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Protective effects of *Mentha longifolia* Methanolic Extracts on Renal biomarkers and Histo-physiology in Paracetamol-intoxicated Rabbit

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Abstract

The present study was designed to investigate the effect of 14 days orals administration of *Mentha Longifolia* methanolic extract on kidney related hematology and histopathology of Kidney in paracetamol intoxicated rabbits. Total of 25 rabbits were randomly divided into five groups (each group containing five rabbits) including normal group, toxic group (paracetamol treated 2g/kg), low group (100mg/kg), median (200mg/kg) and high group (300mg/kg) after paracetamol intoxication. During the present study, administration of high doses of paracetamol significantly increases the level of Urea and creatinine. The methanolic extract of *Mentha longifolia* significantly decreases the level of Urea and creatinine is compared to paracetamol group. The hematological parameters investigated during the present research were improved with the treatment of *Mentha longifolia* methanolic compared with paracetamol intoxicated rabbits. In addition, histopathological assessment of the kidney of different groups was performed. The kidney of paracetamol intoxicated rabbits was seriously affected compared to normal. The methanolic extract actively recovered the aforementioned toxicity in various groups.

Key words: *Mentha longifolia*, Renal Histo-physiology, renal biomarkers, rabbits

1.0 Introduction

Kidney is a vital organ that maintains homeostasis by reabsorbing some necessary components back into the blood and excreting waste products including creatinine, urea and uric acid [1]. It is exposing to a variety of harmful substances, especially bases and organic acids increasing the vulnerability of this important organ to harm [2]. Serum biochemical parameters are the primary indices used to evaluate toxicity [3]. Nephropathy or renal toxicity is a biomarker for renal function and is frequently

associated with a number of metabolic problems, such as imbalances in serum electrolytes, urea, uric acid, and creatinine [4]. There is always a correlation between decreased renal function and elevated levels of creatinine, urea, and uric acid [5].

Numerous substances and medications can result in acute or long-term kidney toxicity [6]. Paracetamol (acetaminophen); APAP) at high dose is hepatotoxic and nephrotoxic in human and animals as well as paracetamol (acetaminophen); APAP) in high doses is harmful to the kidneys and liver in both humans and animals [7]. Because of its close correlation with the use of synthetic pharmaceuticals, kidney disease has emerged as a major global health concern [8]. Medicinal plants are greatly admired worldwide since they are abundant in antioxidants and have no negative side effects [9].

Paracetamol (Acetaminophen or N-acetyl-p-aminophenol or APAP) is commonly used as an analgesic and antipyretic medicine, which causes liver and kidney necrosis in human and animals when used in high doses [10]. Paracetamol overdosing results in vasoconstriction and inhibition of vasodilation in kidneys, which in turn result in decreased blood flow to the renal blood vessels and reduced glomerular filtration rate [11]. Acetaminophen, usually referred to as paracetamol, is a well-known painkiller and fever reducing agent that causes liver and kidney toxicity. When given in a normal amount, paracetamol has no harmful effects, but a high dose can harm the liver [3]. Necrosis results from an increase in NAPQI concentration in situations of paracetamol overdose due to the depletion of glutathione reserves [12]. The *Mentha longifolia* is a household-use medicinal herb. Mentha Longifolia is used because of its antimicrobial properties along with how well it helps with digestion. Both the leaves and blooming stems have antispasmodic and anti-asthmatic characteristics. Tea made from the leaves has been traditionally used to cure fevers, headaches, problems with digestion, and jaundice [13]. They are used to flavor salads and cooked meals. Herb tea is prepared from leaves it has a peppermint taste. Mentha Longifolia can also be used to treat insect stings. Applying a mint compress to the sting relieves pain and irritation while also cooling the area from the outside [13]. The present study aimed to determine the nephroprotective properties of *Mentha longifolia* as traditional medicine with fewer side effects.

2.0 Materials and Methods

2.1 Collection and identification of plant

First, the *Mentha Longifolia* plant was collected from the local streams of Maidan Dir lower (Kumber) in the months of October and November, and the plant was identified by Dr. Gul Rahim Lecturer department of Botany University of Malakand Chakdara.

2.2 Preparation of Plant extract

The collected plant leaves were shade dried for 20 days. After that, the leaves were grinded with electric blender into fine powder. The powder was poured in methanol for five days and was shacked three to four times in a day and then filtered with Whattman filter paper and the remaining methanol was evaporated with rotary evaporator at the department of Pharmacy, University of Malakand. The semi-solid paste was stored at 2-8°C in a refrigerator.

2.3 Dose preparation

The toxic group was treated with 2g/kg body weight via oral route. The plant extract was administered in three different doses (100mg/kg-lower treated group, 200mg/kg-median treated group and 300mg/kg for high treated group). Pure paracetamol was bought from the

District Mardan KPK Pakistan. Then doses were prepared (2g/kg body weight).

2.4 Animals grouping

Twenty five (25) domestic rabbits *(Oryctolagus cuniculus*) of single sex (male) were purchased from local market in city Timergara Dir lower KPK, weighing from 800gm to1042gm and marked them in unique number for identification. All rabbits were divided into five groups (Normal control, toxic category, low, median and high groups). In each group added five rabbits of the same weight. They were housed in a wide room at GDC Lal Qilla Dir Lower, Khyber Pakhtunkhwa, Pakistan. The rabbits were fed on grasses and green leaves, and allowed water. The animals were kept in such condition for one week to acclimate, before starting experiment.

2.5 Animal dissection and collection of blood sample

All rabbits were dissected at end of experiment. Each rabbit was slaughtered and blood samples were collected in Gel tube for biochemical analysis. Gel tube was placed in icebox and sends them to Chakdara hospital Dir lower for different blood testing.

2.6 Determination of Renal Biochemical markers

During the present research some biochemical parameters such as creatinine and blood urea were determined.

2.7 Histopathological assessment

For histopathological analysis kidney from each individual in group was collected and thoroughly rinsed with normal saline and then kept in 10% formalin to prevent any microbial action. The renal tissue of each group was dehydrated in grading ethanol (60%, 70%, 80%, 90%, & 100%). After that the tissues were embedded in paraffin wax and prepared blocks for tissue slices. The tissues were sliced with microtome of 4 µm thickness and then stained with eosin and hematoxylin stains on a glass slide. The slides were then studied for any histological alteration under compound microscope.

2.8 Statistical analysis

The results were showed as means ± standard deviation. One-Way ANOVA was applied for the comparison of different group's data. The significance level was kept as $P<0.05$.

3.0 Results

3.1 Renal Biomarkers

Blood Urea Contents were examined in Normal, Toxic, Low, Medium and High group rabbits. Blood Urea Contents in the high group was higher than toxic, low, medium and almost similar with Normal group. Creatinine was examined in the Normal, Toxic, Low, Medium and High group of rabbits. The Creatinine level in the High group was higher than Toxic, Low, Medium and Normal group as shown in Table 1.

Table 1: Effects of *Mentha Longifolia* on renal biomarkers of rabbits

	Normal	**Toxic**	**Low dose**	**Medium dose**	**High dose**
Urea	23.2±0.2***	29.6± 1.2	27.2 ±0.4*	26.4±0.3**	22.1 ±1.2***
Creatinine	0.4±0.02***	0.9 ±0.3	0.6± 0.9ns	0.5±0.3**	0.4± 1.2***

*$P<0.05$, **$P<0.0$, ***$P<0.00$ & *ns*=non-significant

3.2 Renal Histo-physiology

Kidney section of rabbits belong to normal group, paracetamol/toxic group, low group (treated with

100mg/kg b. w), medium group (treated with 200mg/kg b. w) and high group (treated with 300mg/kg b. w) were also studied during the present research (Fig. 1) after treating paracetamol and *Mentha longifolia* extract. The kidneys of normal group show normal glomeruli, ascending and descending convoluted tubules of nephrons. The kidney of animals group treated with low dose showed no significant difference from control group. The kidney of animals group treated with medium dose demonstrated oedematous changes, mild necrosis, and infiltration as obvious from. While in case of high dose administered extract group, the kidneys exhibited no significant difference from control group except mild inflammatory cells infiltration.

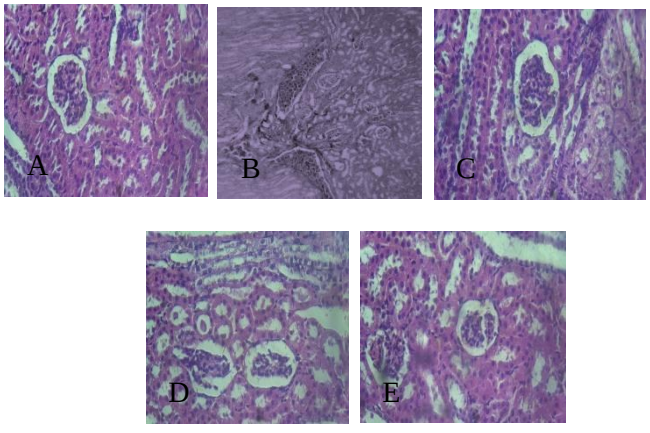

Figure 1: Renal histology of rabbits. (**A**) Normal control, (**B**) Toxic group, (**C**) Low group, (**D**) Median group, and (**E**) High group.

4.0 DISCUSSION

In present study, the effects of 14days oral administration of *Mentha longifolia* methanolic extract on levels of some kidney, heart related hematological parameters and histopathology of Kidney and Heart in paracetamol intoxicated rabbits were studied. The kidney related parameters included the levels of urea clearance and creatinine clearance. The levels of creatinine were measured for calculation urea and creatinine clearance. During the present study, administration of high dose of paracetamol resulted in significantly reduction in levels of urea clearance and creatinine clearance. This indicates damaged kidney after repeated paracetamol overdosing. The photomicrograph of kidney section of paracetamol group showed necrosis and infiltration of inflammatory cells [14]. Urea and creatinine is the waste product of protein metabolism that need to be excreting by kidney, therefore marked increase in serum urea creatinine is indicator of functional damage to the kidney [15]. Reports are available about paracetamol induced nephrotoxicity. During a study on evaluation of nephroprotective diuretic and antioxidant activities of plectra thus amboinicus of paracetamol induced nephrotoxic rats; nephrotoxicity was induced by paracetamol administration. The paracetamol caused elevate levels of serum urea, hemoglobin total leukocyte count creatinine rise body weight reduced levels of neutrophils, granulocytes, uric acid sand platelets concentration [16]. Similarly, during another study on the nephroprotective activity Momordicatuberosa Cogn albino rats, nephrotoxicity was induced by oral administration of paracetamol. Paracetamol administration revealed in increase in blood urea in serum creatinine levels [17]. During a study of nephroprotective effect of curcumin was studied in male Wister albino rates, nephrotoxicity was induced by the administration of a single dose of paracetamol. Paracetamol administration caused elevated levels of renal malondialdehyde market depletion of glutathione level antioxidant enzyme activity and deteriorated the renal functional as assessed by the increased plasma urea and creatinine levels [4]. During the present study of *Mentha Longifolia* methanolic extract (300mg\kg) showed nephroprotective activity the rabbits

treated with extract at a dose of 300mg/kg b.w. show in significant increase in the urea clearance level as compared to normal group during the study of urea clearance level, the extract at a dose of 300mg/kg caused a significant increase in urea clearance level as compared to paracetamol control rabbit group ($p<0.03$.). During the study of creatinine clearance level, the extract at dose of 300mg/kg caused in significant increase in creatinine clearance level at compared top paracetamol normal group ($p<0.03$) thus the oral administration of methanol extract of *Mentha longifolia* repaired are maintained the histology of the kidneys of paracetamol intoxicated rabbits this was confirmed by observing and comparing photomicrograph kidney section of extract treated group rabbits with those normal control rabbits. The kidney of animals group treated with low dose showed no significant difference from control group. The kidney of animals group treated with low dose showed no significant difference from control group. While in case of high dose administered extract group, the kidneys exhibited no significant difference from control group except mild inflammatory cells infiltration. Literature has shown medicinal plant with the nephroprotective property to mediate their protection via antioxidant and or free radical scavenging activities due the high concentration of flavonoids and alkaloids they contain [18]. Saponins have been reported to protect liver and kidney against carbon tetrachloride intoxication [19]. Many plants have been reported for its nephroprotective effects during fruit extract of *Solanum xanthocarpum* was studied for its nephroprotective activities Gentamicin intoxicated rodents. Treated with plant extract caused reduction in the elevated levels of plasma urine urea and creatinine levels [2]. Another study the ethanolic extract of leaves of *Buteamono sperma* was investigated for its nephroprotective activity in rats with Gentamicin induced toxicity. Treatment with extract caused significant decrease in the elevated levels of creatinine, serum urea, blood urea nitrogen along with significant increase urea volume [20]. During a study, methanolic extract of leaves and stem bark of the *Celtis occidentalis* and *Canarium schweinfurthii* on acetaminophen induced renal injuries in rats was reported for its nephroprotective activities [21]. Treatments extract caused a significant reduction in elevated levels of blood urea and serum creatinine [22]. Similarly, during another study of ethanoic extract of the leaves of the *Elephaentophus scaber* was also reported for its nephroprotective actions in Gentamicin nephrotoxic rats. Treatment with extract results in the reduction of elevated levels of serum urea and creatinine [23]. During the present research, the effects of 14 days oral administration of *Mentha longifolia* methanolic extract on some hematological parameters of paracetamol-intoxicated rabbits were also studied. Several reports are available about the effects of paracetamol overdosing parameters. During the study on the effects of *Maytenus emarginata* leaves ethanolic extract of paracetamol intoxicated albino Wistar rats, chronic administration of paracetamol caused a significantly decrease in total red blood cells count, hemoglobin concentration and hematocrit value [6].

5.0 CONCLUSION

From the result of the present study, it was concluded that the Methanolic extract of *Mentha Longifolia* is nephroprotective and show beneficial on in paracetamol intoxicated rabbits.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

References

[1] Rosner, M.h., 2011. An overview of renal physiology Practical Urology: essential principles and practice. Springer, pp.

[2] Bennett WA, Verpooten GA, editors. Clinical Nephrotoxins. 2. London: Kluwar Academy Burcham, P.C., 2014 Target-organ toxicity: liver and kidney an introduction to Toxicology.

[3] Lee, W. M. (2017). Acetaminophen (APAP) hepatotoxicity—isn't it time for APAP to go away ? *Journal of h*, *epatology 67*(6), 1324-1331.

[4] Gao, D., Wang, S., Lin, Y., & Sun, Z. (2020). In vivo AAV delivery of glutathione reductase gene attenuates anti-aging gene klotho deficiency-induced kidney damage. *Redox Biology*, *37*, 101692.

[5] Swem, T. F., Aba, P. E., & Udem, S. C. (2020). Effect of hydro-methanol stem bark extract of Burkea africana on erythrocyte osmotic fragility and haematological parameters in acetaminophen-poisoned rats. *Clinical Phytoscience*, *6*, 1-7.

[6] Johnson, M., Olufunmilayo, L. A., Anthony, D. O., & Olusoji, E. O. (2015). Hepatoprotective effect of ethanolic leaf extract of Vernonia amygdalina and Azadirachta indica against acetaminophen-induced hepatotoxicity in Sprague-Dawley male albino rats. *American Journal of Pharmacological Sciences*, *3*(3), 79-86.

[7] Yousef, M.I., Omar, S.A., El-Guendi, M.I., Abdelmegid, L.A., 2010. Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat.

[8] Levitt, D.G., Levitt, M.D., 2016. Human serum albumin homeostasis: a new look at the roles of synthesis, catabolism, renal and gastrointestinal excretion, and clinical value of serum albumin measurements. Int. J General Med.

[9] Sen, T., Samanta, S.K., 2014. Medicinal plants, human health and biodiversity: A broad review Biotechnological applications of biodiversity. Springer, pp. 59–110.

[10] Ozkaya, O., Genc, G., Bek, K., & Sullu, Y. (2010). A case of acetaminophen (paracetamol) causing renal failure without liver damage in a child and review of literature. *Renal failure*, *32*(9), 1125-1127.

[11] Zagaria, M.A.E. Vigilance Regarding Drug-Induced Acute Kidney Injury in Ambulatory Older Adults. US Pharm. 2020, 45, 32–35.

[12] Bessems, J. G., & Vermeulen, N. P. (2001).Paracetamol(acetaminophen)induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. *Critical reviews in toxicology*, *31*(1), 55-138.Tseng, C. H., Chong, C. K.,

[13] Abbas, S R., & Nisar, M. (2020). Herbal significance of horse mint (Mentha Longifolia) a review. Journal of biotechnological science ,8 138-143.

[14] Karthivashan, G., Kura, A. U., Arulselvan, P., Isa, N. M., & Fakurazi, S. (2016). The modulatory effect of Moringa oleifera leaf extract on endogenous antioxidant systems and inflammatory markers in an acetaminophen-induced nephrotoxic mice model. *PeerJ*, *4*, e2127.

[15] Orinya, O. A., Adenkola, A. Y., & Ogbe, R. J. (2016). Haematological and biochemical studies on the effect of diclofenac sodium on Wistar Rattus norvegicus.

International Journal of Biological and Chemical Sciences, *10*(5), 2231-2242.

[16] Taşkömür, A. T., & Erten, Ö. (2021). The role of cystatin C, neutrophil-lymphocyte ratio and platelet-lymphocyte ratio in the evaluation of kidney function in women with preeclampsia. *Taiwanese Journal of Obstetrics and Gynecology*, *60*(4), 615-620.

[17] El-Mashad, A. E. R., El-Mahdy, H., El Amrousy, D., & Elgendy, M. (2017). Comparative study of the efficacy and safety of paracetamol, ibuprofen, and indomethacin in closure of patent ductus arteriosus in preterm neonates. *European journal of pediatrics*, *176*, 233-240.

[18] Darkwah, W. K., Ao, Y., Adinortey, M. B., Weremfo, A., Abrokwah, F. K., & Afriyie, E. (2018). Total phenolic, flavonoid and alkaloid contents, oxidative DNA damage protective and antioxidant properties of methanol and aqueous extracts of Dissotis rotundifolia whole plant. *Free Radicals and Antioxidants*, *8*(2), 82-88.

[19] Hira, K., Sultana, V., Ara, J., & Ehteshamul-Haque, S. (2017). Protective role of Sargassum species in liver and kidney dysfunctions and associated disorders in rats intoxicated with carbon tetrachloride and acetaminophen. *Pak J Pharm Sci*, *30*(3), 721-728.

[20] Mestry, S. N., Dhodi, J. B., Kumbhar, S. B., & Juvekar, A. R. (2017). Attenuation of diabetic nephropathy in streptozotocin-induced diabetic rats by Punica granatum Linn. leaves extract. *Journal of traditional and complementary medicine*, *7*(3), 273-280.

[21] Muhsin, A., Dilnaz, Zahoor, M., Ullah, R., & Alotaibi, A. (2025). Effect of Celtis occidentalis L. extract on induced nephrotoxicity by mitigating inflammation, oxidative stress, and nephrotoxic nephritis in rabbits. *Discover Medicine*, *2*(1), 118.

[22] Osukoya, O. A., Oyinloye, B. E., Ajiboye, B. O., Olokode, K. A., & Adeola, H. A. (2021). Nephroprotective and anti-inflammatory potential of aqueous extract from Persea americana seeds against cadmium-induced nephrotoxicity in Wistar rats. *Biometals*, *34*(5), 1141-1153.

[23] Bolkiny, Y., Tousson, E., El-Atrsh, A., Akela, M., & Farg, E. (2019). Costus root extract alleviates blood biochemical derangements of experimentally-induced hypo-and hyperthyroidism in mice. *Annual Research & Review in Biology*, *31*(5), 1-10.