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Assessment of Therapeutic Potential of *Moringa oleifera* Leaves on Renal Impairment in Dogs in Braj Bhoomi Region of Uttar Pradesh, India

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Abstract: The present study was conducted to evaluate the therapeutic potential of *Moringa oleifera* leaves on renal impairment in dogs. For this, a total of 3881 dogs, irrespective of age, breed and sex presented to veterinary clinical complex, DUVASU, Mathura were examined. Total 287 dogs showed clinical signs concern to renal impairment, out of which 43 dogs were found positive for renal impairment by various clinical markers and laboratory tests. Twelve found positive for renal impairment during screening were taken for therapeutic study and randomly allocated into two groups, each group having six animals. In group B conventional treatment was given and in group C conventional treatment with combination of *Moringa oleifera*@ 30mg kg^{-1} body weight PO (per os-orally**)** was administered for 14 days. Six apparently healthy dogs were placed in healthy control group (group A) after thorough physical examination and various diagnostic tests. Treatment with conventional drugs as well as combination therapy with *Moringa oleifera* significantly reduced the concentration of Hb, TEC, PCV, Platelets as well as activities of the activities of serum creatinine, BUN, ALP, AST, GGT and renal biomarker protein cystatin-C and SDMA in renal impairment dogs. Based on results of the study it was concluded that *Moringa oleifera* may be advised as adjunct therapy along with conventional treatment for early recovery in renal impairment in dog.

Key words: Renal impairment; *Moringa oleifera;* semi-arid.

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Renal impairment is one of the important constraints in human and animal health globally since last two decades, irrespective of geographical, racial and cultural boundaries (Panigrahi *et al.,* 2016). Conservative medical management of renal impairment consists of the supportive and symptomatic treatment. Renal impairment aims to treat anomalies in the fluid, electrolyte, acid-base, endocrine, and nutritional balance. The goals of medical management are to reduce clinical uremia symptoms; lessen disturbances brought on by excesses or losses of water, electrolytes, vitamins, and minerals; support enough nutrition by supplying daily protein, calorie, mineral and other nutrient requirements and slow down the development of renal

failure (Polzin, 2011). Due to progression of CRF (chronic renal failure) to end-stage disease, renal function can be regenerated only by renal replacement therapy i.e. kidney transplantation or dialysis, which is costlier and unaffordable in veterinary cases. Hence, ayurvedic drugs can be used to complement modern medicines to reverse kidney damage in animals (Patel *et al.,* 2011). Indeed, renal impairment is a multifactorial disease and involves in alteration of several biochemical pathways. Therefore, the present situation demands a newer approach of therapy. Several recent studies have highlighted the effectiveness of medicinal plants and natural compounds for treatment and management of renal impairment (Kamboj, 2000). Moreover, herbal remedies are known to contain multiple constituents, acting through multiple pathways such as antioxidant, analgesic, diuretic, pH neutralizing, etc.

Moringa oleifera, belonging to family Moringaceae, is commonly known as horseradish tree or drumstick tree and is native to India. It is known as a miracle tree because of its versatility in medicine and therapy. The pharmacological properties of moringa and its constituents include antibacterial, antioxidant, anti-inflammatory, anticancer, antiulcer, antidiabetic, antiproliferative, antispasmodic, and many more properties (Menon *et al.,* 2019). It aids in the prevention of numerous diseases that affect the liver, eyes, gastrointestinal tract, respiratory system, cardiovascular system, kidney and other organs. Study shown that, it helps in lowering the blood levels of urea and creatinine (Gopalakrishnan *et al.,* 2016). Taking into consideration of the above properties a study was conducted to access the efficacy of *Moringa oleifera* leaves on renal impairments in dogs

Materials and Methods

Study area and selection of animals: Brajbhoomi is a part of the Ganges-Yamuna-Doab (Ganges valley and upper Indus) region lies well within the golden triangle of Delhi-Jaipur-Agra covering an area of about 3,800 km^2. The area stretches from Mathura, Jaleswar, Agra, Hathras, Aligarh and Farrukhabad district of Uttar Pradesh, India. Dogs presented to VCC, DUVASU, Mathura from above Braj Bhoomi region, of any breed, of any sex, of any age group, with history of renal impairment and clinical symptoms like polyuria, polydipsia, weight loss, growth restriction, in-appetence, and vomiting etc. were chosen for the experimental study.

Selection of animals: The present study was undertaken in the Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura in association with veterinary clinical complex (VCC), DUVASU, Mathura. Dogs presented to VCC, DUVASU, Mathura of any breed, of any sex, of any age group, with history of renal impairment and clinical symptoms like polyuria, polydipsia, weight loss, growth restriction, in-appetence, and vomiting etc. were chosen for the experimental study. Dogs having clinical findings of renal impairment were screened for the experimental trial based on urine analysis, hematology, serum biochemistry and advanced diagnostic imaging techniques.

Collection and processing of plant material: Fresh green leaves of *Moringa oleifera* was taken for the study. The plant was collected from medicinal herb garden, DUVASU, Mathura. The plant material was cut into pieces; washed with distilled water to remove the dirt, air dried and uniformly powdered using an electric grinder.

Hydro-alcoholic extract preparation: The powdered plant material was placed inside a clean container. The extraction solvent (Ethanol: distilled water 1:1) was then poured on top of the drug material at room temperature (37˚C), soaked, and kept for 72 hrs. Then the extract was filtered using filter paper (Whatman no. 40). The solvent was removed by using rotary evaporator. The extract was dried *in vacuo* and stored refrigerated.

Capsules preparation: After extraction of Moringa leaf extract, it was mixed with the vehicle and homogenous mixture was formed with the help of mortar and pestle then the formed powder was filled in the gelatin capsules at different doses i.e. 450 mg, 500 mg, and 600 mg according to the body weight.

Therapeutic trial: Six apparently healthy dogs were placed in healthy control group (group A) after thorough physical examination and various diagnostic tests. Twelve dogs screened for renal impairment were randomly divided into two groups (group B and group C) containing six animals each. Group B animals were treated with the conventional treatment (as per clinical

Table 1. Urine profile in dogs before and after treatment

Parameter	Groups	Day 0	Day 7	Day 14
Specific gravity	Group A	1.01±0.003	1.01±0.0009	1.01±0.003
	Group B	1.01±0.003	1.01±0.002	1.01±0.003
	Group C	1.01±0.002	1.02±0.003	1.01±0.003
Urine pH	Group A	6.13^{aA}±0.07	6.26^{aA}±0.11	6.35^{aA}±0.11
	Group B	7.23^{bB}±0.37	6.60^{aA}±0.17	6.00^{aA}±0.18
	Group C	7.13^{bB}±0.20	6.21^{aA}±0.20	5.83^{aA}±0.16

Group A: healthy control group; Group B: conventional treatment group and Group C: conventional treatment + *Moringa oleifera.* Values (Mean±SE) within same column for a particular parameter (capital letters) and in same row (small letter) bearing similar superscript do not differ at P<0.05.

findings and requirement) for14 days. Group C animals were treated with the combination of *Moringa oleifera* leaves powder along with conventional treatment for 14 days. Therapeutic efficacy was evaluated at weekly interval for 14 days by urine analysis, hemato-biochemical analysis and renal diagnostic biomarkers *viz*. cystatin C and SDMA.

The data was statistically analyzed using Statistical Package for the Social Sciences (SPSS software IBM, version 20.0). Significance has been taken at p <0.05

Results and Discussions

A total of 3881 dogs, irrespective of age, breed and sex presented to VCC, DUVASU, Mathura were examined. Total 287 dogs showed clinical signs concern to renal impairment, out of which 43 dogs were found positive for renal impairment by various clinical markers and laboratory tests. The overall occurrence (hospital based prevalence) of renal impairment in total dog population was 1.107% (43/3881)

The variations in mean of specific gravity of urine between different groups were non-significant in our study (Table 1). These observations are in agreement with those of Pareta *et al.* (2011). However higher values of specific gravity of urine in renal impairment were observed by Puri *et al.* (2015). The oliguric phase of renal failure, protein-losing and nephrotoxic nephrosis and early renal dysfunction are all conditions that cause the specific gravity of urine in renal failure dogs to remain unchanged.

There was significant increase in urinary pH in renal impairment dogs (group B and group C) as compared to apparently healthy control dogs (group A) before administration of any treatment (Table 1). Conventional treatment as well as combination of *Moringa oleifera* significantly decreased the concentration of urinary pH in dogs. These findings were in contrast to Thade (2019) who reported non-significant variation in the value of urinary pH. It could be because urinary pH is affected by meals and digestion, which causes it to change throughout the day and retained urine with a varied pH can conceal changes in urine pH at any moment (Hekmatynia *et al.,* 2019).

In the present study, mean hemoglobin, packed cell volume and total erythrocyte count were significantly lower in dogs with renal impairment (group B and group C) than those of apparently healthy dogs (group A) before initiation of any treatment (Table 2). Whereas, after treating the animals with conventional treatment as well as combination of *Moringa oleifera*, the values increased significantly. A similar increase in hemoglobin, packed cell volume and total erythrocyte count has also been reported in Sirohi goat kids in another study (Meel *et al*., 2018). However, these observations are in contrast with Nurhayati *et al.* (2023) who reported non-significant change in hemoglobin, packed cell volume and total erythrocyte count in male Wistar rats. The reduced ability of the kidney to induce erythropoiesis with the hormone erythropoietin might be responsible for the decline in these parameters in renal impairment dogs. Reduced erythropoiesis fails to stimulate the bone marrow from producing enough red blood cells, ultimately resulting in a drop in hemoglobin levels. Anemia could be due to reduced erythropoietin formation resulting in decreased total erythrocyte count. The low packed cell volume before treatment might be due to the decreased hemoglobin value as it is directly correlated with hemoglobin concentration (Oburai *et al.,* 2015).

Table 2. Hematology profile in dogs before and after treatment

Parameters	Groups	Day 0	Day 7	Day 14
Haemoglobin (g/dL)	Group A	14.11^{aB}±0.33	14.10^{aB}±0.46	14.23^{aB}±0.45
	Group B	08.95^{aA}±0.36	09.70^{aA}±0.25	11.63^{bA}±0.57
	Group C	08.58^{aA}±0.19	10.24^{bA}±0.20	11.93^{cA}±0.28
Total erythrocyte count(TEC) ($\times10^6$/µl)	Group A	06.50^{aC}±0.18	06.61^{aC}±0.25	06.58^{aC}±0.20
	Group B	03.73^{aA}±0.19	04.34^{bA}±0.17	04.63^{bA}±0.15
	Group C	04.27^{aB}±0.14	05.11^{bB}±0.16	05.96^{cB}±0.16
Packed cell volume (PCV) (%)	Group A	42.76^{aC}±0.41	42.74^{aC}±0.41	42.99^{aB}±0.44
	Group B	25.28^{aA}±1.06	30.92^{bA}±0.75	35.47^{cA}±0.51
	Group C	28.71^{aB}±1.10	33.87^{bB}±1.01	37.16^{cA}±0.71
Platelet count ($\times10^5$/µL)	Group A	289.83^{aB}±2.58	289.98^{aA}±2.71	289.10^{aA}±3.30
	Group B	187.67^{aA}±34.60	194.83^{aA}±14.18	257.00^{bA}±17.46
	Group C	188.17^{aA}±3.08	241.33^{bA}±10.97	286.83^{cA}±3.97
Total leukocyte count TLC ($\times10^3$/µl)	Group A	11.76^{aA}±0.47	11.80^{aA}±0.49	11.93^{aA} ±0.49
	Group B	24.56^{bB}±5.85	18.09^{aA}±2.13	13.78^{aA} ±0.62
	Group C	18.16^{aB}±1.00	17.15^{aA}±0.65	13.98^{aA} ±0.68

Group A: healthy control group; Group B: conventional treatment group and Group C: conventional treatment + *Moringa oleifera*. Values (Mean±SE) within same column for a particular parameter (capital letters) and in same row (small letter) bearing similar superscript do not differ at $P<0.05$.

In present study, mean total leucocyte count increased significantly in dogs with renal impairment than the apparently healthy dogs before initiation of any treatment. Whereas, after treating the dogs with conventional treatment as well as combination of *Moringa oleifera*, the values were decreased significantly. A similar increase in total leucocyte count has also been reported by Oladele *et al.* (2011), who studied the evaluation of anti-inflammatory and membrane stabilizing effects of aqueous root extract of *Boerhaavia diffusa* in rats. These observations are in contrast with Meel *et al.* (2018) who reported non-significant change in total leucocyte count in Sirohi goat kids and in rabbits, respectively. In renal impairment, infection or massive tissue damage can lead to an increase in leukocytes. Hence, it indicates presence of inflammation, having either infectious or non-infectious origin.

In the present study, mean platelet count decreased significantly in dogs with renal impairment than apparently healthy dogs before initiation of any treatment. Whereas, after treating the dogs with conventional treatment as well as combination of *Moring aoleifera*, the values were increased in all the treatment groups. A similar significant increase in platelet count has also been reported in rats and rabbits in another study (Hisham *et al*., 2012). However, these observations are in contrast with those of Nurhayati *et al.* (2023) who reported non-significant change in platelet count in male wistar rats. In the present study no significant differences were recorded in mean concentration of differential leucocyte count in per cent (neutrophils, lymphocyte, monocyte, eosinophils), MCV, MCH, MCHC, either between the groups or within groups at different observation periods. Similar findings have been reported by Terzungwe *et al*. (2013) in rabbits.

A significant increase in concentration of BUN and creatinine, was recorded in dogs with renal impairment (group B and group C) as compared to apparently healthy control dogs (group A) before administration of any treatment. Conventional therapy as well as combination of *Moringa oleifera* significantly decreased the concentration of BUN and creatinine in affected dogs. A similar decrease in BUN and creatinine has also been reported in other studies. Akinrinde *et al*. (2020) reported decreased concentration of BUN and creatinine by administering methanol extract of *Moringa oleifera* leaves on acute kidney injury induced by ischemia-reperfusion in rats. However, these results are in contrast with those of Terzungwe *et al.* (2013) who observed non-significant differences in mean concentration of BUN and creatinine. Increase in BUN and creatinine levels could be attributed to

Table 3. Biochemical profile in dogs before and after treatment

Parameters	Groups	Day 0	Day 7	Day 14
Blood Urea Nitrogen (BUN) (mg/dL)	Group A	15.55^{aA}±1.14	15.78^{aA}±1.19	15.92^{aA}±1.23
	Group B	70.01^{cB}±2.54	48.87^{bB}±11.21	29.08^{aA}±3.03
	Group C	62.09^{bB}±4.43	27.75^{aA}±3.67	27.63^{aA}±1.68
Creatinine (mg/dL)	Group A	0.89^{aA}±0.12	0.89^{aA}±0.11	0.90^{aA}±0.11
	Group B	5.31^{bC}±0.27	4.29^{bC}±0.61	3.39^{aC}±0.75
	Group C	4.08^{bB}±0.31	3.11^{abB}±0.24	2.19^{aB}±0.28
Total protein (gm/dl)	Group A	6.18^{aA}±0.24	6.05^{aA}±0.25	6.05^{aA}±0.23
	Group B	4.93^{aA}±0.40	6.01^{abA}±0.41	6.83^{bA}±0.58
	Group C	5.87^{aA}±0.60	6.06^{aA}±0.57	6.19^{aA}±0.44
Albumin (gm/dl)	Group A	3.06^{aA}±0.15	2.99^{aA}±0.17	2.80^{aA}±0.12
	Group B	2.58^{aA}±0.31	3.29^{abA}±0.32	3.80^{bB}±0.36
	Group C	2.84^{aA}±0.29	3.36^{aA}±0.21	3.55^{aAB}±0.19
Globulin (gm/dl)	Group A	3.12±0.33	3.05±0.32	3.25±0.24
	Group B	2.42±0.34	2.61±0.42	2.95±0.48
	Group C	3.02±0.67	2.70±0.76	2.63±0.57
A:G ratio	Group A	1.05±.13	1.05±0.14	0.89±0.08
	Group B	1.17±.22	1.50±0.36	1.63±0.42
	Group C	1.42±.51	4.29±2.32	2.42±1.11
Gamma-glutamyltransferase (U/L)	Group A	11.68^{aA}±0.25	11.75^{aA}±0.25	11.86^{aA}±0.31
	Group B	16.64^{bB}±1.14	17.02^{bB}±0.97	12.96^{aA}±0.80
	Group C	15.74^{bB}±1.13	13.57^{abA}±0.92	11.74^{aA}±0.31

Group A: healthy control group; Group B: conventional treatment group and Group C: conventional treatment + *Moringa oleifera.* Values (Mean±SE) within same column for a particular parameter (capital letters) and in same row (small letter) bearing similar superscript do not differ at $P<0.05$.

decreased kidney function. The reduction in the BUN and creatinine levels (post-treatment) can be explained due to the renoprotective, anti-inflammatory and antioxidant effects of the Moringa leaves (Gopalakrishnan *et al.,* 2016).

There was significantly decreased concentration of TP and albumin in renal impairment dogs as compared to apparently healthy control dogs before administration of any treatment (Table 3). The conventional therapy as well as combination with *Moringa oleifera* significantly increased the TP and albumin concentration in dogs. These findings are in agreement with Meel *et al.* (2018) who studied the effect of *Moringa oleifera* leaves feeding on sirohi goat kids. However, these findings are in contrast with Terzungwe *et al.* (2013) who reported non-significant differences in mean concentration of total protein and albumin. Proteinuria is a severe side effect of damaged kidneys' inability to filter the body's waste because proteins and nutrients are reabsorbed back into the circulation due to their molecular weight. However, there are some herbal supplements that may be used to protect the injured kidneys and reduce structural damage. In present study, no significant difference was recorded in mean concentration of globulin either between the groups or within groups at different observation periods of study before and after therapy. A similar result has also been reported by Terzungwe *et al*. (2013) in rabbits.

In present study, GGT significantly increased in renal impairment dogs before the treatment on day 0, whereas, after treating the dogs with conventional treatment as well as combination of *Moringa oleifera*, the values of GGT were significantly decreased. These results are in agreement with Younis *et al.* (2022) who reported significantly decreased concentration of GGT in rats. The decrease in enzyme levels in the *M. oleifera* treated group might indicate a repairing effect of phyto-chemicals that are found in high concentrations in the *M. oleifera* leaves (Younis *et al.,* 2022). GGT levels in the blood may increase in renal impairment dogs due to a number of factors like damage to the proximal tubules because they are responsible

Table 4. Serum Cystatin C and SDMA concentration in dogs before and after treatment

Parameter	Groups	Day 0	Day 7	Day 14
Serum Cystatin C (mg/L)	Group A	1.08^{aA}±0.16	1.10^{aA}±0.16	0.80^{aA}±0.22
	Group B	4.00^{bB}±0.34	3.66^{aB}±0.59	2.71^{aB}±0.17
	Group C	4.24^{bB}±0.44	3.11^{aB}±0.18	2.74^{aB}±0.54
SDMA (**µg**/dl)	Group A	207.98^{aA}±2.78	209.53^{aA}±3.94	210.47^{aA}±3.052
	Group B	377.50^{bB}±22.65	348.85^{bB}±44.74	243.90^{aA}±30.66
	Group C	452.63^{bC}±42.91	352.10^{bB}±44.90	228.58^{aA}±40.18

Group A: healthy control group; Group B: conventional treatment group and Group C: conventional treatment + *Moringa oleifera.* Values (Mean±SE) within same column for a particular parameter (capital letters) and in same row (small letter) bearing similar superscript do not differ at P<0.05.

for filtering waste products out of the blood. When these tubes are damaged, GGT can leak into the blood (Garry *et al.,* 1990).

There was significantly increased concentration of cystatin C in dogs suffering from renal impairment as compared to apparently healthy dogs before administration of any treatment (Table 4). Conventional treatment as well as combination of *Moringa oleifera* significantly decreased the concentration of cystatin C in affected dogs. Increase in serum cystatin C activity in kidney dysfunction is in accordance with the findings of Iwasa *et al.* (2019). In the contrary, these results are in contrast with Nwakuilite *et al.* (2020), who reported non-significant differences in mean concentration of cystatin C after the treatment in streptozocin induced diabetic rats treated with *Moringa oleifera* leaf powder. This rise in serum Cystatin C might be triggered by renal failure, which causes a decline in glomerular filtration rate. Prior to the onset of renal symptoms, elevated blood Cystatin C concentrations have been shown to identify pathological changes in renal failure.

In the present investigation, serum SDMA activity of both the renal impairment groups (group B and group C) were significantly higher (Table 4) than that of apparently healthy control group on the day of presentation (Day 0). However, there was significant decrease in serum SDMA on day 14 post-treatment in both the treatment groups. The present result of increase in serum SDMA in renal dysfunction is in accordance with the findings of Dahlem *et al.* (2017). Significantly higher SDMA concentrations could be explained by a decreased glomerular filtration rate caused due to renal hypo-perfusion and/or intrinsic renal damage. Serum creatinine concentrations are directly correlated with age and muscle mass of the animal. However, no such correlation is observed in case of SDMA. Thus, SDMA is a more sensitive biomarker for assessing renal impairment than creatinine (Hall *et al.,* 2015).

Conclusions

Based on results of this study both conventional therapy as well as combination of Moringa oleifera with conventional therapy was found effective against renal impairment in dog as evidenced by restoration of urine pH, BUN, creatinine, AST, ALP, GGT as well as concentration of serum Cystatin C and SDMA. Moringa oleifera may be advised as adjunct therapy along with conventional treatment for early recovery in renal impairment in dog.

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