Vol. 5 No. 1 (2026)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

Diuretic Potential of Methanolic Extract of Malva neglecta Wallr; an Animal Based Pharmacokinetic Study

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Keywords: Diuretics, Malva neglecta Wallr, Furosemide, Excretion, Pharmacology.

Received on 27 Jan 2026

Accepted on 5 Mar 2026

Published on 12 Mar 2026

Abstract

Herbal medicines are mostly used to treat many pathophysiological problems. The use of *Malva neglecta Wallr (MNW)*, specifically aerial parts are traditionally claimed to be useful in urinary tract inflammation. This vital study mainly focused to investigate diuretic effect of methanol extract of *MNW* in albino rats, which were not investigated previously. In this vital study methanol extracts of *MNW* were administered to experimental rats orally at doses of 300, 600 and 900 mg/kg. Furosemide (20 mg/kg) was used as positive control in study. The diuretic effect of the *MNW* extract was evaluated by quantification of urine volume, sodium, potassium and chloride content. Moreover, saluretic natriuretic index, diuretic index and pH was evaluated. The findings suggested that urine volume was significantly increased in all treatment groups of methanol extract in comparison to control group. While the excretion of sodium, potassium and chloride were also increased by extract at all doses but more significant at 900 mg/kg. There were no significant changes in the pH of urine after administration of the *MNW* extract. Protein

excretion increases in urine by 25% values after extract administration. The diuretic effect of the extract was comparable to that of the reference standard (furosemide) and the methanol had the additional advantage of a potassium-conserving effect.
Through significant findings of this study, it has been concluded that the extract of *MNW* produced notable diuretic effect which appeared to be comparable to that produced by the reference diuretic furosemide. This vital study provides a quantitative basis for explaining the folkloric use of *MNW* as a diuretic agent.

Introduction

Diuretics are the drugs which increase the outflow of the urine. They are also used for the removal of waste products and poisons from the body through the renal excretory system (Cuthbert & Clark, 2024). Diuretics are indicated in different conditions such as hypertension (HTN) (Dahar et al., 2021) and edematous states, hepatic cirrhosis, congestive heart failure (CHF) (Coppola et al., 2025), and chronic renal failure (CRF) (Sadki et al., 2010) . The purpose of most clinically useful diuretics is to reduce the extracellular fluid volume by reducing total-body NaCl contents (Goodman et al., 2006). Diuretics not only alter the excretion of sodium (Na^+), but also may make possible excretion of other cations (e.g., K^+, H^+, Ca^{2+}, and Mg^{2+}), anions (e.g., Cl^-, HCO^{3-}, and $H2PO^{4-}$), and uric acid (Siddiqui et al., 2018).
Traditional medicinal plants are used word widely in the treatment of some kidney disorders (Trullàs et al., 2024) and have extended use as diuretic agents (Wright et al., 2007) (Freitas et al., 2011). *Malva neglecta* (MN) belongs to *Malvaceae* family and most commonly known by different names such as, 'button weed', 'common mallow', 'cheese weed', 'cheese plant', 'round leaf mallow', 'dwarf mallow', 'toluk', 'sonchal', 'khabasi', 'berberu' (Irfan et al., 2021). The family *Malvaceae* consists of almost 120 genera and 1700 - 2000 species (Dalar et al., 2012). MN plant is growing annually. The stem is pilose and ascending to erect with simple setae. Leaves are very shallowly lobed, pilose, and crenate. Flowers are borne in fascicles in the axils of the leaf. Epicalyx segment is linear. Sepals are triangular; 2-4.5 mm petals are pink to white, 8-15mm maricarps pilose and smooth (Seyyednejad et al., 2010). The aerial parts of the plant are used in diarrhea, kidney stones, inflammation of respiratory tract, urinary inflammation (Jan et al., 2020). The present study was designed to determine diuretic activity of ME of MNW in rats.

MATERIAL AND METHODS

Herbal Material

The aerial parts of MNW were collected from fields in Gujarat, Pakistan which was authenticated by the taxonomist belongs to the Department of Botany, University of Faisalabad the voucher specimen (75-1-2018) was deposited at the herbarium.

Preparation of Herbal Extract

Aerial parts of the plant were washed to remove dust and superfluous material with distilled water and shade-dried for two weeks. Then it was crushed into powder form. The coarse powder (1kg) was subjected to maceration with 1500 70% methanol for seven days in air air-tight container with occasional shake at room temperature. The macerate was passed through muslin cloth and then filtered through Whatman filter paper no. 1 and filtrate was collected in a rotary vacuum evaporator at 35 ^{O}C and high pressure. Then concentrated ME was stored at 4^{O}C in amber colored glass container. The dried ME of MNW was placed in a desiccator for storing and the weighed amount was triturated with Tween 80 freshly before administration.

Experimental Animals

Healthy Wilstar adult male albino rats weighing about 150-230 g were housed in polypropylene cages and maintained at 24 ± 2°C less than 12 hours light/dark and 41 ± 5% humidity. They were fed with a standard rat pellet diet and water *ad libitum*. The experimental protocol was approved by the animal ethical committee of Government College University Faisalabad-Pakistan under Ref No. GCUF/ERC/1982.

Reference Drug

Furosemide (Sanofi Aventis, Pakistan Ltd), a loop diuretic, was used as reference drug. It was dissolved in water before administration.

Phytochemical Screening

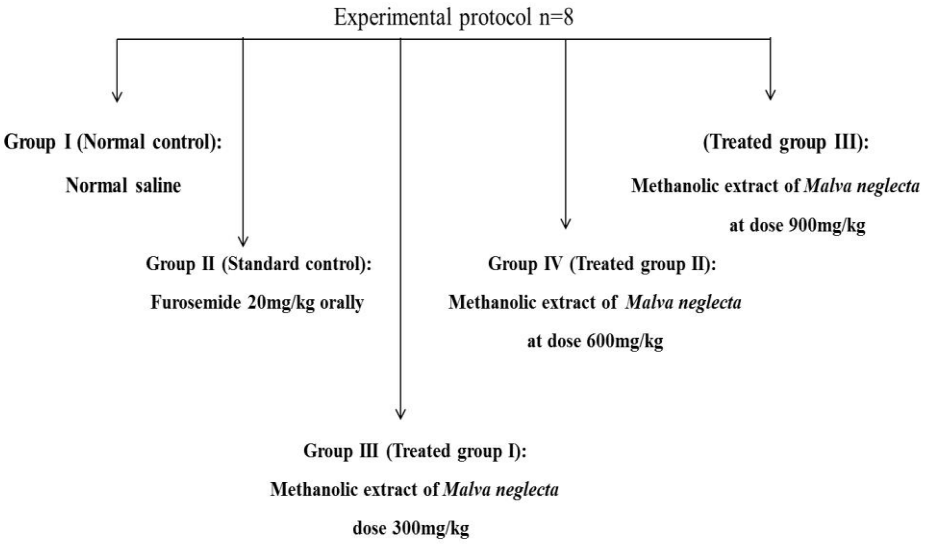
ME of MNW was treated with various reagents which showed the presence of various phytochemical constituents (Poh & Stanslas, 2024).

Assessment of Oral Acute Toxicity

The ME of MNW was evaluated for its toxicity in healthy albino rats by using the limit test dose of 2000 mg/kg body weight according to OECD guidelines no. 425. After ME administration, the rats were carefully and continuously observed for behavioral, neurological, autonomic, or physical changes for the first 4 hours. and then for the next 24 hours. Subsequently, they were kept under close observation for up to 14 days to monitor the presence of any signs of morbidity or mortality (Sadki et al., 2010).

Assessment of Diuretic Activity

Healthy albino rats weighing between 120-250 g were divided into five groups (n=8). Each animal was placed in a separate metabolic cage provided with a wire mesh bottom and a funnel to collect the urine. Stainless steel sieves are placed in the funnel to allow the urine to pass and retain feces. The rats were fed with water *ad libitum* and a standard diet. All animals were fastened for 12 hrs. before the diuretic study. After dosing of standard drug furosemide; 20 mg/kg, orally and ME, animals were administered 5ml 0.9% normal saline solution per 100 g body weight. Cumulative urine output was collected and measured at the 6 h, 12 h, and 24 h time intervals. The electrolytes (Na^+, k^+, and Cl^-) concentration and pH were determined in the 24-hour urine sample (Maghrani et al., 2005).



Assessment of Body weight

Animal's body weight (g) was calculated on day first before starting treatment, and at the last day of treatment, to ensure the difference in their body weight.

Assessment of urinary excretion

Urinary excretion is calculated by dividing the total urine excretion and total liquid administration (Dose + 0.9 % N/S) (Nedi et al., 2004).

$$\boldsymbol{Urinary\ excretion} = \frac{\boldsymbol{Total\ urinary\ output}}{\boldsymbol{Total\ liquid\ administration}} * \mathbf{100}$$

Assessment of urine electrolytes and diuretic index

Concentration of sodium, potassium, and chloride in urine were measured. Moreover, diuretic index was calculated by dividing the urinary excretion (U.E) of the test group by the urinary excretion control group.

$$\boldsymbol{Diuretic\ index} = \frac{\boldsymbol{U.E\ in\ test\ group}}{\boldsymbol{U.E\ in\ control\ group}}$$

Assessment of Lipschitz value

Lipschitz value is defined as the mean urine volume of the test group divided by the mean urine volume of the standard group. A saluretic drug like hydrochlorothiazide shows Lipschitz values around 1.7 – 1.8, whereas loop diuretics like furosemide and bumetanide reach values of 4.0 and more (Mekonnen et al., 2010).

$$\boldsymbol{Lipschitz\ value} = \frac{\boldsymbol{Mean\ urine\ value\ of\ test\ group}}{\boldsymbol{Mean\ urine\ value\ \ of\ control\ group}}$$

Assessment of Saluretic activity

Excretion of electrolytes with water excretion is an important therapy for ascites and peripheral edema in CHF as well as for the management of HTN. For the calculation, saluretic activity rats were modified in such a way that potassium, chloride, and osmolality were measured in addition to sodium and water (Shivhare et al., 2012). The sum of sodium (Na^+) and chloride (Cl^-) excretion is calculated as a parameter for saluretic activity.

$$\boldsymbol{Saluretic\ activity} = \frac{\boldsymbol{U.E\ of\ electrolyte\ of\ test\ group}}{\boldsymbol{U.E\ of\ electrolyte\ of\ control\ group}}$$

Assessment of Natriuretic activity

The ratio Na^+/K^+ is calculated by using the natriuretic formula. Values greater the 2.0 indicate the favorable natriuretic effect and a ratio greater the 10.0 indicates the K^+ sparing effect (Hemalatha et al., 2013).

$$\boldsymbol{Natriuretic\ index} = \frac{\boldsymbol{Urinary\ excretion\ of\ \ Cl-}}{\boldsymbol{Urinary\ excretion\ of\ \ K+}}$$

Assessment of Ion quotient effect

The ratio Cl^-, Na^+, and K^+ (ion quotient) is calculated for the estimation of carbonic anhydrase inhibition. Carbonic anhydrase inhibition can be excluded at ratios between 1.0 and 0.8. With slight to decreasing ratios strong carbonic anhydrase inhibition can be assumed (Nedi et al., 2004).

$$\boldsymbol{Ion\ quotient} = \frac{\boldsymbol{Urinary\ excretion\ of\ \ Cl-}}{\boldsymbol{Urinary\ excretion\ of\ Na+\ and\ K+}}$$

Assessment of other urine parameters

Other urine parameters such as urobilinogen, bilirubin, glucose, protein, ketones, nitrite, and blood were also calculated to check the effect of ME of MNW on these parameters.

Statistical analysis

Data were expressed as means ± SEM. One-way ANOVA followed by Dunnett's multiple comparison post hoc tests was applied by using Graph-pad Prism version 6.01.

RESULTS

Phytochemical Screening

ME was treated with various reagents which showed the presence of various phytochemical constituents with different qualitative reagents in Table 1.

Table 1: List of phytochemical constituents present in plant extract

Name of phytochemical constituents	Methanolic extract
Lipids	+
Proteins	+
Carbohydrates	+
Alkaloids	+
Flavonoids	+
Polyphenolics	+
Glycosaponins	+
Tannins	-
Steroids	-
Phyto-sterols	-

Present (+), Absent (-)

MNW decreases body weight enhanced urine excretion

Physical body weight was calculated before and after treatment of MNW. The findings show that after use of MNW body weight of study animals were decreases significantly ($P<0.01$, $P<0.001$) as compared to the control group. Meanwhile the urine excretion was significantly increased in a dose-dependent manner. As depicts in Table 2 the control group showed a 2.7% decrease whereas the standard drug decreased body weight by 78.5%. ME displayed a maximum decrease i.e. 59.90% in body weight at 900mg/kg dose.

Table 2: Effect of *Malva neglecta* extract on change in body weight in dose dose-dependent manner

Groups	Treatments	Doses	Initial body weight	Final body weight Final body weight	Body weight change
I	Normal control (0.9 % N/S)	1ml/100g	263 ± 2.00	256 ± 1.00	7 ± 1.00
II	Standard control (Furosemide)	20mg/Kg	260 ± 3.50$^{\#}$	239 ± 4.00$^{\#}$	21.5 ± 0.500ns

III-V	Methanolic extract of *M. neglecta*	300mg/Kg	230 ± 8.00*	220 ± 8.00*	5 ± 0.00ns
		600mg/Kg	210 ± 2.50**	195.5 ± 1.50**	16 ± 1.50**
		900mg/Kg	215 ± 8.00*	198.5 ± 11.5*	17.5 ± 3.50**

*All of the data obtained from the experimental groups have been compared control group. Values are expressed as MEAN ± SEM, one-way ANOVA followed by Dunnets't' test. Where *P value <0.05, **P value <0.01, ***P value <0.001 compared to standard control and ### P<0.001, ## P<0.01, # P<0.05 compared to normal control.*

MNW enhanced Urine Volume/ outflow

Urine out flow was measured after treatment of MNW to albino rats, the findings shows that, cumulative urine out flow was significantly increased (P<0.05), and cumulative urine volume was observed 67.36% with standard furosemide; however, ME at 300-, 600-, and 900 mg/kg caused a 21.50%, 19.94%, and 17.48% increase respectively (Table 3).

Table 3: Effect of *Malva neglecta* extract on urine volume

Groups	Treatments	Doses	% saline loaded	Urine volume (mL/6 h)	Urine volume (mL/12 h)	Cumulative urine volume (mL/24 h)
I	Control	-	67.1 ± 3.00*	4.04± 0.18	5.53 ± 0.44	8.06 ± 0.25
II	Standard	20mg/Kg	108.3 ± 4.6###	5.94±0.44##	8.44±0.48###	13.49 ± 0.21###
III-V	Methanolic extract of *M. neglecta*	300 mg/Kg	81.2 ± 6.9*	4.16±0.36**	7.14 ± 0.34*	10.59 ± 0.24***
		600 mg/Kg	85.1 ± 6.2**	5.05±0.44**	6.83 ± 0.31**	11.07 ± 0.36***
		900 mg/Kg	95.6 ± 5.5**	4.52± 0.71**	6.56± 0.18***	11.94 ± 0.11***

*All of the data obtained from the experimental groups have been compared control group. Values are expressed as MEAN ± SEM, One-way ANOVA followed by Dunnets't' test. Where *P value <0.05, **P value <0.01, ***P value <0.001 compared to standard control and ### P<0.001, ## P<0.01, # P<0.05 compared to normal control.*

MNW increases Sodium, Potassium and Chloride excretion and Diuretic index

After use of MNW, electrolytes excretion (Na^+, K^+, and Cl^-) was significantly increased, which denotes loop diuretic activity of ME. The findings showed that dose-dependent increase in electrolytes excretion with the highest value at 900 mg/kg dose with ME. Diuretic activity is categorized as good when the diuretic index is >1.50, moderate when the diuretic index is 1-1.50, mild in the case where the diuretic index is 1-0.72, and nil with <0.72 diuretic index value. Diuretic index values of treatment groups displayed

the diuretic effect of the ME and the highest effect was observed at 900 mg/kg dose of ME (Table 4).

Table 4: Effect of *Malva neglecta extract* on urine electrolytes, pH, and diuretic index

Groups	Treatments	Doses	Diuretic index	pH	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)
I	Normal control (0.9 % N/S)	1mL/100 g	-	6.46 ± 0.13	95.0 ± 1.00	45.23 ± 0.06	161.8±0.66
II	Standard control (Furosemide)	20mg/Kg	3.30 ± 0.67$^{\#\#}$	7.76 ± 0.30$^{\#\#\#}$	106.4 ± 0.51$^{\#\#\#}$	46.5 ± 0.26$^{\#\#\#}$	202 ± 0.71$^{\#\#\#}$
III-V	Methanolic extract of *M. neglecta*	300mg/Kg	1.18 ± 0.02***	6.62 ± 0.19^{ns}	104 ± 1.14***	46.9 ± 0.22***	183.4 ± 1.33***
		600mg/Kg	1.41 ± 0.03***	7.05 ± 0.34**	109.0 ± 1.45***	68.1 ± 0.11***	196 ± 1.50***
		900mg/Kg	2.28 ± 0.01**	7.9 ± 0.24***	167.8 ± 5.34***	65.24 ± 0.20**	283.8 ± 1.77***

*All of the data obtained from the experimental groups have been compared control group. Values are expressed as MEAN ± SEM, one-way ANOVA followed by Dunnets't' test. Where *P value <0.05, **P value <0.01, ***P value <0.001 compared to standard control and $^{\#\#\#}$ P<0.001, $^{\#\#}$ P<0.01, $^{\#}$ P<0.05 compared to normal control.*

Effects of MNW on Lipschitz value, Saluretic index and Natriuretic index of Electrolytes

Lipschitz value shows the diuretic activity, the outcomes of this study showed in treatment groups, the ME of MNW showed Lipschitz values ranging from 0.78 to 0.88. Meanwhile dose-dependent increase in diuretic activity was found with ME. i.e. ME exhibited 72%, 80%, and 87% diuretic activity at 300-, 600-, and 900 mg/kg dose levels. (Table 5). The saluretic activities of ME showed like hydrochlorothiazide Lipschitz values around 1.8. ME of MNW showed a natriuretic index range from 1.30 to 2.07, representing a favorable natriuretic effect. ME of MNW represented a value above 1.0, so they have weak carbonic anhydrase activity.

Table 5: Effect of *Malva neglecta* extract on the saluretic index, natriuretic index, and ion quotient

Groups	Treatments	Doses	Lipsehitz value	Saluretic index			Natriuretic index	Ion quotient
				Na^+	K^+	Cl^-		
I	Normal control (0.9 % N/S)	1mL/100 g	-	-	-	-	2.10 ± 0.03	1.15 ± 0.01

II	Standard control (Furosemide)	20mg/Kg	-	1.13	1.40	1.76	1.25± 0.01$^{###}$	1.30 ± 0.02$^{###}$
III-V	Methanolic extract of *M. neglecta*	300mg/Kg	0.78	1.20	0.81	1.68	2.07 ± 0.03^{ns}	1.22 ± 0.11**
		600mg/Kg	0.82	1.15	1.02	1.53	1.50 ± 0.02***	1.11 ± 0.01*
		900mg/Kg	0.88	1.81	1.30	1.70	1.30 ± 0.03***	1.20 ± 0.01*

*All of the data obtained from the experimental groups have been compared control group. Values are expressed as MEAN ±SEM, one-way ANOVA followed by Dunnets't' test. Where *P value <0.05, **P value <0.01, ***P value <0.001 compared to standard control and $^{###}$ P<0.001, $^{##}$ P<0.01, $^{#}$ P<0.05 compared to normal control.*

MNW enhanced Urobilinogen, and Specific gravity of Urine

Investigation of urobilinogen and specific gravity (SG), and other biochemical materials like glucose, proteins, ketones etc. in urine has great importance. These parameters also investigated after use of MW of MNW. The findings show that after use of MNW protein excretion increase in urine by 25% as compared to standard drug furosemide. Meanwhile the control group have urobilinogen value of 0.5, but ME increases the urine values of urobilinogen. Similarly, the specific gravity of urine also increased in the animal group which was treated with ME of MNW (Table 6).

Table 6: Effect of ME of MNW on Urobilinogen, Specific gravity of Urine and other urine parameters

Groups	**Treatments**	**Doses**	**Parameters**							
			Urobilinogen	**Bilirubin**	**Glucose**	**Protein**	**ketones**	**Nitrite**	**Blood**	**S.G**
I	Normal control (0.9 % N/S)	1mL/100g	0.5	N	N	N	N	N	N	1.003 ± 0.00
II	Standard control (Furosemide)	20mg/Kg	0.5$^{###}$	N	N	+	N	N	N	1.007 ± 0.00
III-V	Methanolic extract of *M. neglecta*	300mg/Kg	1.0NS	N	N	+	N	N	N	1.013 ± 0.01*
		600mg/Kg	1.0NS	N	N	++	N	N	N	1.017 ±

								0.00***
900mg/Kg	1.0NS	N	N	+	N	N	N	1.015 ± 0.01***

*All of the data obtained from the experimental groups have been compared control group. Values are expressed as MEAN ± SEM, one-way ANOVA followed by Dunnets't' test. Where *P value <0.05, **P value <0.01, ***P value <0.001 compared to standard control and ### P<0.001, ## P<0.01, # P<0.05 compared to normal control.*

DISCUSSION

To evaluate the diuretic effect of ME of MNW administered at doses 300, 600, 900 mg/kg, orally and standard drug at dose 20 mg/kg, orally and compared with normal control group. To evaluate the diuretic effect important parameters were calculated such as saline-loaded excretion, urine excretion, and electrolyte excretion (Na+, K+, and Cl-). ME significantly increased the Na^+, K^+, and Cl^- excretion as compared to the control group. Cumulative urine outflow was also increased significantly and shows a significant diuretic activity of ME of MNW.

Diuretics increases urine volume with increase excretion of electrolytes (Na^+, K^+, and Cl^-), therefore diuretics used in critical malaise such as CRF, CHF, nephritis, pregnancy toxemia, open-angle glaucoma, HTN, and liver cirrhosis (Bove et al., 2018). Diuretics are most commonly used in critical patient's acute renal failure/injuries (Montejano-Rodríguez et al., 2013). Solute reabsorption is a selective process and can occur by carrier proteins or by simple diffusion in the tubular epithelium. Reabsorption of water is taken passively by the process of osmosis. The substances that are reabsorbed enter into the bloodstream (Bagshaw et al., 2007). The present study suggested a significant diuretic activity of ME of MNW. All the results demonstrated that oral administration of ME of MNW significantly showed diuretic activity.

CONCLUSION

The *ME of* MNW exhibited significant diuretic activity and could be utilized as an anti-diuretic medicinal plant in urinary tract inflammation other urinary problems. This study confirms that ME of MNW can be used as diuretic; however further investigations are essential to find out the exact mechanism responsible for diuretic activity.

Disclosure Statement

No potential conflict of interest was reported by the author (s).

Contribution of Authors

All authors contributed equally in this study.

Funding

No any funding granted from anywhere.

Data Availability Statement (DAS)

The study's original contributions are contained in the article. The corresponding author can be contacted for additional questions.

Statement of Ethics

Animal handling ethics protocol was followed as per approved institutional guidelines. The study was carried out in compliance with institutional protocols.

AI Generative Statement
The author (s) of this study declared that AI wasn't used to create this manuscript.

Abbreviations Used
ME (Methanol Extract), MNW (*Malva neglecta Wallr),* OECD (Organization of Economic Corporation and Development), U.E (Urinary excretion), HTN (Hypertension), CRF (Chronic renal failure), CHF (Congestive heart failure), N/S (Normal Saline), B.w (By weight)

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