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Assessment of Diuretic Activity of Hydroalcoholic Extract of *Matricaria chamomilla* (Linn.) and Its Phytochemical Analysis with Emphasis on Toxicology

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Abstract

While the traditional medicines are derived from medicinal plants, minerals and organic matter, the herbal drugs are prepared from medicinal plants only. This current research work will be very beneficial in figuring out the Matricaria chamomilla leaves extract diuretic effect by determining the ions and electrolyte excreted by the animals using ion selective channel inhibition. The findings from the phytochemical screening indicated that afterwards, it was checked to see if alkaloids were not present by examining the absence of turbidity or formation of precipitates. The colour changed from violet to blue or green in some samples indicated the presence of steroids. An interface with a reddish-brown coloration was formed in the presence of terpenoids, as positive result. Red coloration identifies the presence of flavonoids (Shinado's test). A colour change was observed in the test tube, which indicated in the presence of tannins. Acute toxicity studies will be performed for the species Matricaria chamomilla which may have not shown any toxicity, the study results could demonstrate their relevance to the human body. Diuretic activity of the herb Matricaria chamomilla has some beneficial effect on the selected animals, the herb Matricaria chamomilla may be selected for the further studies to prove its clinical relevancy.

Keywords: Diuresis effect, hydro alcoholic extract, *Matricaria chamomilla* (Linn.), phytochemical studies

INTRODUCTION

The word diuretic has a Greek stem, diu (through) *ουρειη* (to urinate), and a diuretic is defined as any substance that increases urine flow and thereby water excretion. Diuretics are among the most commonly used drugs and the majority act by reducing sodium chloride reabsorption at different sites in the nephron, thereby increasing urinary sodium, and consequently, water loss.

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Traditional Chinese medicine (TCM) has been used for a long time in China and recorded for more than 5000 years. Numerous herbs have been used in TCM [1]. This field is becoming an integral part of various traditional medicine and modern medicine systems globally. In modern medicine, it is primarily used to prevent various diseases. Therefore, TCM is gaining popularity worldwide and supports human health greatly [2].

Chamomile is an annual or perennial plant belonging to the family Asteraceae. The plant improves the appetite and relieves painful swellings

and sweating [3]. Chamomile is native to temperate regions of Asia and Europe, and cultivated worldwide for its high medicinal, cosmetics and food value [4]. It has been used for thousands of years in Greece, Rome and ancient Egypt. In China, the detailed use of this plant was first recorded in Uyghur medicine. Veteran doctors of TCM believe that preparations containing chamomile have a calming effect. In addition, the plant is also used in other traditional, homoeopathic and Unani preparations [5, 6]. There are two main varieties of chamomile: *Matricaria chamomilia* L. (MC) and *Anthemis nobilis* (L.) All. (CN). *Matricaria chamomilia* L. belongs to the genus *Matricaria*. It is an annual plant, and the flowering period is from May to July in China. *Chamaemelum nobile* (L.) All. is a perennial plant of the genus *Chamaemelum*. The flowering period is from April to May in China [7, 8]. *Matricaria chamomilia* L. is relatively common and has been researched and used widely. At present, 26 countries around the world have included this plant in their pharmacopoeia. Chamomile flower heads are commonly used for medicinal purposes [9]. Chamomile contains flavonoids, coumarins, volatile oils, terpenes, sterols, organic acids, and polysaccharides, among other compounds. Having a wide array of compounds, chamomile exhibits various pharmacological activities such as anticancer, anti-infective, anti-inflammatory, antioxidant, hypoglycaemic, hypotensive, hypolipidaemic, antiallergic, antidepressant, and neuroprotective effects, and others [3–8]. In general, this plant has outstanding research value. Still, there are very few reviews of it in the literature. This article provides a comprehensive review of the botanical characteristics and distribution, traditional uses, chemical constituents, pharmacological effects, and quality control methods of chamomile.

The *Matricaria chamomilla* is lovely annual plant with very cheerful aromatic white daisy flowers. It is easy to grow and maintain preferring well drained soils preferably sandy. It can grow on poor soil and needs little extra care, tolerates mild to medium acid soils (up to 4.8). Deer normally do not eat it. Flowers are fairly long lasting giving a good showing in any garden. Flowers are used in tea and herbal medicines.

The use of traditional medicines as a diuretic agent proportionality was day by day increasing continually in recent years. The diuretic activity of a number of plant extracts used as diuretic agents in ethnomedicine has been confirmed in experimental animals. However, despite the widespread use of *Matricaria chamomilla* in traditional medicine, there is a paucity of data supporting its use as a diuretic agent. World Health Organization has estimated that over 75% of the world's population still relies on plant-derived medicines, usually obtained from traditional healers, for basic health-care needs. Based on the above reasons present study is, therefore an attempt to evaluate the Hydro Alcoholic Extract of *Matricaria chamomilla* by diuretic efficacy.

MATERIALS AND METHODS

Plant Collection

Fresh herbal plant materials were collected from Vattamalai. The collected plants material (leaves and/or dried flowers) were air-dried in darkness at room temperature (20ºC+/-2ºC). The dried plant material was cut up and stored in tightly sealed dark containers until needed.

Extraction of Plants

Dried plant material approximately 5 g was coarsely crushed in small pieces of 2–5 mm using the cylindrical crusher and extracted with water and ethanol, respectively. Each infusion was prepared using 5 g of dried herbal tea in 200 ml of distilled water and (equivalent to 1 teacup) at approximately 100ºC+/-1ºC for 10 min. Each aqueous extract was filtered through a paper filter and stored under refrigeration in glass flasks tapered with screw plastic lid. Both the infusion time and the solvent initial temperature used in this study have been described by several authors as efficient conditions to extract phytochemical compounds, such phenolic and flavonoid compounds from herbs.

Phytochemical Test

Chemical tests performed in the screening and identification of phytochemical constituents in the tested medicinal plants were carried out in extracts as well as powder specimens using the standard procedures.

Maeyer's Reagent

0.355 g of mercuric chloride was dissolved in 60 ml of distilled water. 5.0 g of potassium iodide was dissolved in 20 ml of distilled water. Both solutions were mixed and volume was raised to 100 ml with distilled water.

Dragendorff's Reagent

Solution A: 1.7 g of basic bismuth nitrate and 20 g of tartaric acid were dissolved in 80 ml of distilled water. Solution B: 16 g of potassium iodide was dissolved in 40 ml of distilled water. Both solutions (A and B) were mixed in 1:1 ratio.

Test for Alkaloids

About 0.5 to 0.6 g of the methanolic plant extract was mixed in 8 ml of 1% HCl, warmed and filtered. 2 ml of the filtrate were treated separately with both reagents (Mayer's and Dragendorff's).

Test for Steroids

About 0.5 g of the methanolic extract fraction of each plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid.

Test for Terpenoids

An aliquot 0.5 ml of methanolic extract was mixed with 2 ml of CHCl3 in a test tube. 3 ml of concentrated H_2SO_4 was carefully added to the mixture to form a layer.

Test for Flavonoids

To the substance in alcohol, a few magnesium turnings and few drops of concentrated Hydrochloric acid were added and boiled for five minutes.

Test for Tannins

The 0.5 g of powdered sample of each medicinal plant leaves was boiled in 20 ml of distilled water in a test tube and then filtered. The filtration method used here was the normal.

Experimental Design

Adult female Swiss mice weighing between 20–30 g were used to calculate LD50, and female and male Wistar rats with an average weight of 180–220 g were used in the study. The animals were obtained from the animal house at J.K.K. Natarajah Kumarapalayam. The experimental protocol was approved by the Institutional Animal Ethical Committee. These animals were used to evaluate the diuretic activity of HAEMC. The animals were maintained under standard husbandry conditions for an acclimatization period of 15 days before performing the experiments. All rats were housed in metabolic cages, six in each and temperature maintained at 22 ± 2ºC.

Acute Toxicity Study

Acute oral toxicity study of the *Matricaria chamomilla* L. water extract was performed according to OECD 423 guidelines. Four groups of adult female Swiss mice (n = 6) were kept fasting for 3–4 h and provided only water. One control group treated with the vehicle (distilled water), three groups treated with MCWE at 300 and 2000 mg/kg body weight by gastric intubations. The rodents were observed for behavioural and neurological symptoms continuously for the first 4 h after dosing. The number of survivors was noted after 24 h and these animals were then maintained for further 14 days with observations made daily. If mortality was observed in two out of three mice, then the dose administered was assigned as toxic dose. If mortality was observed in one mouse, the same dose was repeated again to confirm the toxic dose. No mortality or morbidity was observed in MCWE treated rodents up to 2000 mg/kg. Hence 200 mg/kg and 400 mg/kg doses were taken for efficacy studies.

The animals were kept fasted after a 16–19 hours overnight afterwards, blood samples were collected from each animals by retro-orbital puncture under diethyl ether anesthesia at days intervals 0, 7 and 15.

The blood samples were put in EDTA (Ethylenediaminetetraacetic acid) tubes and used for determining the biochemical parameters and into heparinized tubes for hematological parameters.

Screening for Diuretic Activity (Lipschitz test)

Animals required

Species / common name	:	Wistar rats
Age / weight / size	:	3 months / 180–200 g
Gender	:	Male
Number to be used	:	18
Number of days each animal will be housed	:	60 days

The hydro-ethanolic extract of *Matricaria chamomilla* leaves will be evaluated for its diuretic activity with slight modifications of and other methods. Male Wistar rats will be weighing between 180 and 200 g. Divided into four groups, (n=6). Animals of group I received distilled water (vehicle) which served as a control. Group II received furosemide 20 mg/kg p.o served as standard. Group III serve as a low dose aqueous ethanolic extract of *Matricaria chamomilla* of IV- serve as a high dose aqueous ethanolic extract *Matricaria chamomilla*. Four animals will be placed in standard metabolic cages. To be collect the urine samples different time intervals such as 5 and 24 h. Observe the parameters such as urine volume, PH, Urinary Na^+, K^+ and Cl^-.

Determination of Hematological Parameters

The blood samples were analyzed for red blood cell (RBC) count, hemoglobin (Hb) levels, hematocrit (HCT), platelets (PLT), white blood cell (WBC) count, differential WBC count (Neutrophils (N), Lymphocytes (L), Monocytes (M), Eosinophils (E)), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MH) and mean corpuscular hemoglobin concentration (MCHC).

Determination of Serum Biochemical Parameters

The blood samples were centrifuged at 4000 rpm for 10 minutes to obtain serum for the following investigations: Alanine amino transferase (ALT), Aspartate amino transferase (AST), Urea, Creatinine, Glucose.

Histopathology

All rats were sacrificed at the end of the study period and subjected to detailed gross necropsy. Vital organs such as heart, kidneys, liver, lung and spleen were isolated from all rats, weighed and examined for any large lesions. All tissues were preserved in 10% buffered formaldehyde solution for histopathological examination. The tissues were embedded in paraffin, and then sectioned, stained with haematoxylin and eosin and were examined microscopically.

Statistical Analysis

Experimental results were expressed as mean±SEM (n=6). Statistical analysis was performed with one way ANOVA followed by Dunnetts ‘t’ test using Graph Pad Prism software.

RESULT AND DISCUSSION

Phytochemical Analysis

The findings from the phytochemical screening indicated the absence of alkaloids, as evidenced by the lack of turbidity or formation of precipitates. Some samples exhibited a color change from violet to blue or green, indicating the presence of steroids. The colour changed from violet to blue or green in some samples indicated the presence of steroids. An interface with a reddish brown coloration was formed in the presence of terpenoids, as positive result. Red coloration identifies the presence of flavonoids (Shinado’s test). A colour change was observed in the test tube, which indicated in the presence of tannins.

Table 1. Phytochemical analysis.

S.N.	Phytochemicals	*M. chamomilla*
1	Alkaloids	-
2	Steroid s	+
3	Terpenoids	+
4	Flavonoids	+
5	Tannins	+

+, Presence of the compound
-, Absence of compound

Acute Toxicity

Oral administration of the *Matricaria chamomilla* extract up to dose 2000 mg/kg in mice did not cause any mortality or any toxicity during the experimentation period. However, mice after receiving *Matricaria chamomilla* extract exhibited a normal action for all the group of animals, monitored haematological, biochemical, histopatological and also no death was observed. *Matricaria chamomilla* at a dosage of 2000 mg/kg body weight orally was deemed safe for consumption and medicinal use in accordance with OECD guidelines No. 423. Based on this study, doses of 200 and 400 mg/kg were selected for evaluating diuretic action.

As per OECD 423 guidelines perform the above dosing to the 3 hours fasted animals to give the special observation for 24 hours to find the grooming sniffing and rearing characteristics for all the groups but other characteristics absent (mortality, excess urination, hair loss, convulsion and locomotion).

Daily observation of food intake was normal for all the groups of animals up to 15 days when compared to standard clinical animal data by the above parameter indicated that the given doses of extract have not induce the diabetes mellitus and pancreas dysfunction related diseases.

Water intake was normal for all the groups of mice up to 15 days, when compared to typical scientific animal data by the above parameter, points out that the given drug does not induce the diabetes mellitus, pancreas dysfunction related diseases and other GIT associated diseases.

Observation of daily body weight showed no changes in any group of mice compared to standard scientific data for mice. These results indicated that the given doses of extract did not induce obesity or increase cholesterol levels. Additionally, no hair loss was observed in any mouse of all groups after extract administration, suggesting no induction of cancer or immunological disorders. Observed parameters indicated no excess urination in any group of animals, indicating that the extract doses did not induce urinary system-related disorders.

Haematological

By the 15 days study indicated that the Table 2 clarified parameters such as RBC, Haemoglobin, Haematocrit, Platelets WBC, Neutrophils, Lymphocytes, Monocytes, Eosinophils, MCV, MCH and MCHC were normal for after the drug at the day of zero. After seven days of treatment, the above parameters remained unchanged compared to day zero. Mild variations were observed on the 15th day compared to day zero. Comparison within the control group at different intervals (0, 7, and 15 days) indicated that the administered drug did not produce any toxic effects.

Biochemical

Biochemical study results expressed that the Table 3 elucidate parameters such as ASAT (U/I), ALAT (U/I), Urea (mg/l), Creatinine (mg/l), Glucose (g/l) were normal for after the drug at the day of zero. After the seven days treatment, above parameters such as glucose, urea and creatinine are not changed when compared to zero day. At the day of 15^{th} milder of biochemical variations were observed when compared to zero day. In control group, comparison indicated that the different days intervals 0, 7 and 15 days above parameters are similar by the indication administered drug not to produce any toxic

effect. After the drug treatment ASAT (U/I) and ALAT (U/I) levels were moderately increased when compared to control group, which indicated extract have antioxidant property.

Histopathology

Various organs histopathology report was revealed that the kidney liver, stomach, heart, spleen, lungs were not cellular changes observed different dose levels 300 and 2000 mg/kg.

Mortality

In the observation of 15 days, mortality rate was zero (Alive percentage -100) by the indication to founded cookies LD_{50} value 2000 mg/kg for 1 in 10^{th} (200 mg/kg), 1 in 5^{th} (100 mg/kg) and 1 in 5^{th} (400 mg/kg) doses, which may be used for specific animal model like diabetic and bioavailability studies as shown in Table 2, Figure 1 and Table 3.

Diuretics Action (Lipschitz Test)

The results of the evaluations carried out on the extracts listed in Table 4 shows the urinary volume (ml/100 g/8 h) and electrolyte (Na+ and K+) content (mequiv/100 g/8h) of the urine of the animals. Urine volume (Figure 2). Table 1 shows that the reference diuretic, HCTZ, increased urine volume by 54%. The Hydro Alcoholic Extract of *Matricaria chamomilla* also caused an increase in urine volume. For the Hydro Alcoholic *Extract* of *Matricaria chamomilla*, the increase at doses of 400 mg/kg body weight was 18% ($P < 0.01$) and 41 % ($P < 0.001$), respectively, compared to the control group as seen in Figures 3 to 18.

Table 2. Hematological values of rats in the acute toxicity study of hydro alcoholic extract of *Matricaria chamomilla.*

	0 Day			**7th Day**		**15th Day**	
Parameters	***Normal***	***300 mg/kg***	***2000 mg/kg***	***300 mg/kg***	***2000 mg/kg***	***300 mg/kg***	***2000 mg/kg***
RBC	7.89 ± 0.73	7.7 ± 0.60	7.69 ± 0.71	7.66 ± 0.35	8.14 ± 0.64	8.08 ± 0.6	8.38 ± 0.86
Haemoglobin	14.58 ± 0.73	14.55 ± 0.26	14.26 ± 0.31	14.07 ± 0.74	14.25 ± 0.54	14.8 ± 0.64	14.71 ± 0.64
Haematocrit	39.75 ± 0.79	40.08 ± 0.48	40.93 ± 0.65	40.75 ± 0.77	41.65 ± 0.82	42.65 ± 0.93	42.26 ± 0.97
Platelets	623 ± 63	594.00 ± 71	585.00 ± 87	631 ± 63	597 ± 93	599 ± 81	597 ± 91
WBC	10.54. ± 2.1	10.62 ± 0.45	11.13 ± 0.88	11.06 ± 0.8	11.75 ± 0.70	10.83 ± 0.97	11.85 ± 0.45
Neutrophils	20.75 ± 1.89	20.8 ± 0.32	20.62 ± 0.88	21.12 ± 0.2	20.28 ± 0.96	21.17 ± 0.29	20.34 ± 1.08
Lymphocytes	70.51 ± 2.66	70.98 ± 2.06	70.56 ± 2.01	70.81 ± 2.77	69.03 ± 3.07	68.1 ± 3.61	67.91 ± 3.93
Monocytes	2.03 ± 0.17	2.01 ± 0.08	1.98 ± 0.09	2.05 ± 0.18	2.00 ± 0.18	2.00 ± 0.3	2.03 ± 0.33
Eosinophils	1.7 ± 0.43	1.7 ± 0.37	1.7 ± 0.36	1.73 ± 0.34	1.73 ± 0.35	1.75 ± 0.32	1.75 ± 0.34
MCV	51.47 ± 1.25	51.8 ± 0.79	51.7 ± 0.84	51.02 ± 1.65	51.37 ± 0.86	51.92 ± 1.98	51.5 ± 0.84
MCH	18.34 ± 0.69	17.75 ± 0.63	18.15 ± 0.65	17.87 ± 0.66	17.97 ± 0.82	18.46 ± 0.88	18.33 ± 1.82
MCHC	34.17 ± 0.47	34.2 ± 0.36	34.3 ± 0.36	34.03 ± 0.77	34.46 ± 0.88	34.2 ± 0.49	34.01 ± 0.87

Table 3. Biochemical values of rats in the chronic toxicity study of hydro alcoholic extract of *Matricaria chamomilla.*

Treatment Schedule							
	0 Day		**7th Day**			**15th Day**	
Parameters	*Normal*	*300 mg/kg*	*2000 mg/kg*	*300 mg/kg*	*2000 mg/kg*	*300 mg/kg*	*2000 mg/kg*
ASAT (U/I)	84 ± 2.1	85 ± 1.9	83 ± 1.7	83 ± 2.0	85 ± 2.1	84 ± 1.9	85± 2.3
ALAT (U/I)	65.83 ± 4.3	61.33 ± 4.6	65.66 ± 3.65	64.55 ± 4.15	65.16 ± 4.18	66.55 ± 4.11	64.83 ± 3.22
Urea (mg/l)	0.26 ± 0.05	0.26 ± 0.06	0.28 ± 0.03	0.25 ± 0.09	0.26 ± 0.07	0.34 ± 0.04	0.35 ± 0.06
Creatinine (mg/l)	5 ± 0.63	4.73 ± 0.42	4.96 ± 0.27	5.03 ± 0.28	5.11 ± 0.20	4.88 ± 0.49	4.92 ± 0.17
Glucose (g/l)	1.52 ± 0.12	1.02 ± 0.08	1.15 ± 0.11	1.08 ± 0.4	1.11 ± 0.39	1.07 ± 0.45	1.12 ± 0.35

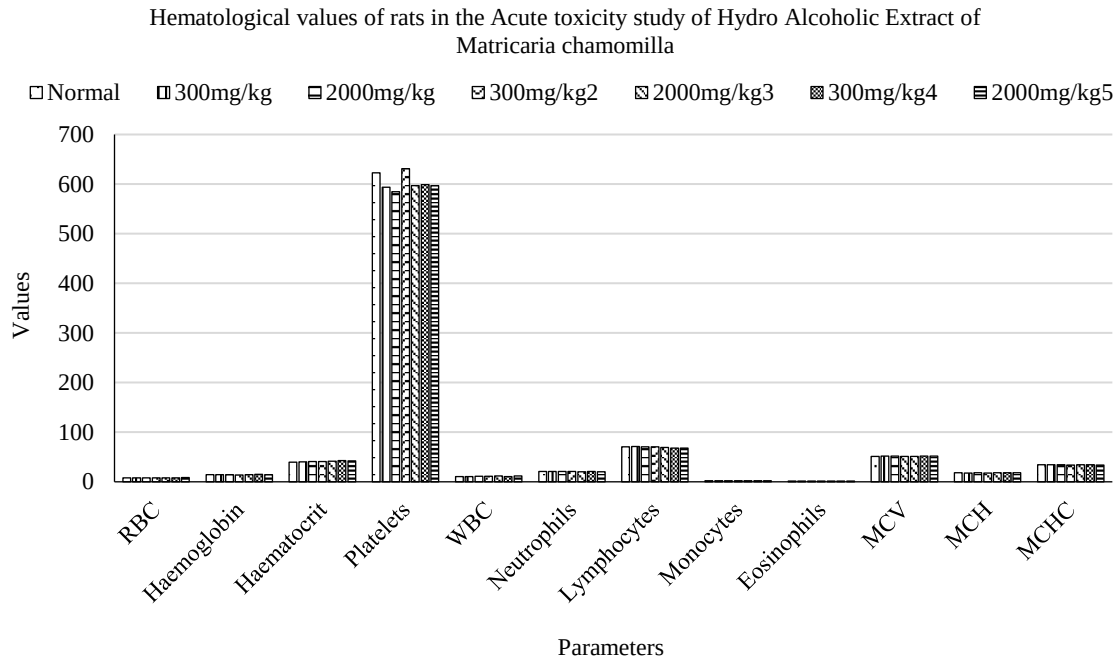

Figure 1. Heamatological values of rats.

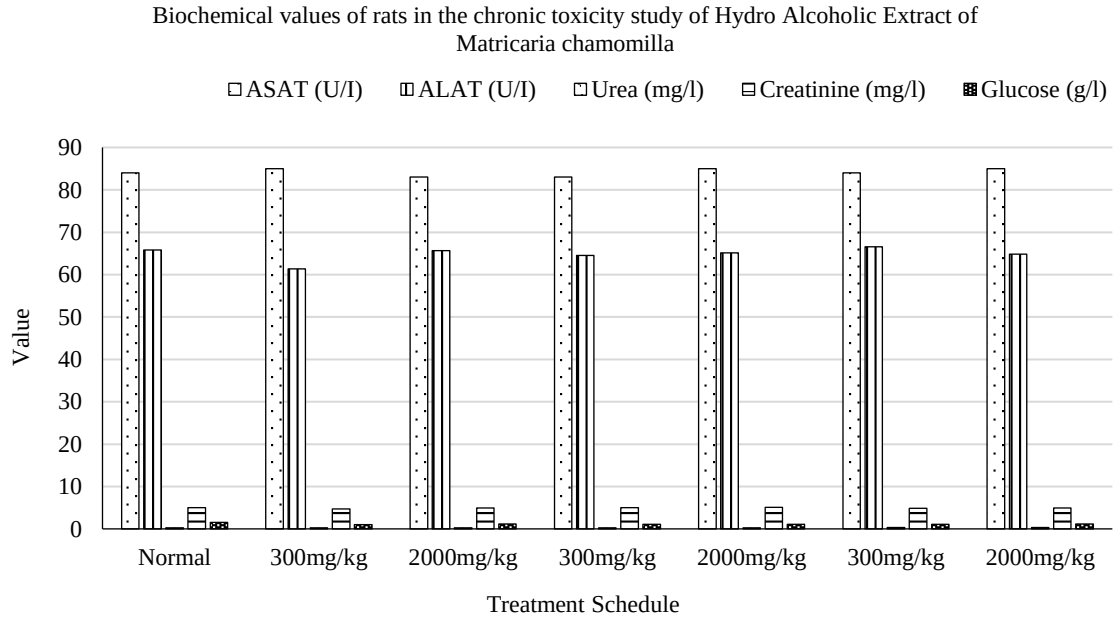

Figure 2. Biochemical values of rats.

Table 4. Diuretic effect of hydro alcoholic extract of *Matricaria chamomilla* Lipschitz test method.

S.N.	Groups	Total urine Vol (ml/kg BW/5 h)	Na+ mmol/L	K+ mmol/L	Cl− mmol/L
1	Control (10 ml/kg BW)	13.22±0.32	107.42±0.75	48.00±0.32	76.99±0.23
2	Standard (Frusemide 10 mg/kg BW)	21.00±0.03***	189.01+0.64***	82.03±0.44***	128.07±0.24***
3	HA EMC (400 mg/kg BW)	17.45±0.41***	178.93±0.73***	81.32±0.35***	113.06±0.06***

*HA EMC: Hydro Alcoholic Extract of Matricaria chamomilla, n = 6, values expressed as mean ± SEM. Significant at P < 0.001*** compared with the control group (one way ANOVA followed by Dunnett's 't' test)*

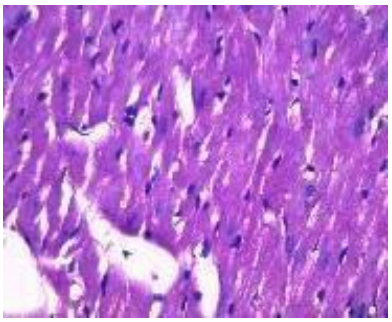

Figure 3. Control heart.

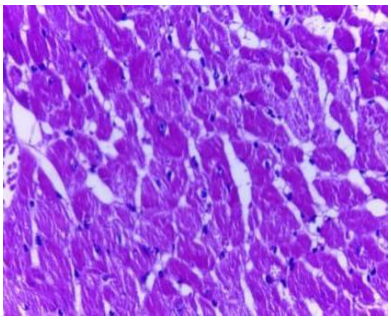

Figure 4. 300 mg/kg heart.

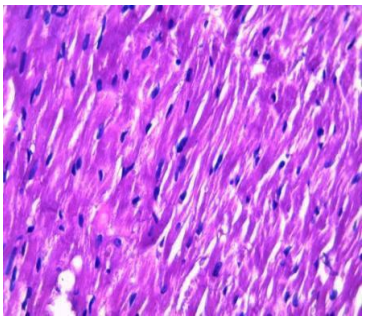

Figure 5. 2000 mg/kg heart.

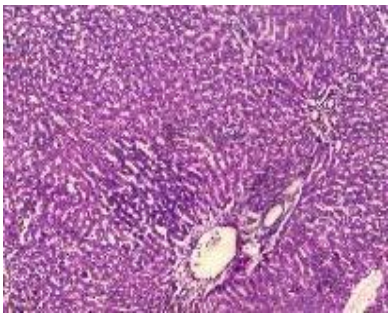

Figure 6. Control liver.

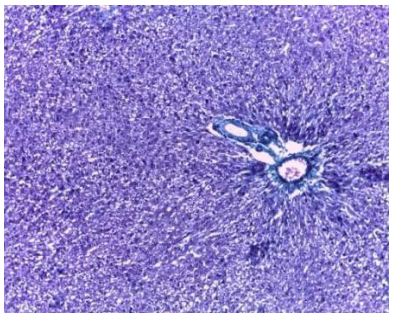

Figure 7. 300 mg/kg liver.

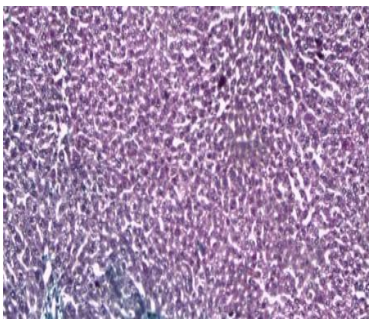

Figure 8. 2000 mg/kg liver.

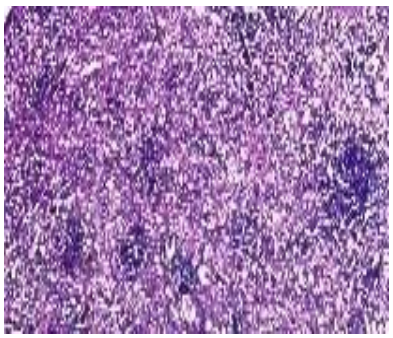

Figure 9. Control spleen.

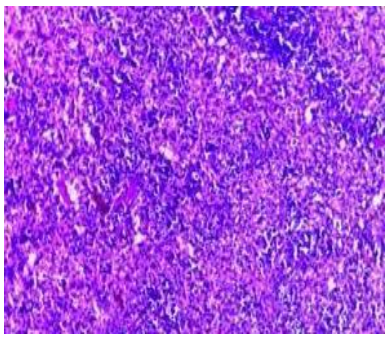

Figure 10. 300 mg/kg spleen.

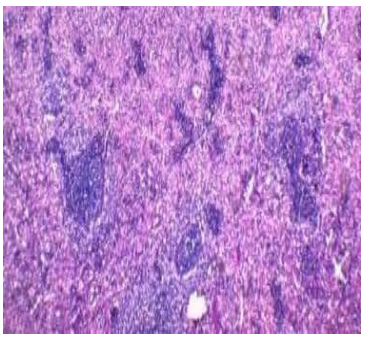

Figure 11. 2000 mg/kg spleen.

Figure 12. Control lungs.

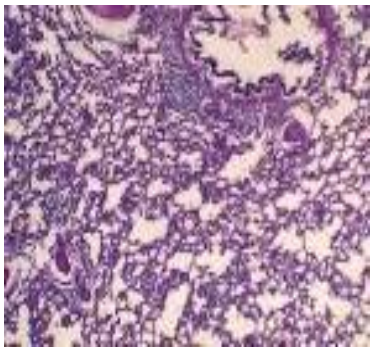

Figure 13. 300 mg/kg lungs.

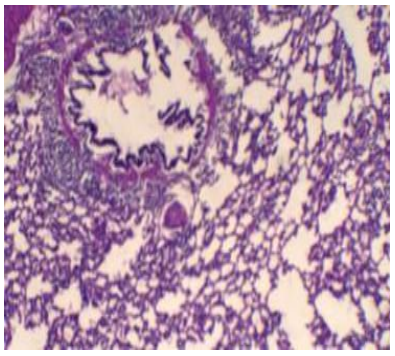

Figure 14. 2000 mg/kg lungs.

The result obtained with evaluation of diuretic activity of HAEMC were shown in Table 4 and Figure 18. From the result, the extract of HAEMC significantly increased the urine output and increase the excretion of electrolytes (Na+, K+ and Cl-) when compared to control group. A comparison was made with the standard drug furosemide, the excretion of urine output significantly increased in the extract at the dose level of 400 mg/kg HAEMC.

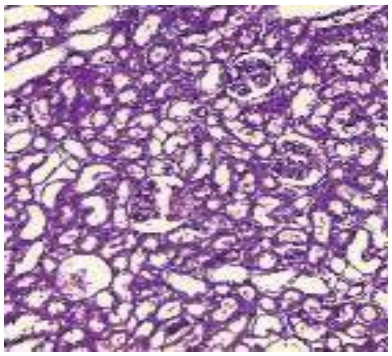

Figure 15. Control kidney.

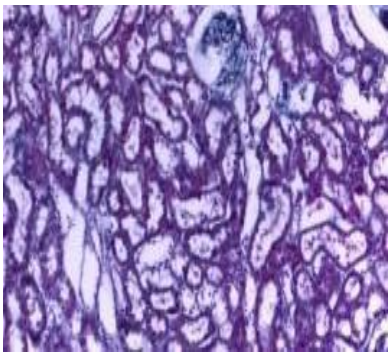

Figure 16. 300 mg/kg kidney.

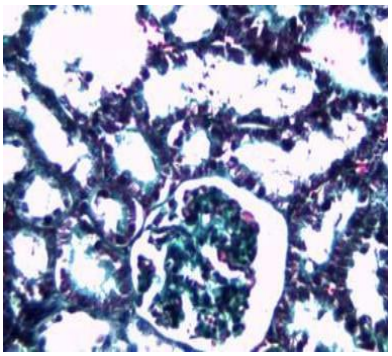

Figure 17. 2000 mg/kg kidney.

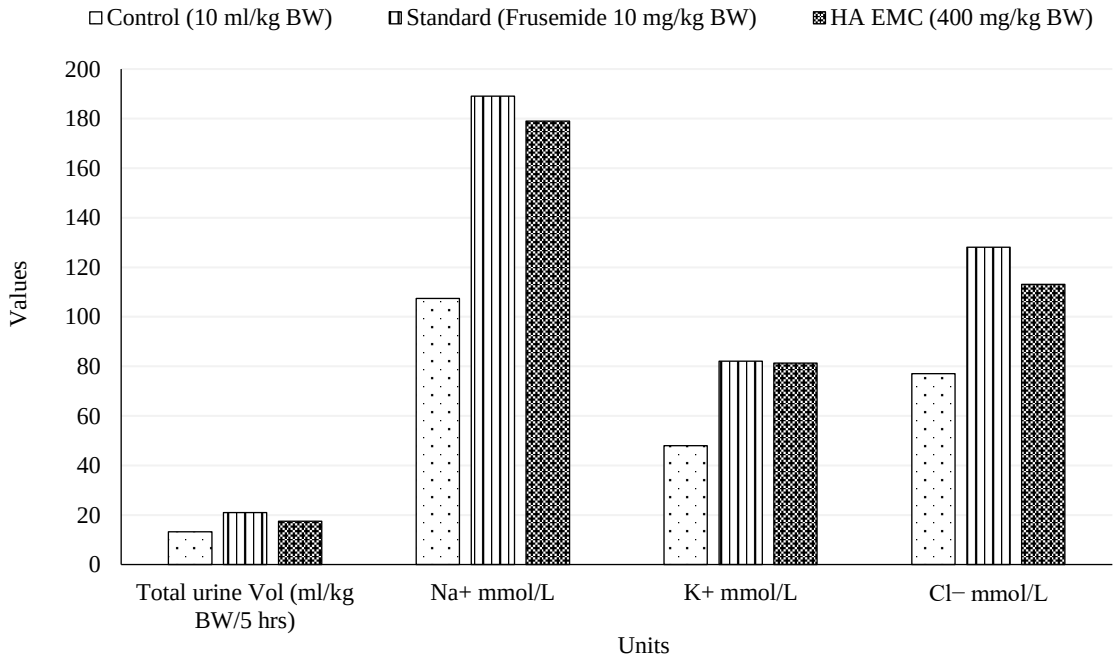

Figure 18. Diuretic effect of hydro alcoholic extract of *Matricaria chamomilla.*

CONCLUSION

While the traditional medicines are derived from medicinal plants, minerals and organic matter, the herbal drugs are prepared from medicinal plants only. Use of plants as a source of medicine has been inherited and is an important component of the health care system in India. In the Indian systems of medicine, most practitioners formulate and dispense their own recipes, hence this requires proper documentation and research. The major hindrance in the amalgamation of herbal medicines into modern medical practices is the lack of scientific and 170 clinical data and better understanding of efficacy and safety of the herbal products. Traditionally, herbs that stimulate the kidneys were used to reduce edema. In this investigation, the medicinal plant Hydro Alcoholic Extract of *Matricaria chamomilla* was used. This research work will be beneficial in determining the diuretic effect of *Matricaria chamomilla* leaf extract by analyzing the ions and electrolytes excreted by the animals using ion-selective channel inhibition. Acute toxicity studies indicated no toxicity for *Matricaria chamomilla*, which may be further validated by human studies. The diuretic activity of *Matricaria chamomilla* showed beneficial effects on the selected animals, suggesting its potential for further clinical studies.

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