1.	Foreign Organic Matter	0.2	± 2.0%
2.	Loss on drying	2.3	± 10%
3.	Total Ash	8.6	± 10%
4.	Acid Insoluble Ash	2.4	± 3.0%
5.	Water Soluble Ash	4.8	---
6.	Alcohol soluble Extractive	18.6	± 0.5%
7.	Water soluble Extractive	23.2	± 9.0%

% yield, consistency and colour of extractof Boerhaavia diffusa

S.NO	Extract	Colour	Consistency	% Yield
1	Alcoholic	Dark brown	Semi-solid	9.72

Phyto-chemical screening

S.NO.	CHEMICAL TESTS	ALCOHOLIC EXTRACT
1.	Alkaloids Tannic Acid Test	(+)
2.	Glycoside Keller Killiani Test	(+)
3.	Flavonoids Alkaline Reagent Test	(+)
4.	Tannins Vanillin Hydrochloride Test	(+)
5.	Saponins Froth test	(+)
6.	Proteins Ninhydrin test	(+)
7.	Carbohydrates Molisch test	(+)
8.	Quinines Sulfuric Acid test	(-)
9.	Phenols-Ferric Chloride test	(+)
10.	Gum & Mucilage	(-)
11.	Steroids Libermann-Burchard test	(+)
12.	Lipid Stain test	(+)

Urine Analysis

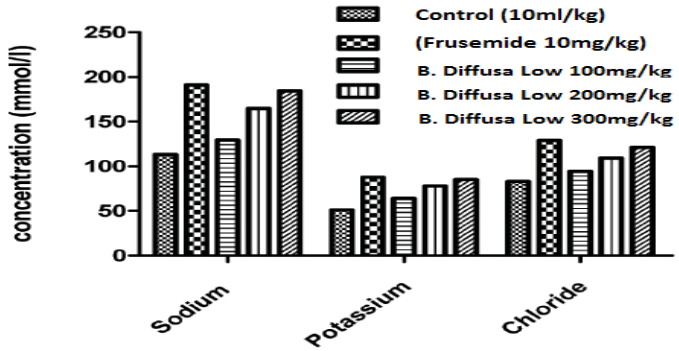
GROUP	VOLUME OF URINE (ML)
Group A (Normal group)	18 ml
Group B (Standatd group)	25 ml
Group C (AEBD 100mg/kg)	20 ml
Group D (AEBD 200mg/kg)	17.7ml

Group E (AEBD 300mg/kg)	22 ml

Statistical analysis

All the values are expressed as mean±standard error of mean (S.E.M.) and analysed for ANOVA (analysis of variance) by employing statistical software, GraphPad, InStat 3. Differences between groups were considered significant at $p < 0.05$ levels.

s.no	Groups	Total Urine Vol (ml/kg b.wt/5 h)	Na+ mmol/L	K+ mmol/L	Cl- mmol/L
1	Control (10 ml/Kg)	13.45±0.02	113.03 + 2.16	51.09 + 1.51	82.95 + 1.42
2	Standard (Frusemide 10 mg/kg b.wt)	22.23±0.01***	191.05+2.09***	87.81+1.60***	129.06+1.67***
3	Alcoholic extract of roots of b.diffusa (100 mg/kg)	15.20±0.02***	129.40+2.80***	64.13+1.82***	94.42 + 1.73***
4	Alcoholic extract of roots of b.diffusa (200 mg/kg)	17.41±0.02***	164.99+2.00***	77.93+2.67***	109.44+1.20***
5	Alcoholic extract of roots of b.diffusa (300 mg/kg)	20.46±0.02***	184.53+2.32***	85.11+1.79***	121.39+2.00***



[Table/Fig-2]: Effect of alcoholic extract of roots of **b.diffusa** on urinary sodium, pot assium, chloride (mmol/l) ions concentration in hydrated rat model in albino rats

Conclusion:-

Results showed that single dose administration of alcoholic extract of roots of *Boerhaavia diffusa as* 100,200 and 300 mg/Kg and standard Furosemide (10 mg/kg) have increased the urinary output along with an increase in concentration of Sodium, Potassium and Chloride ions in urine. Alcoholic extract of roots of *Boerhaavia diffusa* mg/Kg produced a greater diuretic activity which is comparable to that of standard Furosemide (10 mg/kg). In traditional medicine the plant is used for its diuretic activity. This study come up with identification of so many phytoconstituents reported earlier for this diuretic effect in alcoholic extract of roots of *Boerhaavia diffusa*. Thus the study supports and justifies the rationale behind the use of roots of *Boerhaavia diffusa* its diuretic activity.

References:-

1. Abdallah JG, Schrier RW, Edelstein C, et al. Loop diuretic infusion increases thiazide-sensitive Na(+)/Cl(cotransporter abundance: role of aldosterone. J Am Soc Nephrol 2001; 12:1335.
2. Amsler K, Kinne R. Photoinactivation of sodium-potassium-chloride cotransport in LLC-PK1/Cl 4 cells by bumetanide. Am J Physiol 1986; 250:C799.
3. Batlle DC, von Riotte AB, Gaviria M, Grupp M. Amelioration of polyuria by amiloride in patients receiving long-term lithium therapy. N Engl J Med 1985; 312:408.
4. Bock HA, Stein JH. Diuretics and the control of extracellular fluid volume: role of counterregulation. Semin Nephrol 1988; 8:264.
5. Boer WH, Koomans HA, Dorhout Mees EJ. Acute effects of thiazides, with and without carbonic anhydrase inhibiting activity, on lithium and free water clearance in man. Clin Sci (Lond) 1989; 76:539.
6. Bronner F. Renal calcium transport: mechanisms and regulation--an overview. Am J Physiol 1989; 257:F707.
7. Carr MC, Prien EL Jr, Babayan RK. Triamterene nephrolithiasis: renewed attention is warranted. J Urol 1990; 144:1339.
8. Costanzo LS. Localization of diuretic action in microperfused rat distal tubules: Ca and Na transport. Am J Physiol 1985; 248:F527.
9. Dirks JH, Cirksena WJ, Berliner RW. Micropuncture study of the effect of various diuretics on sodium reabsorption by the proximal tubules of the dog. J Clin Invest 1966; 45:1875.
10. Ellison DH, Velázquez H, Wright FS. Adaptation of the distal convoluted tubule of the rat. Structural and functional effects of dietary salt intake and chronic diuretic infusion. J Clin Invest 1989; 83:113.
11. Fairley KF, Woo KT, Birch DF, et al. Triamterene-induced crystalluria and cylinduria: clinical and experimental studies. Clin Nephrol 1986; 26:169.
12. Farge D, Turner MW, Roy DR, Jothy S. Dyazide-induced reversible acute renal failure associated with intracellular crystal deposition. Am J Kidney Dis 1986; 8:445.
13. Friedman PA. Basal and hormone-activated calcium absorption in mouse renal thick ascending limbs. Am J Physiol 1988; 254:F62.
14. Frindt G, Sackin H, Palmer LG. Whole-cell currents in rat cortical collecting tubule: low-Na diet increases amiloride-sensitive conductance. Am J Physiol 1990; 258:F562.
15. Garg LC, Narang N. Effects of hydrochlorothiazide on Na-K-ATPase activity along the rat nephron. Kidney Int 1987; 31:918.
16. Gesek FA, Friedman PA. Mechanism of calcium transport stimulated by chlorothiazide in mouse distal convoluted tubule cells. J Clin Invest 1992; 90:429.
17. Greger R, Velázquez H. The cortical thick ascending limb and early distal convoluted tubule in the urinary concentrating mechanism. Kidney Int 1987; 31:590.
18. Guyton AC. Blood pressure control--special role of the kidneys and body fluids. Science 1991; 252:1813.
19. Hebert SC, Reeves WB, Molony DA, Andreoli TE. The medullary thick limb: function and modulation of the single-effect multiplier. Kidney Int 1987; 31:580.
20. Hoover RS, Poch E, Monroy A, et al. N-Glycosylation at two sites critically alters thiazide binding and activity of the rat thiazide-sensitive Na(+):Cl(-) cotransporter. J Am Soc Nephrol 2003; 14:271.
21. Horisberger JD, Giebisch G. Potassium-sparing diuretics. Ren Physiol 1987; 10:198.
22. Hufnagle KG, Khan SN, Penn D, et al. Renal calcifications: a complication of long-term furosemide therapy in preterm infants. Pediatrics 1982; 70:360.
23. Ikram H, Chan W, Espiner EA, Nicholls MG. Haemodynamic and hormone responses to acute and chronic frusemide therapy in congestive heart failure. Clin Sci (Lond) 1980; 59:443.
24. Kleyman TR, Cragoe EJ Jr. The mechanism of action of amiloride. Semin Nephrol 1988; 8:242.

25. Kunau RT Jr, Weller DR, Webb HL. Clarification of the site of action of chlorothiazide in the rat nephron. J Clin Invest 1975; 56:401.
26. LEAF A, SCHWARTZ WB, RELMAN AS. Oral administration of a potent carbonic anhydrase inhibitor (diamox). I. Changes in electrolyte and acid-base balance. N Engl J Med 1954; 250:759.
27. Leviel F, Hübner CA, Houillier P, et al. The Na+-dependent chloride-bicarbonate exchanger SLC4A8 mediates an electroneutral Na+ reabsorption process in the renal cortical collecting ducts of mice. J Clin Invest 2010; 120:1627.
28. Liu FY, Cogan MG. Angiotensin II: a potent regulator of acidification in the rat early proximal convoluted tubule. J Clin Invest 1987; 80:272.
29. Loon NR, Wilcox CS, Unwin RJ. Mechanism of impaired natriuretic response to furosemide during prolonged therapy. Kidney Int 1989; 36:682.
30. Maronde RF, Milgrom M, Vlachakis ND, Chan L. Response of thiazide-induced hypokalemia to amiloride. JAMA 1983; 249:237.
31. Morgan DB, Davidson C. Hypokalaemia and diuretics: an analysis of publications. Br Med J 1980; 280:905.
32. Nijenhuis T, Hoenderop JG, Loffing J, et al. Thiazide-induced hypocalciuria is accompanied by a decreased expression of Ca2+ transport proteins in kidney. Kidney Int 2003; 64:555.
33. Nomura A, Yasuda H, Minami M, et al. Effect of furosemide in congestive heart failure. Clin Pharmacol Ther 1981; 30:177.
34. O'Grady SM, Palfrey HC, Field M. Characteristics and functions of Na-K-Cl cotransport in epithelial tissues. Am J Physiol 1987; 253:C177.
35. Osborn JL, Holdaas H, Thames MD, DiBona GF. Renal adrenoceptor mediation of antinatriuretic and renin secretion responses to low frequency renal nerve stimulation in the dog. Circ Res 1983; 53:298.
36. Plotkin MD, Kaplan MR, Verlander JW, et al. Localization of the thiazide sensitive Na-Cl cotransporter, rTSC1 in the rat kidney. Kidney Int 1996; 50:174.
37. Preisig PA, Toto RD, Alpern RJ. Carbonic anhydrase inhibitors. Ren Physiol 1987; 10:136.
38. Ram CV, Garrett BN, Kaplan NM. Moderate sodium restriction and various diuretics in the treatment of hypertension. Arch Intern Med 1981; 141:1015.
39. Ridgeway NA, Ginn DR, Alley K. Outpatient conversion of treatment to potassium-sparing diuretics. Am J Med 1986; 80:785.
40. Rouch AJ, Chen L, Troutman SL, Schafer JA. Na+ transport in isolated rat CCD: effects of bradykinin, ANP, clonidine, and hydrochlorothiazide. Am J Physiol 1991; 260:F86.