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***Research Article** (Open Access)*

ANTIHYPERTENSIVE ACTIVITY OF MANGO PEEL AND ITS ACTIVE PHYTOCHEMICHAL MANGIFERIN ON SALT-INDUCED RAT

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ABSTRACT
The ethanolic extract of *Mangifera indica* Linn. has an ethno-medicinal reputation as an antihypertensive agent. The most active biological constituent is mangiferin, followed by benzophenones, phenolic acids, and other antioxidants such as carotenoids, flavonoids, isoquercetin, quercetin, tocopherols and ascorbic acid. Mangiferin is the main contributor to most of the biological activities of its extract. On account of the presence of alkaloids and its antioxidant properties, we hypothesized that *Mangifera indica* may attenuate the development of salt-induced hypertension. Wistar rats (n=6 each) were treated for 7 days as follows: control (Tc) (normal diet + water), salt-loaded (Ts) (3% salt water + normal diet), salt-extract-loaded (Tse) (3% salt water + normal diet + 200mg/kg b.w. extract), salt-mangiferin-loaded (Tsm) (3% salt water + normal diet + 20mg/kg b.w. mangiferin) and salt-Lisinopril loaded (Tsl) (3% salt water + normal diet + 0.8mg/kg b.w. Lisinopril). Their serum and urine electrolyte profiles were measured. The urinary space in bowman capsule and structure of kidney and were studied. The findings indicate that mango peel extract and mangiferin are effective in reducing or attenuating hypertensive effects in salt-induced hypertensive rats. The Tse, Tam and Tsl groups does not differ significantly from the control group.

Keywords: *Mangifera indica, Mango peel, Mangiferin, Hypertension, Electrolyte, Aldosterone*

INTRODUCTION
Hypertension or high blood pressure, is a chronic medical disorder characterized by consistently higher blood pressure inside the arteries. A rise in blood pressure, often known as hypertension, is a condition that occurs when the blood vessels expand in response to constant pressure and have a systolic blood pressure that is greater than or corresponding to 140 mmHg or a diastolic blood pressure that is more than 90 mmHg. Because of this, the heart has to pump blood through the circulatory system at a higher rate than usual (Rout *et al*., 2010). Disorders of the heart and blood arteries are often referred to as cardiovascular disease (CVD). A variety of cardiovascular diseases and conditions, such as coronary artery disease, heart failure, atherosclerosis, renal insufficiency, and myocardial infarction, may lead to hypertension (Oparil, 1999). The oxidative stress that causes hypertension is caused by excessive quantities of oxidants in comparison to antioxidants. According to (Touyz *et al*., 2004), the oxidative stress may have been brought on by a higher formation of reactive oxygen species (ROS) or a lower amount of nitric oxide (NO). Atherosclerosis, hypertension, and congestive heart failure are only a few of the cardiovascular conditions that are thought to be caused by reactive oxygen species, or ROS (Sugamura, 2011). The number of people diagnosed with hypertension rose from 17,307 per 100,000 in 1990 to 20,525 per 100,000 in 2015, according to statistics collected recently from 154 nations (Forouzanfar *et al*., 2017; Egan *et al*., 2019; Hu-mam, 2017). It is estimated that 12.8% of all fatalities are caused by hypertension, making it one of the leading global health problems (WHO, 2015). Several medications from a variety of pharmacological classes are used to manage or treat hypertension. Angiotensin-converting enzyme (ACE) inhibitors, thiazide diuretics, calcium channel blockers, and angiotensin receptor II blockers are the primary types of medicines that may be used to treat hypertension (Munoz-Durango *et al.* 2016). Other drugs, including vasodilators, aldosterone antagonists, beta-blockers, renin inhibitors, central alpha agonists, central adrenergic inhibitors, and central agonists, are

often used as well (Omboni, 2018). The risk of hypertension-related cardiovascular disease events such as heart failure, stroke, nephropathy, and retinopathy are reduced, but not eliminated, by the pharmacological reduction of hypertension in humans with several anti-hypertensive pathways (Rizvi, 2017). Even after treatment, the blood pressure of certain patients is much higher than the normal range. This results in health concerns that are linked to hypertension. When this occurs, it is necessary to make use of a combination of antihypertensive medications in order to bring the blood pressure under control and to protect various organs (Guerrero-Garcia *et al*., 2018; Stewart *et al*., 2019). This is of utmost significance in light of the high incidence of hypertension resistant to treatment around the globe (Noubiap *et al*., 2019). The frequency of true-resistant hypertension was found to be 22.9% among those with chronic kidney disease, 56.0% among those who had had a renal transplant, and 12.3% among the elderly, based on data from 3.2 million patients (Noubiap *et al*., 2019). In this perspective, during the last three decades, massive, coordinated research efforts have focused on traditional herbal medicine with cardio-vascular-protective, antihypertensive, or hypotensive beneficial abilities (Anwar *et al*., 2019; Al Disi *et al*., 2015; Shouk *et al*., 2014; Saleh *et al*., 2016; Fardoun *et al*., 2017; Anwar *et al*., 2017). The usage of medicinal plants is becoming more widely acknowledged as a natural source of biologically active substances with therapeutic effects that may be utilized to enhance human health and prevent and cure specific disorders, such as hypertension (Aekthammarat *et al*., 2019; Ma *et al*., 2020). Traditional medications are utilized today, although little is known about their mechanism(s) of action, complications, toxicity, and complications. Thus, most herbal medications have not been biologically evaluated (Tomassoni *et al*., 2001). *Mangifera indica* is a significant plant used in traditional medicine, and it has a number of applications in the medical field. The C-glucosyl-xanthone known as mangiferin is thought to be responsible for the biological actions of *M. indica* (Shah *et al.*, 2010; Matkowski *et al*., 2013). A specified and standardized blend of substances, such as polyphenols, terpenoids, steroids, fatty acids, and microelements, are present in the standardized aqueous or ethanolic extract of the stem bark, leaves, and mango peels of *M. indica* (Núñez Sellés *et al.*, 2002). Other research has shown success with polyphenols in treating hypertension (Patten *et al*., 2012; Persson, 2012). The inhibitory activity of polyphenols in the baroreflex and the angiotensin-converting enzyme (ACE) in experimentally hypertensive rats was reported (Guerrero *et al*., 2012; Tangney & Rasmussen, 2013). In view of the exposure and the growing need for innovative alternative medicines, we hypothesized that continuous treatment with mango peel extract from *M. indica* might lower the blood pressure of hypertensive animals. Here we report a comprehensive investigation undertaken with mango peel extract and Mangiferin from *Mangifera indica* on salt-induced hypertensive rats.

MATERIALS AND METHODS

Experimental Design:

Four groups of animals (three in each group) received treatment. This experiment was conducted in a set of two. Ts, Tae and Tam groups received 3% salt water as drinking water in ad-libitum. Tae and Tam's groups received a freshly prepared single dose with distilled water via the oral route. After 7 days all the animals were sacrificed by mild ether anesthesia. Blood was collected and centrifuged for serum separation. Organs were collected and preserved for histological investigation.

Groups	Group symbol	Drugs used	Dose
Control	C	----	------
Salt	Ts	Salt (Nacl)	------
Salt+Extract	Tse	Salt + Extract	200 mg/kg b.w. (7 days)
Salt+Mangiferin	Tsm	Salt+ Mangiferin	20. mg/kg b.w. (7 days)
Salt+Lisinopril	Tsl	Salt+ Lisinopril	0.8 mg/kg b.w. (7 days)

Preparation of plant extract:
Mango peels were collected from freshly ripe mango of *Mangifera indica*, Peels were washed, and sun-dried for 2 weeks. peels were ground to powder (500g) and homogenized with 70% ethanol (1000 ml). The powder was solubilized and mixed well with intermittent stirring for 3 days continually. A layer of Whatman filter paper (No. 1) was used to filter the plant-solvent combination after it had been created. In order to evaporate the acquired solvent, Petri dishes were placed in an oven heated to 45–50^0 Celsius and left there for four to five days. The resulting extracts were collected and preserved in a refrigerator for future usage.

Animals:
Before starting the experiments, all the animals will be adapted for an interval of 7-10 days to the usual laboratory environments with 12:12 hrs day and night cycle for each 24 hrs period with ambient room temperature (25±2^{0}C) and relative humidity 55-60%. For the present anti-hypertensive study, adult Albino Wistar rats ranged between the weight of 100g – 150g were considered. Sterile polypropylene cages were used to uphold the rats in the animal house. The animals were provided with a standard diet of rat pellets and were given access 3% salt water on an unlimited basis except control (C) group. Control group received normal diet and normal drinking water in ad-libitum. Every experiment involving animals was carried out with the agreement of the Departmental Ethical Committee as well as in compliance with the standards for the appropriate use of animals in the laboratory.

Experimental Induction of hypertension in Wistar rat:
Individually identifiable animals will be chosen, weighed, and marked. To develop hypertension in Wistar rats weighing 100- 150g, 3% salt (NaCl) water was used as drinking water in ad-libitum to the animals for the whole experimental period (Miura *et al*., 1999; Somova *et al*., 1999).

Instruments and Reagents:
All the biochemical tests of blood and others were done using a semi-automated biochemistry analyzer 'Prietest easy lab' made by Robonik India Pvt. Ltd., A-374, TTC Industrial Area, Mahape, Navi Mumbai-400710, India. Pure mangiferin extracted from *Mangifera indica* was purchased from Sigma-Aldrich Chemicals Private Limited, India. Test kits were purchased from Robonik India Pvt. Ltd. and all other reagents used were of analytical grade.

Collection of blood:
On day eighth, blood was drawn from the patient's heart via a cardiac puncture under a light ether anesthetic following an overnight fast. The blood was then allowed to clot at room temperature for ten minutes. The blood samples were centrifuged for ten minutes at a speed of 3000 rpm. After the serum was separated, it was kept at a temperature of -20^0 Celsius until the biochemical measurements could be made.

Biochemical Estimation:
Blood and urine electrolytes level was assayed by colorometric method (Tietz, 1987). The assay kit was supplied by Robonik India Pvt. Ltd., A-374, TTC Industrial Area, Mahape, Navi Mumbai-400710, India.

Histological assessment:
The rat's kidney was promptly removed and treated in Bouin's solution. Tissues were then dehydrated in graduated alcohol concentrations before being embedded in paraffin. 4–6 µm tissue sections were stained by hematoxylin and eosin (H & E). For each animal, six H & E-stained slides were investigated for histological alterations (Khorsandi and Nejad-Dehbashi, 2014).

ELISA for aldosterone hormone assay:
The enzymatic immunoassay, in a point-to-point method, was used to measure the serum aldosterone concentration. Robonik India Pvt. Ltd., A-374, TTC Industrial Area, Mahape, Navi Mumbai-400710, India, provided the assay kit. The aldosterone enzyme-linked immunosorbent assay (ELISA) is a solid-phase immunoassay that operates in a conventional competitive binding fashion. Unlabeled antigens (found in standards, controls, and samples) compete with enzyme-labeled antigens (conjugates) for the same pool of antibody binding sites on a microwell plate. Non-bound substances are removed using washing and decanting processes. The enzyme substrate is introduced after the washing process. Stopping solution is added to halt the enzymatic process. A microtiter plate reader is used to determine the absorbance value. The amount of aldosterone present in a sample has a negative correlation with the

intensity of the resulting coloration. Aldosterone levels in a sample can be read through the use of a standard curve, which is plotted using a known set of standards (Ahmadi *et al*., 2014).

Statistical Analysis:

The data is presented as (SEM). Students t-tests were used to statistically analyse all the data at the 1% and 5% levels of significance. Using the SPSS 12.0 edition and Microsoft Excel software, mean, standard deviation (SD), standard error of the mean (SEM), and other statistical computations were performed (Das, 2002; Negi, 2008).

RESULT AND DISCUSSION

High arterial blood pressure, often known as hypertension, is a common and serious health problem that often goes undiagnosed for a long time. It is either categorised as primary (essential) or secondary. Ninety to ninety-five percent of cases are prime hypertension, which has no identifiable medical aetiology (Carretero and Oparil, 2000). Secondary hypertension, which affects the arteries, kidneys, heart, or endocrine system, accounts for the remaining 5 to 10% of cases (Beevers *et al*., 2001). The most frequent environmental element that leads to the aetiology of hypertension is an excess of dietary salt (Aviv ,2001; Frolich and Varagic, 2005; Gu *et al*., 1998; Sacks *et al*., 2001; Meneton *et al*., 2005). High salt consumption is associated with increased blood pressure in both normotensive and hypertensive individuals, as shown by several epidemiological and genetic studies (Weinberger, 1988; Intersalt, 1998; Poulter *et al.*,1990; Forte, 1989; Lifton, 1996). Animals' blood pressure elevated when they consumed a lot of salt over the long term (Corbett, 1979; Cherchovich, 1976). A variety of genetic variations and alterations in ion channels and associated proteins in the kidney have been linked to altered sodium metabolism and/or salt-sensitive hypertension, there is still some debate over which of these factors is most important, the exact mechanisms by which dietary salt induces hypertension remain unknown. Mutations affecting mineralocorticoid production and plasma levels, renal inflammation, oxidative stress, and intra-renal angiotension activity all play a role in the development of salt-sensitive hypertension (Lifton *et al*., 2001; Gu *et al*., 2006; Rodriguez-Iturbe and Vaziri, 2004; Vaziri *et al*., 2006). Additionally, dietary salt-induced hypertension is linked to a decrease in renal VEGF (vascular endothelial growth factor) expression (Gu *et al*., 2006). Traditional antihypertensive medications typically come with a lot of adverse effects. Due to their better tolerance and fewer side effects, herbal medicines are used for primary healthcare by about 75–80% of the world's population, especially in developing nations. Numerous rigorous efforts have been made over the past three decades to identify native medicinal plants with hypotensive and antihypertensive therapeutic potential (Tabassum and Ahmad, 2011).

The experimental result of serum and urine Na^+, K^+ concentration, and urine volume are shown in Table 1, Figure no. 1 and Figure no. 2. Serum electrolytes analysis reveal that the hyponatrimia is found in all the treated groups except Ts ($p<0.05$) group when compared to the control value. The study of serum K^+ concentration reveals that there is no significant difference in all the treated groups compared to control except Ts ($p<0.01$) and is statistically significant. Urine electrolyte analysis reveal that the hypernatrimia is found in all the treated groups i.e., Ts ($p<0.05$), Tsm ($p<0.01$) and Tsl ($p<0.01$) except when compared to the control value and there is no significant difference in between Tse and Tc groups. The study of urine K^+ concentration reveals that hypokalemia is found in all the treated groups i.e., Ts ($p<0.01$), Tse ($p<0.05$), Tsm ($p<0.01$) and Tsl ($p<0.01$) and are statistically significant. In case of serum and urine Na^+ K^+ ratio there is no significant difference in between Control (Tc) and Extract (Tse) group. Higher ratio of Na^+ K^+ are found in Ts ($p<0.05$) group and are statistically significant. Our results suggest that in the 3% salt induced hypertensive rat, the serum Na^+ is higher and low K^+ i.e., its Na^+ K^+ ratio is high. The results of this investigation suggest that a high K+ concentration or high Na+/K+ ratio in mango peel extract, mangiferin, and lisinopril may be responsible for their ability to reduce salt-induced hypertension. The capacity of plant/fruit-based diets to prevent hypertension is connected with the high potassium content or high Na+ K+ ratio, as revealed in the Dietary Strategy to Stop Hypertension trial and other comparable studies (Appel *et al*., 2005; Morris & Tangney, 2014). These characteristics were present in animal feed that consisted mostly of fruit/plant-based -diet, making hypertension very uncommon, if not nonexistent, since the kidneys could readily preserve the little sodium and remove the overabundance of potassium (O'Shaughnessy, 2004). A high-sodium diet has been shown to stimulate the sodium pump and open potassium channels. The high concentration of serum Na+ in the 3% salt-induced hypertensive

rat may act in the same way (Amberg *et al*., 2003; Haddy *et al*. 2006). Increased serum potassium levels lead to endothelial cell hyperpolarization, which eventually extends to the vascular smooth muscle (VSM), causing a decrease in intracellular calcium and causing vasodilation (Amberg *et al*., 2003; Haddy *et al*. 2006; Joseph *et al*., 2013; Sonkusare *et al*., 2005; Daghbouche-Rubio *et al*., 2022) and a drop in blood pressure. For analysis of the urine, samples were collected early in the morning from all the treated groups and it was found that the volume of urine collected was significantly higher in Ts ($p<0.01$) and Tsl ($p<0.05$) groups compared to the control. A major rise in urine volume occurred in treatment salt (Ts) group and indicate the rising of the blood pressure (Kim, 2000). In Tse and Tam groups shows a reduced urine volume than Ts group which indicates the increased renal tubular reabsorption of sodium (Convertino, 1991).

Table 1: Effect of mango peel extract and mangiferin on Urine volume; Serum and Urine Na^+, K^+ in salt-induced rat.

Groups	Urine Volume (ml)(24hr)	Serum Na^+ (mmol/l)	Serum K^+ (mmol/l)	Serum Na^+ K^+ Ratio	Urine Na^+ (mmol/l)	Urine K^+ (mmol/l)	Urine Na^+ K^+ Ratio
Control (Tc)	2.16±0.10	146.28±3.27	5.24±0.33	28.68±2.48	99.01±2.68	25.94±0.11	3.81±0.10
Treatment Salt (Ts)	4.26±0.08**	162.32±4.57*	4.18±0.19**	39.49±1.78*	121.19±4.94*	23.39±0.22**	5.18±0.24**
Treatment Salt Extract (Tse)	1.96±0.11#	141.09±1.83**	4.89±0.31#.	29.49±0.36#	109.69±5.40#	24.46±0.48*	4.48±0.21**
Treatment Salt Mangiferin (Tsm)	2.31±0.07#	139.23±1.8**	5.05±0.46#	28.54±2.38#	111.4±3.28**	24.18±0.28**	4.61±0.17*
Treatment Salt Lisinopril (Tsl)	4.60±0.36*	143.09±3.32#	5.48±0.61#	26.65±1.62#	113.16±3.45**	24.18±0.28**	4.31±0.14**

*Values are mean ± SEM (n=6); **= Significant at 1% level, *= Significant at 5% level and # = Not significant*

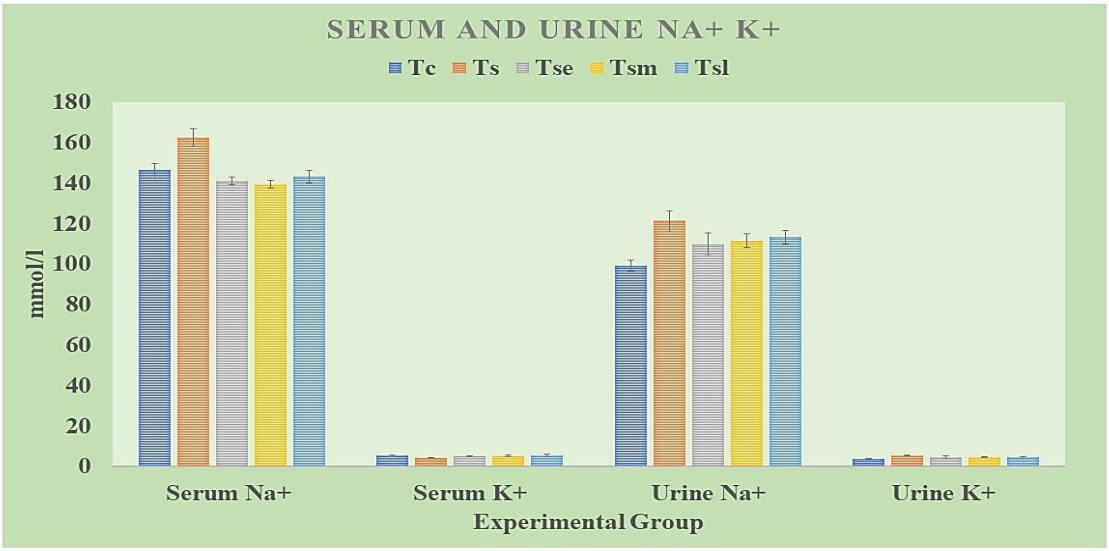

Figure 1: Effect of mango peel extract and mangiferin on serum and urine Na^+, K^+ in salt-induced hypertensive rat

Serum and urine Na^+ levels are increased in the Ts groups due to high salt and decreased in the Tse, Tsm, and Tsl groups. Serum K^+ decreases in the Ts group and increases in the Tse, Tsm, and Tsl groups. Levels of urine K^+ increase in the Ts group and decrease in the Tse, Tsm, and Tsl groups.

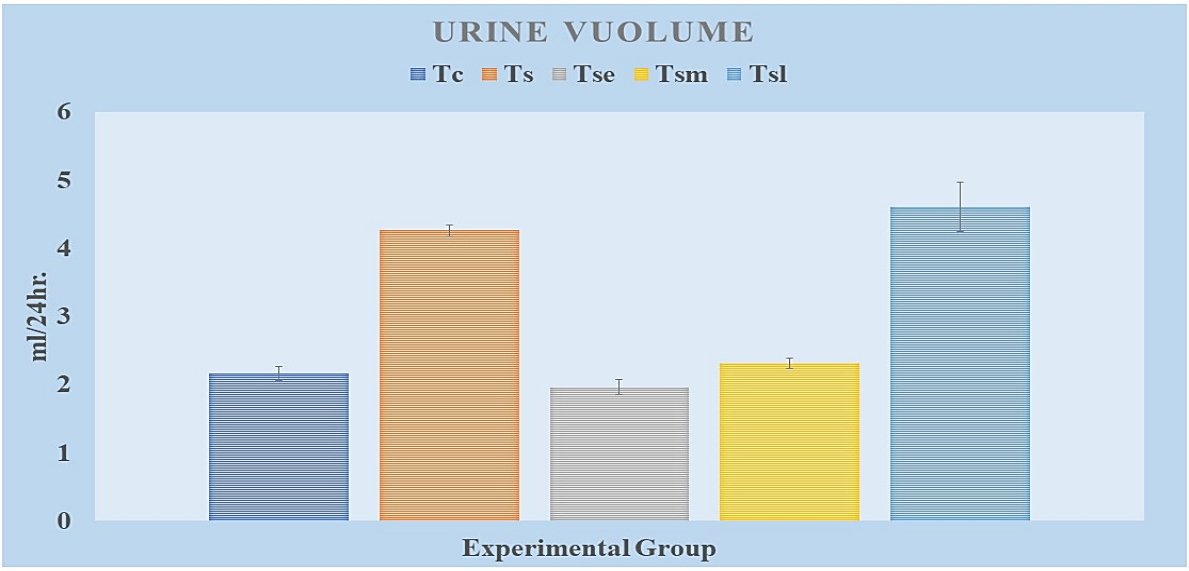

Figure 2: Effect of mango peel extract and mangiferin on urine volume in salt-induced hypertensive rat

The volume of urine increases in the Ts and Tsl groups and decreases in the Tse and Tsm groups.

The histological structures of the kidney of the control and various treated groups are described in figure no-3. The large renal corpuscles consist of a tuft of capillaries that form a round glomerulus (G), and an outer wall, the Bowman's capsule (BC). Urinary space or urine filled area is present between the glomerulus and the capsule. In Salt (Ts) group, the number of Bowman's capsules is also significantly decreased in compared to the control one. Some cells within the kidney tissues are with degenerating nuclei due to treatment of high salt. Narrow urinary space (NUS) or less urine filled area is present between the glomerulus and the capsule. After 2 weeks of 4.0% NaCl diet, David (2006) documented histological alterations in kidneys collected from vehicle and chronic administration of mycophenolate mofetil (MMF) treated salt Dahl SS rats. In mango peel extract (Tse) and Mangiferin (Tsm) groups, the recovery of kidney structure in all aspects is found in this group. The cells are with round shaped normal nuclei. Wide urinary space (WUS) area is present between the glomerulus and the capsule. In Tsl group, Medullary region remains same as in control. The cells present in the kidney tissues are with more or less with normal nuclei but very few numbers of degenerating nuclei are there. Mixed levels of urine filled area, moderate urinary space (MUS) and narrow urinary space (NUS) are present between the glomerulus and the capsule. Lum *et al*., 2022 provide a thorough review of the reno-protective efficacy of mangiferin against renal diseases using experimental models. In renal tissue of the Tse and Tsm groups, the protection is primarily attributable to the antioxidant actions of mangiferin mediated by the inducible nitric oxide synthase (iNOS) and NF-KB pathways. Mangiferin's antioxidant activity against NaF (Sodium Fluride)-induced nephrotoxicity in normal kidney epithelial cells was further confirmed by recent research by Samadarsi and Dutta (2020). In the kidney tissue number and area of Malpighian corpuscles and area of urinary space have been decreased in high salt treated group compared to control. This compatible finding is also reported by Basu (2011) in male albino mice. Histological changes of Reduction of kidney tissues, Bowman's capsules, glomerulus and urinary spaces were noticed in high salt treated (Ts) group. Wide urinary space between glomerulus and Bowman's capsule are observed in Tse, Tsm and Tsl groups of rats respectively.

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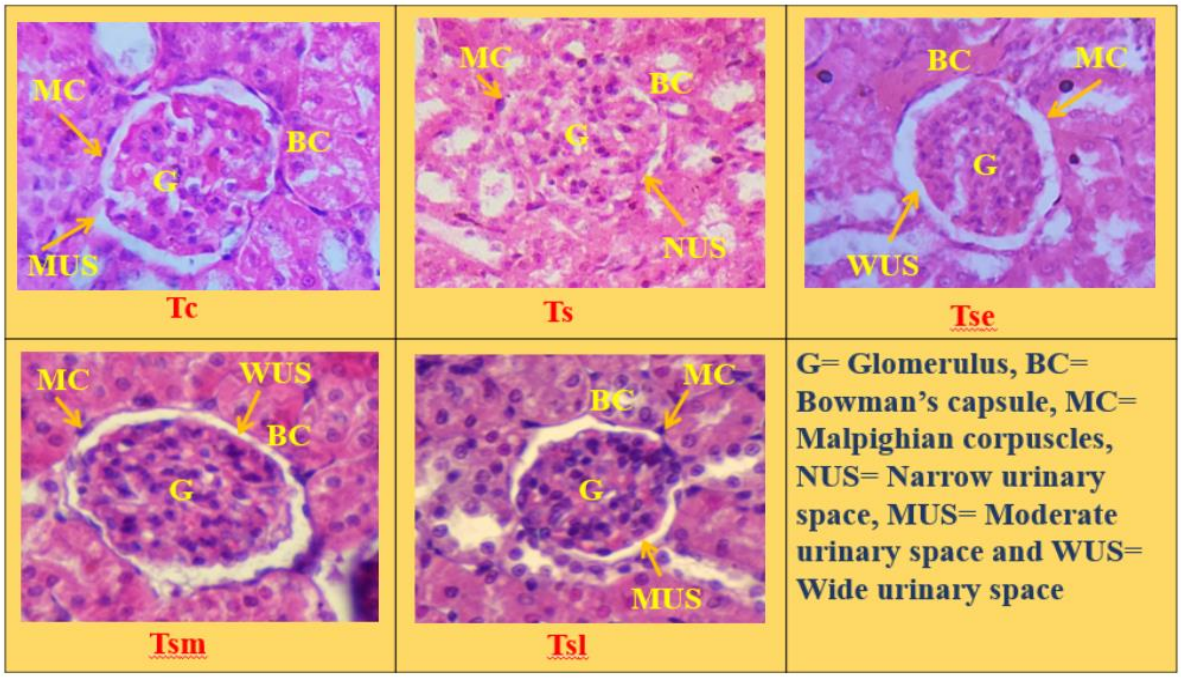

Figure 3: Effect of mango peel extract and mangiferin on kidney structure in salt-induced hypertensive rat.

The urinary spaces in the Bowman capsule are similar to the control group in the Tse and Tsm groups. Tsl groups show moderate urinary space. Ts has a narrow urinary space due to diuretic effects in the presence of high salt levels in serum**.**

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