AMELIORATING ACTION OF ASTRAGALUS MEMBRANACEUS ON KIDNEY DISEASE OF MALE RAT

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Abstract— Aniline, also known as aminobenzene or phenylamine, is an aromatic amine with six carbon atoms, seven hydrogen atoms, and one nitrogen atom. It is used in various chemical syntheses and is poisonous. Aniline is a colorless, slightly yellowish viscous liquid used in dye production and agrochemicals. In vivo, aniline toxicity is caused by hazardous metabolites, oxidative and nitrosative stress, and damage to proteins and nucleic acids. Globally, chronic kidney disease (CKD) is expected to be a serious public health concern. A prevalent plant in traditional Chinese medicine is Astragalus root. In some clinical and animal investigations, it has been shown to have renoprotective benefits, and in China and Japan, it is prescribed to patients with chronic kidney disease. The advancement of CKD has been linked to oxidative stress, according to reports. Since astragalus root has been shown to have anti-oxidative properties, we hypothesized that this is how its renoprotective properties work. For this study on the ameliorating potential of Astragalus, we used Rattus norvegicus as an animal model. The presenting study examined the ameliorating potential of Astragalus in various aspects of rat health, including enzymatic, histological, behavioral, morphological, weight response, hematological, biochemical, and blood and urine analysis..

Index Terms—Kidney, Aniline, Saccharin, Astragallus, Ameliorates

I. INTRODUCTION

Studies on aniline and its chlorinated derivatives show biotransformation pathways like N-oxidation, N-acetylation, and phenyl ring oxidation. Many aniline derivatives cause renal damage in men, but detailed studies on acute effects are lacking. Further research is needed to understand the potential risks of exposure to these chemicals. The purpose of this study was to investigate the acute nephrotoxic potential of aniline. The purpose of this study was to investigate the acute nephrotoxic potential of aniline in male rats. The renal effects of these compounds were studied in vivo. Aniline is used as chemical intermediate in a variety of chemical syntheses. The primary toxicity of aniline is characterized by methemoglobinemia (Anon., 2001). The increased production of methemoglobin (MetHb) in erythrocytes (ferrihemoglobin HbFe31) results from the oxidation of heme iron from the ferrous to the ferric state (Kiese, 1974; McLean et al., 1969). Although MetHb formation is reversible and does not enhance red blood cell destruction per se, it is a component of oxidative injury to red blood cells (Jollow and McMillan, 2001) and is generally involved as a step in Heinz body formation (Harvey, 1989). MetHb is physiologically inactive and cannot bind reversibly with oxygen. Methemoglobinemia is usually a transient effect of intraerythrocytic mechanisms that facilitate the conversion of MetHb back to hemoglobin. However, extended intra-erythrocytic oxidative stress promotes depletion of the antioxidant capacity of the cell, causing dysfunction by reversible interactions between protein thiols and glutathione, which

leads to binding of the hemoglobin to the erythrocyte membrane (Dickinson and Forman, 2002; Jarolim et al., 1990; Marchesi, 1985; Mawatari and Murakami, 2004). The critical role proposed for the RBC membrane skeletal proteins in controlling the morphology of this cell caused by exposure to hemolytic agents has been described in detail (Grossman, Simson, and Jollow, 1992). Aniline is a toxic organic compound with the chemical formula C6H5NH (CH3), used as a solvent and intermediate for dyes, agrochemicals, and manufacturing organic products. It is metabolized quickly at lower doses and can cause methemoglobinemia duration. N-Methylaniline, an aniline derivative and precursor of nitrosamine, is a toxic organic compound with strong methemoglobinogenic potency. It is well absorbed in the respiratory and gastrointestinal tracts and through the skin. Aniline shows a more potent methemoglobinogenic effect than aniline, with higher levels of MetHb observed in rats after intraperitoneal administration. It can cross the placenta to the fetus. Aniline is a toxic aromatic amine widely used in the chemical industry, particularly in the manufacture of dyes, resins, varnishes, perfumes, pigments, herbicides, fungicides, explosives, isocyanates, hydroquinone, and rubber chemicals. Chronic exposure to aniline leads to the development of splenomegaly, increased erythropoietic activity, hyperpigmentation, hyperplasia, and fibrosis. The clinical symptoms of aniline exposure, such as cyanosis, weakness, dizziness, headache, stupor, loss of coordination, and coma, occur rapidly (within 1–3 h) after ingestion or skin contact. Earlier studies have shown that aniline exposure leads to the formation of oxidative and nitrosative stress, which is due to iron overload and the induction of lipid peroxidation. The excess production of free radicals could attack proteins and nucleic acids, leading to structural and functional changes in the kidney. Chronic kidney disease (CKD) is a major public health issue, with an estimated global prevalence. The development of CKD stages is an independent risk factor for mortality and cardiovascular events, and these risks are more than four times higher in patients with advanced CKD stages than in the population with normal kidney function. In addition, patients who receive renal replacement therapy have a lower quality of life and restricted daily activity because dialysis takes a certain amount of time. Therefore, to prevent CKD progression, various clinical strategies, including the control of blood pressure, dietary treatment with renin-angiotensin system (RAS) inhibitors, and the use of sodium-glucose cotransporter-2 inhibitors, are recommended. However, some patients, in whom these treatments are ineffective, reach a state of end-stage kidney disease. For this change in kidney amleriotes caused by the use of *Astragalus* in this study. *Astragalus* root is a commonly used herb in traditional Chinese medicine. It has been reported to have renoprotective effects in some clinical and animal studies and has been prescribed for patients with CKD in Japan and China. Oxidative stress has been reported to play a role in CKD progression. *Astragalus* root, a traditional Chinese medicine, has been found to exhibit anti-oxidative effects and renoprotective effects, potentially due to its renoprotective effect on the Renin Angiotensin System (RAS). This is crucial for the progression of Chronic Kidney Disease (CKD), a global health issue triggered by glucose and lipid metabolism disorders. Studies have shown that *Astragalus* root extract can inhibit angiotensin-converting enzymes (ACE) in hypertensive rats and decrease plasma angiotensin II levels. However, the effectiveness of astragalus in inhibiting RAS remains controversial. Diabetic kidney disease (DKD), a major global health issue, is primarily caused by high glucose levels, oxidative stress, hemodynamic disorders, inflammation, and fibrosis. Early intervention and treatment are essential to slow disease progression and reduce complications. *Astragalus* is a traditional Chinese medicine (TCM). These herbal medicines have been used for the treatment of anemia, chronic cardiovascular disease, and chronic kidney diseases, as recorded by ancient Chinese pharmacopoeias. *Astragalus* has been reported to have good clinical activity with CKD. Thus, in this study, we examined the renoprotective effects and their mechanisms, including oxidative stress and RAS, of *Astragalus* root using male rats

Material and Method.

Animals

Male wistar rats (200–250g) were used in the study and each group contains six rats The

animals were procured from datta meghe sawangi wardha. Rats were placed separately in polypropylene cages with paddy husk as bedding. The animals were maintained under standard laboratory conditions at temperature 23 ± 1°C, relative humidity 45–55, and 12 h light and 12 h dark cycles throughout the experiments.

Experimental protocol

Animals were divided into different groups (*n* = 6). Group I served as normal control and received normal drinking water, orally as vehicle. Group II rats received Aniline 20 mg/kg and *Astragalus membranaceus* 5gm/kg bw daily in drinking water for 30 days.

Biochemical evaluation

General parameters like body weight, kidney weight, were studied at the end of study. At the end of treatment period, blood was withdrawn form heart and serum was separated. Blood sample was used for the estimation of hemoglobin (Sahli's hemometer method), and red blood cell (RBC) and white blood cell (WBC) using hemocytometer and serum sample was used for the estimation of enzyme content and protein content, urea,creatinine and bilirbin also estimate.

Assessment of Histology

For histological study animals were sacrificed after 30 days of treat-men wash with saline solution then put cutted tissue in to fixative then HE stainimg for getting changes.

Statistical analysis

All the values are presented as mean ± standard error of the mean (SEM). Statistical significance between more than two groups was tested using one-way analysis of variance (ANOVA). Differences were considered to be statistical significant when $P < 0.05$.

RESULTS

Effect of Aniline of Effect of Aniline and ameliorating effect of *Astragalus* on body weight, Kidney weight.

As shown in Table 1, at the end of 30th day body weight, water intake and feed consumption was monitored and it was found to be moderately changed in AH-treated group as compared to control group. Whereas fecal matter content was found to be unchanged in AH-treated group. Chronic (30 Days) treatment with *Astragalus* showed significant alteration in body weight, Kidney weight. compared to Aniline-treated rats. The kidneys have several essential homeostatic functions. These functions include waste removal (NH3), fluid/electrolyte balance, metabolic blood acid-base balance, as well as producing/modifying hormones for blood pressure, calcium/potassium homeostasis, and red blood cell production. The renal corpuscle (filtration unit, which comprises the glomerulus and the surrounding glomerular or Bowman's capsule) and tubules (reabsorption and excretion) of the kidney perform the majority of these kidney functions. The primary function of the kidney is to filter blood and form urine. The histological structures of the filtering units of the kidney (renal corpuscles) are crucial for this function. The renal corpuscles are located only in the kidney cortex, with about 1 million per kidney with variation due to race. This unique filtration barrier contains three histological structures: the capillary endothelium of the glomeruli, specialized cells called podocytes, and the fused basement membranes of both of these cells. This filtration barrier allows for the filtration of small molecules such as water, ions, creatinine and glucose, and small proteins (less than **90 kDa**). This structure must prevent the filtration of large proteins present in the blood, such as albumin and immunoglobulins. Effect of Aniline and ameliorating effect of *Astragalus* on Histological structure of Kidney.

Table I

Body and Kidney weight of control, effect of Aniline, *and Astragallus* on rats after 30 days

Sr. No.	Group	**Body weight (gm)** (mean)	Kidney weight. (mean)
1.	Group-1 (normal)	193.00	0.931
2.	Group-2 Aniline	190.66	0.593
3.	Group-3 Astragallus+ Aniline	197.66	0.676

Table 2

Haematological paprameters of control, aniline treated, and amelioration of *Astragalus membranaceus*

Sr. No.	Haematological Parameter	Control Rat(Mean)	Aniline Treated rat(Mean)	Aniline + *Astragalus* Treated rat(Mean)
1	RBC**(million/mm3)**	5.39	5.27	5.83
2	WBC**(thousands /mm^3)**	4.35	4.22	5.08
3	Hemoglobin **(gm/dL)**	10.71	10.21	12.07
4	Bilirubin **(mg/dL)**	4.04	5.24	5.84

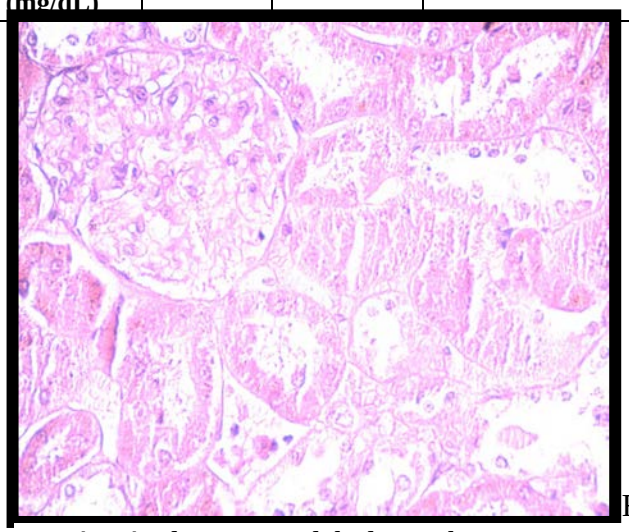

Fig-A Aniline treated kidney showing acute tubular necrosis in renal tubular cells with loss of brush border (red arrows); flattening of the renal tubular cells due to tubular dilation (green arrows); Glomerulo nephritis (yellow arrows)

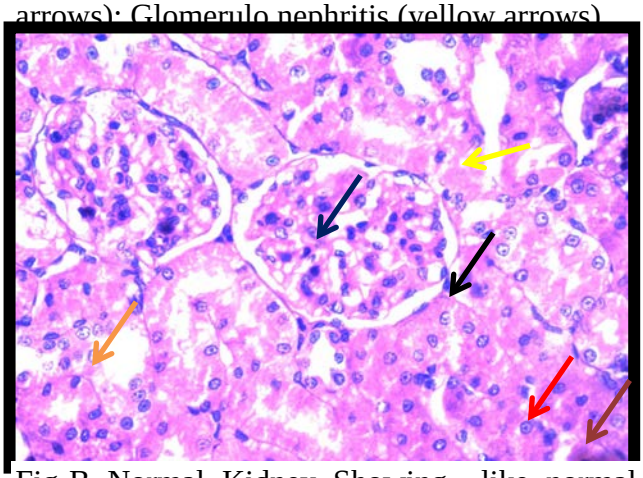

Fig-B Normal Kidney Showing like normal cleared glomerlar capillaries in renal corpuscles (red in color), Glomerular tuft with podocyte (black in color), showing clear bowman capsule (green in color), proximal convolted tubule (purple in color) and distal convolted tubule (yellow in color).

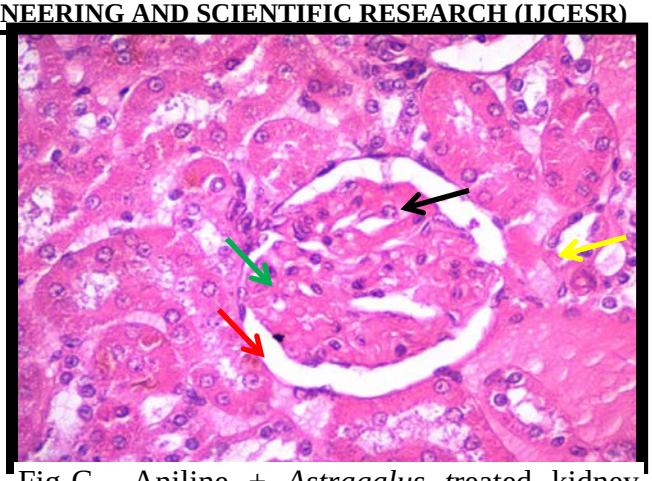

Fig-C Aniline + *Astragalus* treated kidney showing returning of normal cleared glomerlar capillaries in renal corpuscles (black in color), Glomerular tuft with podocyte (green in color), showing clear bowman capsule, proximal convolted tubule (red in color) and distal convolted tubule (yellow in color),

DISCUSSION

In the present study, kidney toxicity was induced by chronic administration of Aniline via drinking water. Toxicity of kidney was confirmed by evaluating the hemoglobin level for alternate days for 30 days. At 30th day, hemoglobin level was significantly decreased.Aniline exposure leads to the development of kidney toxicity in rats. Studies have shown that exposure to aniline produces substantial decreases haemoglobin content in rat as earlier discussed by (Ju¨rgen Pauluhn). *Astragalus* membranacios increase haemoglobin in rats even when is given dosage with aniline that means ameliorates power of *Astragalus* help to increase the Hb. in rats as per discssed in (Nanjia et al). Significant decreased in body weight, organ weight by aniline-treated rats might be due to toxicity of aniline which decreased the food consumption and can be directly correlated to decreased body weight as discssed earlier as by (rehan et al). One of the important features of this study was increase in the weights of in anilne and *Astragalus* combine -treated rats as discussed earlier by (zhanzhu sun et al). Aniline treated rat decrease weight of kidney as earlier discussed in (rankin et al). Astagallus reported to play a major role in increasing the weight of rat. In the present study, *Astragalus* treatments reverse the changes in body weight, The alteration of general parameters suggested the positive effect of

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Astragalus in Aniline toxicity. Changes in the level of hemoglobin, RBC, and WBC were observed. In the present study, Aniline-administered rats displayed reversal normal structure of kidney.Acute tubular necrosis is kidney injury caused by damage to the kidney tubule cells. Glomerulonephritis is inflammation and damage to the filtering part of the kidneys (glomerulus). Acute tubular necrosis is kidney injury caused by damage to the kidney tubule cells Acute tubular necrosis (ATN) results in AKI from a number of processes. Medullary ischemia results from hypoxic injury to the thick limb of the loop of Henle. This leads to sloughing of cells (casts), which block tubular flow. The initiation phase is characterized by an acute decrease in glomerular filtration rate (GFR). The tubule cell damage and cell death that characterize ATN usually result from an acute ischemic or toxic event. Nephrotoxic mechanisms of ATN include direct drug toxicity, intrarenal vasoconstriction, and intratubular obstruction. The recovery phase, in which tubular function recovers, is characterized by an increase in urine volume (if oliguria was present during the maintenance phase) and by a gradual decrease in BUN and serum Cr to their preinjury levels.

II. CONCLUSION

The study reveals that chronic Aniline exposure can lead to kidney toxicity in rats. The study found that the hemoglobin level decreased significantly at the 30th day, indicating that the toxicity of aniline can lead to decreased body weight. The study also found that the combination of Aniline and *Astragalus* treatment reversed the changes in body weight, suggesting a positive effect of *Astragalus* in Aniline toxicity. The study also found that Aniline-administered rats displayed a reversal of normal kidney structure. Acute tubular necrosis (ATN) is a kidney injury caused by damage to kidney tubule cells, and the recovery phase involves increased urine volume and gradual decreases in BUN and serum Cr to pre-injury levels.

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