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To Study Diuretic Activity of Ethanolic Extract of Leaves of Mentha Spicata by Using Experimental Animal

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ABSTRACT: This study was to evaluate the In-vivo Pharmacological studies on leaves of Mentha spicata in validated experimental animal models. The present work deals with the "Study of Diuretic Activity of Ethanolic extract of leaves of Mentha spicata by using experimental animal". Leaves of plant were collected, dried, pulverized, packed and stored in cool place. Morphological studies of leaves and physiochemical characterization of powered drug was done. It was extracted by soxheletion method, yielded was found 11.4%. Phytochemical Screening of Ethanolic Extracts of Mentha spicata extract revealed presence of carbohydrates, Tannins Phenols, Alkaloids, Glycosides, Flavonoids, steroids and proteins. Antioxidant activity was increased as increasing concentration of Mentha spicata extract. Acute oral toxicity study was evaluated on Wistar albino rats. Total 24 Male & female rats were used, standard drug furosemide, 100mg/kg body weight and 200 mg/kg body weight were taken, urine was collected in beaker for 5 hours. Collected urine volume was measured pH was calculated and Concentrations of the different electrolytes Na^+, Cl^- and K^+ Diuretic activity, Saluretic activity, Natriuretic activity, CAI, Diuretic index, Saluretic Index, Natriuretic Index and CAI Index were also calculated.

KEYWORDS: Mentha spicata, Ethanolic, Extract, **Diuretic Activity,** Furosemide,

I. INTRODUCTION

Plant and other natural products have been in use for the human sufferings from time immemorial[1]. The search for new chemical entities obtained by screening natural sources such as plant extracts and microbial fermentation had led to the discovery of many clinically useful drugs that play a major role in the treatment of human diseases[2]. Today, higher plants continue to retain their historical significance as important sources of novel compounds useful either directly as medicinal agents or as lead compounds for synthetic/semi synthetic structural Modification/optimization or biochemical/pharmacological probes[3]. Natural products, in particular herbs, have been used for the treatment of various diseases for thousands of years[4]. The pharmacological behavior of illness began long ago with the use of therapeutic plants. Methods of folk curative during the world commonly used herbs as part of their custom[5]. Ayurved is a medical system primarily skillful in India that has been recognized for almost 5000 years[6].

Diuretics are used to treat a number of cardiovascular and renal related disorders. Allopathic medicines do not have a safe role in this regard. Many indigenous drugs have been claimed to have diuretic effect in traditional medicine but they were not properly investigated[7]. Naturally occurring diuretics are more safe and efficacious. Several plant derived chemical entities have proved to be more efficacious and safe. It remains for the modern scientists to give scientific validation for the herbs claimed for therapeutic activity to make use of herbal potential in a more productive way[8]. The most common adverse effect is electrolyte imbalances such as hypokalemia or hyponatremia and excessive fluid depletion[9]. Hypokalemia is one of the main problems with diuretics and can produce serious metabolic and cardiac issues. Excessive fluid depletion may lead to a reflex increase in cardiac output and vascular resistance due to the baroreceptor reflex being activated. This increased demand on the heart can become an issue for patients, especially those suffering from cardiac disease[10]. Other side effects associated with diuretics are orthostatic hypotension and hyperlipidemia[11]. Diuretics are contraindicated in patients that are diagnosed with gout[12].

The main objective of the study was to evaluate the In-vivo Pharmacological studies on

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leaves of Mentha spicata in validated experimental animal models. The present work deals with the "Study of Diuretic Activity of Ethanolic extract of leaves of Mentha spicata by using experimental animal"

II. MATERIALS AND METHOD

Collection of Plant material: The Leaves of plant Mentha spicata were collected from agriculture area from Gwalior, Madhya Pradesh, India and authenticated by Department of Botany.

Drying and Size Reduction of Plant Material: 1kg of Leaves of Mentha spicata (Lamiaceae) was collected & washed with distilled water to remove dirt and soil. The collected plant leaves was dried under shade at room temperature for 10 to 15 days. The dried plant leaves was powder by using a grinding mill to obtain a coarse powder and then passed through 40 mesh sieve.

Screening of Powder (Physiochemical Analysis)[57]: Physiochemical screening of powdered leaves was done by the standard reported methods. Physiochemical screening was also uses as standardization of the plant, the obtained values of these parameters were useful in the characterization of plant. Screening parameters are loss on drying, total ash value, acid insoluble ash value, water soluble ash value and foaming index.

Extraction of leaves of Mentha spicata[58]: Initially 100 gm of crude powder was taken and packed in a packing paper. This pack was placed in a soxhlet extractor & extract with ethanol, the extraction was carried out until the extract becomes colorless. The extract was then filtered with what man filter papers (No.1) and the filtrate was evaporated to dryness in rotary evaporator at 40 ºC. The obtained crude extract was stored in a refrigerator at 4º C until time of use.

Percentage Yield: The Percentage yield of the extract was calculated by using the following formula:

$$\% \text{ Yield} = \frac{\text{Weight of plant extract obtained}}{\text{Weight of plant matrial used}} \text{ X } 100$$

Phytochemical analysis of crude extract [59,60]: Standard screening test of the extract was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites such as alkaloids, glycosides, carbohydrates, proteins, flavonoids, tannins steroidal compounds, phenolic compounds, flavonoids, and saponins by using standard procedures. The extract obtained from successive Soxhlet extraction method was subjected to preliminary phytochemical analysis in order to identify the nature of constituents present in the powdered plant materials leaves of Mentha spicata.

Anti-oxidant activity by DPPH method

(a) Preparation of standard solution: Required quantity of Ascorbic acid was dissolved in methanol to give the concentration of 100, 200, 300, 400 and 500µg/ml

(b) Preparation of test sample: Stock solutions of samples were prepared by dissolving extract (10 mg) in of methanol (10 ml) to give concentration of 1mg/ml or 1000 µg/ml. Separately all the samples were diluted in 10 ml volumetric flask to give (100, 200, 300, 400 and 500 µg/ml concentration. Then closed by closers and wrapped by aluminium foil to protect sample dilutions from the light and stored at cool and dry place.

(c) Preparation of DPPH solution: 3.94 mg of 2, 2- diphenyl-1-picryhydrazyl radical (DPPH), a stable radical was dissolved in methanol (100ml) to give a 100 µm solution: it was protected from light by covering the test tubes with aluminium foil.

Estimation of DPPH scavenging activity[61]: 150µl DPPH solution was added to 3 ml methanol and absorbance was taken immediately at 517 nm for control reading. Diluted test sample with methanol up to 3 ml. 150µl DPPH solution was added to each test tube. Absorbance was taken at 517 nm in UV-visible spectrophotometer (Systronics 2203) after 15 min using methanol as a blank. The free radical scavenging activity (FRSA) (% antiradical activity) was calculated using the following equation:

$$\%\,(\text{FRSA}) = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{\text{Absorbance of control}} \times 100$$

Each experiment was carried out in triplicate and results are expressed as mean and percent antiradical activity.

In-vivo Pharmacological screening of extracts

Preparation of extract suspension: Accurately weighed amount (1 g) of each Crude extracts of leaves of Mentha spicata was dissolved in 10 ml of suspension of 0.5% PVP. It is ready suspension for oral introduction.

Chemicals and drugs: Furosemide tablet Lasix 40mg, Sanofi Aventis Pharmaceuticals, India), Normal saline and Urine electrolytes (sodium, potassium and chloride) were determined by Ion Selective Electrode method as described by the user

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instruction manual of Roche 9180 electrolyte analyzer (Roche, Roche Diagnostics Pvt. Ltd, Gurgaon, Haryana).

Animals care and Handling: The animal experimental protocol was approved by the Institutional Animals Ethical Committee (IAEC), Bhopal, (M.P). Approval Number, ABCD/IAEC/ July 2021/07. Male & female Wistar albino rats (140-170g) were provided by Institution, Bhopal Madhya Pradesh, India. The animals were housed in standard conditions of temperature (25 ± 2^0C) and 12:12 h light-dark cycle. The rats were fed with commercial diet and water ad Libitum. The experiment was approved by the Institutional Ethics Committee, (M. P.).

Acute oral toxicity study (OECD guideline 425)**:** Acute oral toxicity study was evaluated as per OECD guidelines (425) on Wistar albino rats. Before experimentation rats were fasted overnight with water ad libitum. Three animals were selected which receives dose of 2000mg/kg. All three animals were received dose of 2000 mg/kg body weight of ethanolic extract of Mentha spicata leaves extract by gavage using oral canula (limit test). Animals were observed individually for any toxicity sign of gross changes like convulsion, tremor, circling, depression, and mortality after dosing for 24 hours with special attention given during the first 4 hours, and thereafter, 24 hours, Administered dose was found tolerable (as no death found). Therefore, two dose levels 100 mg/kg & 200 mg/kg was selected for further activity.

Observations : Animals are observed individually for any toxicity sign of gross changes like convulsion, tremor, circling, depression, and mortality after dosing for 24 hours, with special attention given during the first 4 hours, and thereafter, 24 hours, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. No significant signs were noticed in animals. Hence administered dose was found tolerable as no death was found. Therefore, 2000mg/kg dose of extract of Mentha spicata was considered maximum safe dose.

Diuretic activity (Experimental design) [62]: The rats were divided into four groups (n=6). **Group-I** (Controlled group) administered 0.5ml blank suspension, **Group-II** (Standard group) rats receiving Furosemide orally at 10 mg/kg body weight in saline, **Group-III** (test group) rats receiving dose of leaves of Mentha spicata (MS) Extract of 100 mg/kg body weight, **Groups-IV** (test group) rats receiving dose of leaves of Mentha spicata (MS) Extract of 200 mg/kg body weight. Furosemide was used as the standard Diuretic throughout the experimentation. Immediately after the administration the animals were kept in diuretic cages (3 per cage) specially designed to separate urine and fecal matter and kept at room temperature of 25 ± 0.5º C throughout the experiment. The total volume of urine was collected at the end of 5hrs after dosing. During this period no water and food was made available to animals.

Biochemical estimation: Following Parameter were calculated:
Statistics

Total urine volume: The urine volume was measured and was expressed in ml/5h.
Urine pH: Urine pH was obtained by digital pH meter after 2 times dilution.
Concentrations of the different electrolytes (Na^+, Cl^- and K^+): The concentrations of the electrolytes Na+, Cl^- and K+ (cations), in urine was expressed in terms of mmol/L.
Diuretic index:

$$\textbf{Diuretic Index} = \frac{\textbf{Volume of Urinary excretion of test group}}{\textbf{Volume of Urinary excretion of control group}}$$

Diuretic activity:

$$\textbf{Diuretic Activity} = \frac{\textbf{Diuretic action of test Drug}}{\textbf{Diuretic action of standard drug}}$$

Saluretic activity:
Saluretic activity = $Na^+ + Cl^-$
Natriuretic activity:
Natriuretic activity = Na^+ / K^+
Natriuretic effects > 2
Potassium sparing effect > 10
Estimation of CA inhibition (CAI):
CAI = $Cl^- / (Na^+ + K^+)$
Inhibition can be excluded at ratio between 1to 0.8 with decreasing ratio to strong carbonic anhydrase inhibition can be assumed.
Saluretic Index:

$$\textbf{Saliuretic Index} = \frac{\textbf{Saluretic activity of test group}}{\textbf{Saluretic activity of control group}}$$

Natriuretic Index:

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Natriuretic Index

$$= \frac{\textbf{Natriuretic activity of test group}}{\textbf{Natriuretic activity of control group}}$$

CAI Index:

$$\textbf{CAI Index} = \frac{\textbf{CAI of test group}}{\textbf{CAI of control group}}$$

III. RESULTS AND DISCUSSION

Diuretic activity of the leaves of plant Mentha spicata extract was evaluated. Leaves of plant were collected from agriculture area from Gwalior (M.P.) and dried under shade then pulverized, packed in zip locked polythene and stored in cool. Morphological studies of leaves reveals there were green colored, menthol odor and ovate with serrated margin shaped 6-9 cm long and 1.5-3 cm broad in size with Rough surface. Powered drug was screened for physiochemical characterization found as shown as table no. 1.

Table No. 1:- Physiochemical analysis of powder of Mentha spicata

S. No.	Parameters	Leaf (%)
1	Loss on drying	9.4
2	Total ash value	8.3
3	Acid insoluble ash value	3.4
4	Water soluble ash value	1.7
5	Foaming index	9(ml)

Mentha spicata was extracted with ethanol by soxheletion method, yielded as semisolid dark green material was found 11.4%. Phytochemical Screening of Ethanolic Extracts of Mentha spicata extract revealed presence of carbohydrates, Tannins Phenols, Alkaloids, Glycosides, Flavonoids, steroids and proteins. Antioxidant activity was increased as increasing concentration of Mentha spicata extract. DPPH -assay method was used and Ascorbic acid taken as standard. 50 % inhibition was found at 300ppm concentration of Mentha spicata extract as shown in table no. 2.

Table No.2:- Antioxidant activity by DPPH assay

	% Inhibition				
Conc. (µg/ml)	100	200	300	400	500
Standard (Ascorbic acid)	54.84	43.28	34.61	32.33	25.41
Mentha spicata extract Leaf	40.17	67.88	50.40	44.56	34.95

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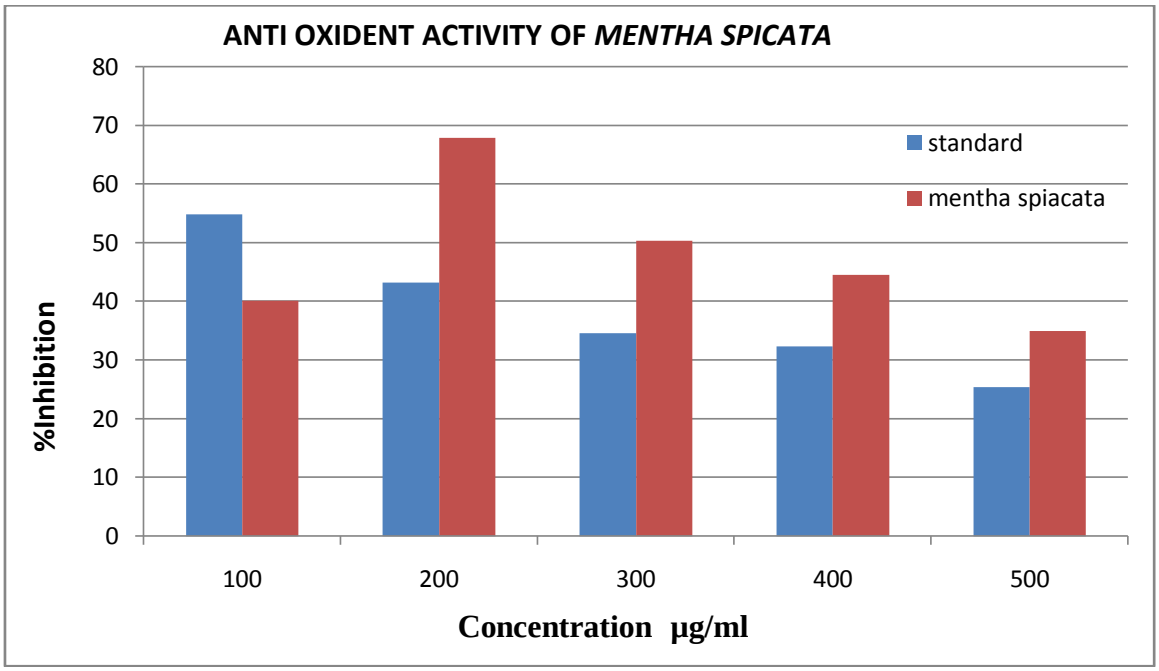

Fig. no. 1: Antioxidant activity of extracts of of Mentha spicata by DPPH assay

For the In-vivo Pharmacological screening extract was dissolved in 0.5% PVP suspension. Acute oral toxicity study was evaluated as per OECD guidelines (425) on Wistar albino rats all are living and no sign of any behavioral defects. Total 24 Male & female Wistar albino rats were used and divided in four groups, control, treated with standard drug furosemide, 100mg/kg body weight and 200 mg/kg body weight. After treatment with drug and extracts rat were kept in metabolic case and urine was collected in beaker for 5 hours. Collected urine volume was measured pH was calculated and Concentrations of the different electrolytes Na^+, Cl^- and K^+ Diuretic activity, Saluretic activity, Natriuretic activity, CAI, Diuretic index, Saluretic Index, Natriuretic Index and CAI Index were also calculated.

Urine volume collected were 2.14 ml/5h for control group, 18.32 ml/5h for standard drug treated, 10.42 for Mentha spicata (MS) Extract (100 mg/kg) and 14.12 for Mentha spicata (MS) Extract (200 mg/kg). pH for all groups was about 6. Concentrations of the electrolytes Na^+, Cl^- and K^+ in mmol/L was obtained by electrolyte analyzer as shown in table no. 3:

Table No. 3: Electrolytes Analysis in Animal Urine Sample

Group	Treatment	Urine volume ml/5h	Urine pH	Concentrations of the electrolytes (mmol/L)		
				Na^+	K^+	Cl^-
Group-I	Control	2.14 ± 0.26	5.8	135.34 ± 3.21	51.64 ± 1.72	20.52 ± 1.24
Group-II	Standard (Furosemide)	18.32 ± 0.52	6.1	152.32 ± 4.21	70.63 ± 2.64	55.63 ± 1.76
Group-III	Mentha spicata (MS) Extract (100 mg/kg)	10.42 ± 0.21	5.9	143.88 ± 3.45	67.82 ± 2.31	38.41 ± 2.14
Group-IV	Mentha spicata (MS) Extract (200 mg/kg)	14.12 ± 0.13	5.6	148.56 ± 2.75	68.53 ± 1.85	39.45 ± 1.74

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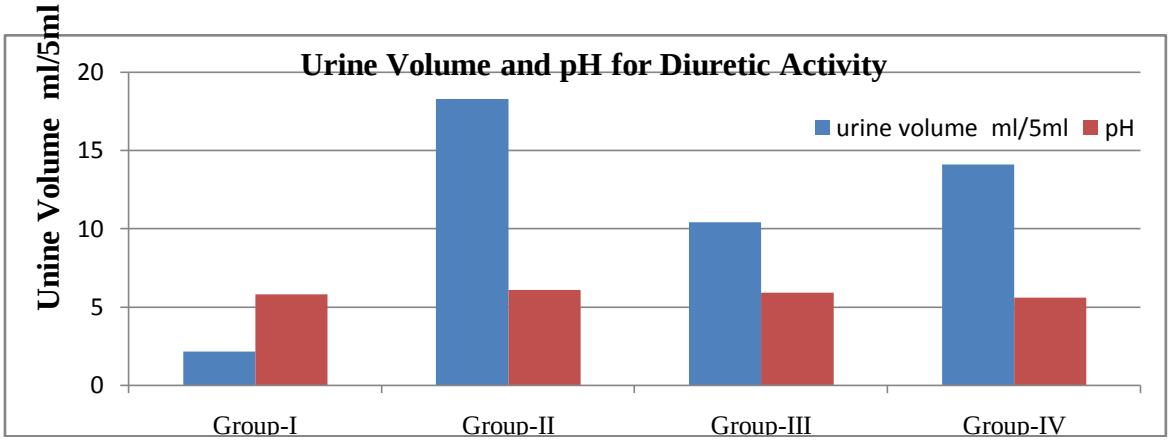

Figure no.2: Urine volume and pH for Diuretic activity of Mentha spicata

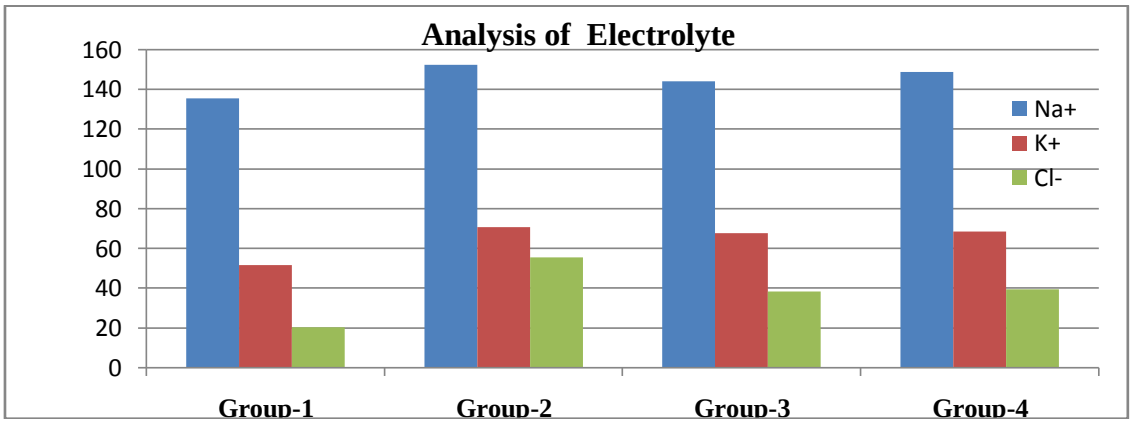

Figure no.3: Analysis of Electrolyte Concentration for Diuretic activity of Mentha spicata

Diuretic activity for Mentha spicata (MS) Extract 100 mg/kg and 200 mg/kg were calculated 0.57 and 0.77 or 57% and 77% respectively as compared to standard drug furosemide. Natriuretic activity greater than 2 showed extract exhibited natriuretic activity. CAI values near 0.20 also point the extract exhibited strong carbonic anhydrase inhibition can be assumed. Over all study showed that diuretic activity increases in dose dependent manner. Mentha spicata (MS) extract 200 mg/kg was 77% Diuretic as compared to standard drug furosemide.

Table no.4: Diuretic, Saluretic, Natriuretic activity and CAI of Animal Urine Sample

Group	Treatment	Diuretic activity	Saluretic activity	Natriuretic activity	CAI
Group-I	Control	0.12	155.86	2.62	0.11
Group-II	Standard (Furosemide)	-	207.95	2.16	0.25
Group-III	Mentha spicata (MS) Extract (100 mg/kg)	0.57	182.29	2.12	0.18
Group-IV	Mentha spicata (MS) Extract (200 mg/kg)	0.77	188.01	2.17	0.21

Table no. 5: Diuretic, Saluretic, Natriuretic Index and CAI Index of Animal Urine Sample

Group	Treatment	Diuretic Index	Saluretic Index	Natriuretic Index	CAI Index
Group-I	Control	1	1	1	1

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Group-II	Standard (Furosemide)	8.56	1.34	0.82	2.27
Group-III	Mentha spicata (MS) Extract (100 mg/kg)	4.87	1.17	0.81	1.64
Group-IV	Mentha spicata (MS) Extract (200 mg/kg)	6.59	1.21	0.82	1.91

IV. CONCLUSION

In conclusion, the present study demonstrates antioxidant activity and Diuretic activity. It seems ethanolic extract of leaf Mentha spicata able to significantly increase in volume of urine and concentration of electrolytes was obtained. Thus, antioxidant activity and Diuretic activity property of the extracts definitely attributed to the phytoconstituents they contain, which may be either due to their individual or additive effect that fastens the process of these activities. At this stage, it is difficult to say which component(s) of the extract are responsible for the above activities. However further studies are required to separate the phyto constituents responsible for the diuretic activity and to confirm the safe use in renal disorders. Further investigations also needed for evaluation these actions.

CONFLICT OF INTEREST

There are no conflicts of interests.

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