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Evaluation of Nephroprotective activity of aqueous seed extract of Macrotyloma uniflorum against Gentamicin induced Nephrotoxicity in Wistar rats.

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ABSTRACT: One of the lesser-known species of grain legumes is horsegram (Macrotyloma uniflorum). Horsegram is commonly used as a feed for cattle, and the complete seeds are used. However, in India, the seed is typically eaten whole, either as sprouts or as part of a meal. The ayurvedic practise of using horsegram to treat kidney stones, piles and oedema is based on anecdotal evidence rather than scientific proof. This investigation focuses on the preventive effects of Macrotyloma uniflorum aqueous seed extract against gentamicin-induced nephrotoxicity in albino rats. In this model, nephrotoxicity was generated by intramuscular injection of 40 mg/kg gentamicin. There were 14 days of data collection. Significant effects were seen with Macrotyloma uniflorum extracts at 250 mg/kg and 500 mg/kg on body weight, urine volume, urine ph, kidney weight, serum creatinine, urea, uric acid, and total protein.

KEYWORDS: Macrotyloma uniflorum, Gentamicin, Nephrotoxicity, Silymarin, Phytochemicals

I. INTRODUCTION

Macrotyloma uniflorum is a kind of bean that doesn't get much attention. Although the horse gramme is most commonly used as a horse feed, it is also a popular culinary ingredient. Horse gramme is widely used as a health food in traditional Ayurvedic cooking. People with jaundice or fluid retention are often given this supplement as part of a weight loss plan. [1] Despite being high in protein (20%), it is primarily consumed by farmers and people with low incomes due to the unappealing flavour and texture of cooked products. As a result, it has mostly been overlooked as a useful food legume. India is home to most of the world's horse gramme fields. It's also grown in places like Malaysia, the West Indies, and Sri Lanka. It is cultivated in the lowlands of Uttaranchal and Himachal Pradesh, as well as in the states of Karnataka, Andhra Pradesh, Orissa, Tamil Nadu, Madhya Pradesh, Chhattisgarh, Bihar, West Bengal, and Jharkhand. In various regions of India, it is taken either as a whole seed, as sprouts, or as a full meal. It has been proposed that these legumes have therapeutic use.

The nutritional value of horse gramme as a source of protein and other nutrients has been acknowledged. It's a great source of iron, molybdenum, and calcium, and its nutritional value is on par with that of other regularly produced pulse crops. Per 100 grammes of dry matter, horse gramme seeds have 321 kcal in calories, 28.7 milligrammes of calcium, 311.3 milligrammes of phosphorus, 6.77 milligrammes of iron, 5.3 milligrammes of riboflavin, and 0.4 milligrammes of thiamine. However, the nutritional quality can be affected in a variety of ways by things like genotype, soil, fertiliser application, cultural practises, weather and climatic factors, post-harvest handling and storage. [2]Despite the fact that horse gramme seed is low in fat and a rich source of protein, dietary fibre, and a range of micro-nutrients and phytochemicals, it is still a mostly untapped food legume, eaten primarily by rural communities in remote places and those with little financial resources.

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II. MATERIAL AND METHODS

Plant Material and Extraction

Plant seeds was collected from tripolia bazar JAIPUR. The plant seeds were authenticated as Macrotyloma uniflorum by Botanical Survey of India Jodhpur, Rajasthan. Macrotyloma uniflorum seeds powder was extracted by aqueous extraction method in soxhlet apparatus at 35-40^{0}C temperature, 500g of the powder was mixed with distilled water. The mixture was filtered in Buchner funnel and dried over water bath till dryness and percentage yield was determined. This extract yielded 26.3% (w/w).

Experimental Animals

The experimental protocol was approved by the Institutional Animal Ethics Committee CPCSEA No. - 2005/PO/RcBT/S/18CPCSEA, and healthy adult Wistar rats of either sex weighing 150-250 g were obtained. [3] The animals were kept in standard conditions of temperature (242 C), relative humidity (30%-70%), and light:dark cycle (12:12). The animals were fed a standard pellet diet and had free access to water.

Chemicals

Silymarin was provided as a free sample by Alchem International Pvt. Ltd. Gurgaon, India while Gentamicin sulfate was purchased from Manus Aktteva Biopharma in Gujarat , India.

Phytochemical Screening

Preliminary tests will be carried out for the presence or absence of phytoconstituents like Alkaloids, Carbohydrates, Flavonoids, Glycosides, Reducing sugars, Saponins, sterols, Anthocyanins, Terpenes and Tannins [3].

Evaluation of Nephroprotective activity

Experimental Procedure

Induction of Nephrotoxicity by Gentamicin.

In this paradigm, nephrotoxicity was generated by administering 40 mg/kg of gentamicin intramuscularly to the animals. There were 14 days of data collection [4].

Preparation of Test Drug

The test substances were formulated with 2% tween 80. Both the standard drug and the test drug was administered via oral gavage, orally, at a dose of 0.4 ml/kg body weight. All medications were freshly prepared prior to administration [4].

III. EXPERIMENTAL PROCEDURE

Induction of Nephrotoxicity by Gentamicin

Five groups of six rats each will be used for the study. Group I consists of animals orally administered with saline for 14 days. Group II animals were treated with only gentamicin (40mg/kg body wt., s.c.) for 14 days, blood was withdrawn on the 14th day. Group III animals administered with gentamicin for 14 days (40mg/kg body wt., s.c.) and treated with plant extract 250 mg/kg per oral dose for 14 days. Group IV was administered with gentamicin for 14 days (40mg/kg body wt., s.c.) and treated with plant extract 500 mg/kg per oral dose for 14 days. Group V was administered with Standard drug formulation silymarin for 14 days (200mg/kg body wt., orally.) only. The 3rd and 4th groups were studied for preventive regimen whereas 5th group is studied for standard regimen. Blood was withdrawn from the rat by retro orbital puncture method and animals were sacrificed for isolation of organs on 15th day. Then Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters [3].

Biochemical parameters

On respective day of completion of studies, blood was collected from rats by retro orbital puncture method and subjected to Biochemical parameters i.e., Estimation of Blood urea, Creatinine, Uric acid, Total protein was analyzed [3].

Statistical Analysis

The obtained results were expressed as Mean ± SEM. Comparison between control and treatment groups were performed by one way analysis of variance (ANOVA) followed by Dunnet's test. The statistical significance criterion was $p< 0.05$ (95% level). $P<0.05$ was considered as significant.

IV. RESULTS AND DISCUSSIONS

Preliminary Phytochemical Screening

Alkaloids, carbohydrates, flavonoids, glycosides, saponins, sterols, anthocyanins, terpenoids, proteins and tannins were all tested for in preliminary phytochemical analysis.

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Table: 1 Results of phytochemical tests of aqueous extract of Macrotyloma uniflorum.

Phytochemical constituents	Presence in aqueous extract of Macrotyloma uniflorum
Alkaloids	+
Anthocyanins	–
Carbohydrates	+
Flavonoids	+
Glycosides	+
Reducing sugars	+
Saponin	+
Steroids	+
Terpinoids	+
Tannins	+
Proteins	+

+ = presence, - = absence

Gentamicin Induced Model

In the second group of animals that were given gentamicin, the levels of blood urea, creatinine, uric acid, total protein, and urine urea, uric acid, and creatinine were all much higher than in the first group, which is a sign of serious kidney damage. The concentrations of Serum Urea, Creatinine, Uric acid, Total protein, and Urine Urea, Uric acid, and Creatinine were all lower in the MUE-treated groups (groups 3 and 4) than in the gentamicin-treated group (group 2). Compared to normal animals (group 1), SOD and glutathione peroxidase were much less active in animals that had been given gentamicin (group 2). When compared to rats that were given gentamicin, rats in groups 3 and 4 that were given a liquid extract of MUE were much less likely to have their SOD and GPx levels drop. Still, lipid peroxidase activity

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went up a lot in animals that were given gentamicin (2nd). The amount of lipid peroxidase didn't go up in groups 3 and 4 because they were given a liquid extract of MU. So, its antioxidant action strongly stops lipid peroxidation in a single organ. When groups 3 and 4 were given a liquid extract of MU, they were well protected from the poisoning caused by gentamicin.

Compared to normal animals (group 1), the weight of the kidneys in animals given gentamicin was much higher, and the weight of the kidneys in animals given a fluid extract was significantly lower ($p<0.001$). Comparing the control groups to the normal control group, there is a significant ($p<0.01$) rise in urine pH and a drop in pee volume. But in the groups that were given extracts, the ph of the urine went down significantly ($p<0.05$ for ethanol extract) and the amount of urine made went up [5].

Table 2: Treatment of rats with 40 mg/kg/day subcutaneous gentamicin and MUE for 24 days: Effect on blood creatinine, urea, uric acid, and Total protein Nephrotoxicity from Gentamicin: A Model

Group	Drug Treatment	Creatinine	Urea	Uric acid	Total protein
Control	2ml/Kg distilled water	0.55±0.006	20.63±1.07	1.79±0.04	8.20±0.04
Induced	40mg/kg	0.9±0.009***	35.28±0.77***	2.69±0.04***	6.77±0.02***
Preventive (test-1)	250mg/kg	0.73±0.008***	31.49±0.54***	2.39±0.05***	7.27±0.05***
Preventive (test-2)	500mg/kg	0.68±0.02***	26.25±0.77***	1.96±0.05	7.87±0.20**
Standard silymarin	3.00 ml/kg	0.60±0.02***	22.2±1.33***	1.80±0.02***	8.02±0.20***

Table 3: Effect of subcutaneous gentamicin 40 mg/kg/day and MUE on Urine Creatinine, Urea, and Uric Acid in Rats Treated for 24 Days -Gentamicin Model

Group	Drug Treatment	Creatinine	Urea	Uric acid	Urinary Protein
Control	2ml/Kg distilled water	9.22±0.03	24.19±0.28	4.16±0.02	0.322 ±0.03
Induced	40mg/kg	15.25±0.02***	48.04±0.21***	8.26±0.04***	0.444 ±0.02***
Preventive test-1	250mg/kg	12.27±0.07***	42.02±0.36***	4.26±0.02***	0.296 ±0.05***
Preventive test-2	500mg/kg	10.20±0.02***	32.23±.31***	3.76±0.07	0.229 ±0.04
Standard silymarin	3.00 ml/kg	10.02±0.02***	26.06±0.68**	4.29±0.04***	0.233 ±0.03***

Table 4: Effect of subcutaneous gentamicin 40 mg/kg/day and MUE on Body weight, Urine Volume, Urine Ph, and Kidney Weight in Rats Treated for Twenty-four Days -Gentamicin Model

Group	Drug Treatment	Body weight	Urine volume	Urine pH	Kidney weight

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Normal	2ml/Kg distilled water	186.66±0.88	25.1±0.45	5.9±0.22	0.95±0.01
Induced Gentamicin	40mg/kg	150.66±1.23***	15.39±0.22*	8.06±0.17***	1.60±0.08**
Preventive (test-1)	250mg/kg	163.83±1.12***	20.1±0.32*	7.54±0.21*	1.41±0.08**
Preventive (test-2)	500mg/kg	168±0.94**	23.03±0.26	6.43±0.22**	1.30±0.04***
Standard silymarin	3.00 ml/kg	180.83±1.01***	24.61±0.30*	6.26±0.17***	1.15±0.05***

Treatment with gentamicin significantly reduced SOD and glutathione peroxidase activities in mice compared to controls (group 1). Groups 3 and 4 of rats treated with MUE had much less of a drop in SOD and GST activity compared to those in group 2 of rats treated with gentamicin. Treatment with the aqueous seeds extract of M. Uniflorum protected the rats from the gentamicin-induced side effects of increased urinary creatinine, serum creatinine, blood urea, blood urea nitrogen, and kidney weights compared to normal rats [4].

Acute tubular necrosis, elevated levels of blood urea, and serum creatinine are common side effects of the nephrotoxicity caused by gentamicin. Therefore, research on drug-induced nephrotoxicity in both animals and humans has relied on these biochemical parameters. In the current investigation, drug-induced nephrotoxicity was created by giving intraperitoneal injections of gentamicin daily for 24 days. The histological features of tubulonephritis and the circulation levels of urea, creatinine, uric acid, and total protein in model Control (group 2) rats are significantly higher than in untreated rats (group 1). When compared to the toxicant group, the extract significantly lowers urea, creatinine, and uric acid levels in both treatment groups. In renal diseases, serum urea accumulates because the body's production of serum urea outpaces its ability to eliminate it. The nephrotoxicity was measured by the serum urea and creatinine levels. The breakdown of creatinine in body tissues is an in vivo source of creatinine. As a result, the concentration of urea in the blood is often seen as a more accurate reflection of kidney health than the quantity of creatinine. However, there is a slight increase in uric acid in the toxicant group compared to the control group. Uric acid levels are dramatically decreased in both treatment groups after oral administration of plant extract compared to the toxicant group. Although most of the drug is filtered out by the kidney's glomeruli, a small amount is actively transported into the proximal tubules, where it causes proximal tubular injury and abnormalities in renal circulation that lead to a drop in GFR [5].

V. CONCLUSION

In a rat model of Gentamicin-induced nephrotoxicity, the current study established the MUE's dose-dependent nephroprotective efficacy. Biochemical tests on the blood and urine, as well as histopathological examination of the kidneys, showed that pretreatment with MU extract reduced kidney damage in a dose-dependent manner. It has diuretic and antioxidant properties that shield the kidneys from damage caused by free radicals. The research supports the use of this compound in treating urinary tract disorders. This proves the effectiveness of the herb as a Folk remedy for nephrotoxicity. The extract of Macrotyloma uniflorum seeds protected kidney cells from oxidative stress and damage. In conclusion, MUE has the ability to safeguard kidney function in those who have been exposed to gentamicin.

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