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Original Article

Nephroprotective Effect of *Ficus Dalhousiae Miq* Leaf Methanolic Extract in Albino Wistar Rats

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ABSTRACT

The aim of the present study was to evaluate nephroprotective effect of Ficus dalhousiae miq leaf methanolic extract (FDLME) in Albino wistar rats.The nephrotoxicity was induced by gentamicin and Acetaminophen. Wistar albino rats weighing 150 - 200 gm were utilised for the study. The preliminary phytochemical screening of the methanolic extract of leaf showed the presence of various phytoconstituents namely alkaloids, flavanoids glycosides and sterols. The nephroprotective effect of FDLME was evident by the significant decrease in the elevated levels of serum markers such as urea, uric acid and creatinine($p<0.001$) in both the experimental models.

Keywords : FDLME, Albino rats, Nephroprotective.

1. INTRODUCTION

Herbal medicines have recently attached much attention as alternative medicine useful for treatment and prevention of life-style disorders. However, relatively very little knowledge is available about their mode of action [1]. The earlier recorded use of herbal remedies comes from Hippocrates, who advocated the use of simple plants such as garlic, neem etc. India is one of the ancient country in the world with its own use of traditional medicine. Other cultures, which do not have a well recorded history such as the native peoples of Africa, South America, North America and the indigenous tribes of Australia, have also used plants for medicinal purposes. Herbal remidies have therapeutic effects which are acceptable treatments for diseases and their symptoms. Demand for medicinal plants is progressively

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rising in industrialized nations as it is in developing countries[2].

The World Health Organization (WHO) estimates that about 80% of the developing World's population meet their primary healthcare needs through traditional medicine.

Human beings are exposed intentionally or unintentionally to a variety of adverse chemicals which can harm the kidneys like drugs,natural products, industrial chemicals etc.[3]The nephrotoxicants produce a variety of clinical syndromes such as acute renal failure, chronic renal failure, nephritic syndrome, hypertension , renal failure etc.In the present investigation an initiative has been taken to carry out preliminary phytochemical screening and nephroprotective activity of Ficus dalhousiae Miq leaf methanolic extract.

2. MATERIAL AND METHODS

Ficus dalhousiae was collected from Chitoor district of Andhra Pradesh. The plant was identified, and authenticated by comparing with voucher specimen available at Survey of medicinal plants & collection unit, Department of Botany, Sri Venkateswara University, Tirupathi by Field Botanist Dr. Madhava shetty. The leaves of the plant were shade dried. The dried material was then pulverized separately into coarse powder by a mechanical grinder. The resulting powder was then used for extraction. NaOH ,Formalin 10% and Tween 80 were procured from Asha analytical Pvt Ltd,Uppal,Hyderabad.

Preparation of methanolic Extract

The powdered drug was dried and packed well in Soxhlet apparatus and extracted with 1500 ml of methanol for seven days. The extract was concentrated and dried using Rotary flash evaporator. It was kept in a dessicator until used.

Experimental Animals

Swiss Albino rats of either sex were obtained from Mahaveer enterprises, Hyd(169/CPCSEA/1999). The rats were divided randomly into 5 groups of 6 rats each for each model of weigh between 180-200 gm. The animals were housed separately in different cages. The animals were left for 48 hrs to acclimatize to the laboratory conditions. They were maintained in standard laboratory conditions of temperature 22±2^{o}c, humidity, 12 hours light and dark cycles fed with standard pellet diet (Hindustan lever, Bangalore) and adequate tap water.

Methods

Gentamicin-induced nephrotoxicity model

Experimental design: Rats were divided into five groups, each group consisting of six animals. [4]

Group 1: Control with normal saline (5 ml/Kg)

Group 2: Gentamycin (80 mg/kg/body weight, i.p.), daily for 10 days

Group 3: Methanolic extract of Ficus dalhousiae (200mg/kg/body weight, p.o) and simultaneously administered gentamicin (80 mg/kg/body weight, i.p.), daily for 10 days.

Group 4: Methanolic extract of Ficus dalhousiae leaves (400mg/kg/body Weight,p.o.) and simultaneously administered gentamicin (80 mg/kg/body weight, i.p.), daily for 10 days.

Group 5: Silymarin (25mg/kg/body Weight, p.o.) and simultaneously administered gentamicin (80 mg/kg/body weight, i.p.), daily for 10 days.

Acetaminophen-induced nephrotoxicity model[5]

The rats were randomly divided into 5 groups of 6 rats per group.

Group I which served as positive control administered daily, single intraperitoneal 200mg/kg,i.p. acetaminophen 1 hour after oral administration of normal saline.

Groups II which served as the negative and was intraperitoneally administered 10 ml/kg of body weight normal saline And Acetaminophen 200mg/kg,i.p.

Groups III – Extract (200mg/kg/body weight, p.o) 1 hour before single, daily intraperitoneal injection acetaminophen 200mg/kg,i.p for 14 days.

Groups IV rats were orally dosed with single, daily 400 mg/kg of Extract 1 hour before single, daily intraperitoneal injection of acetaminophen 200mg/kg,i.p for 14 days.

Groups V rats were orally dosed with Silymarin (25 mg/kg/body Weight, p.o.) 1 hour before single, daily intraperitoneal injection of acetaminophen 200mg/kg,i.p for 14 days.

At the end of experimental period, all the animals were sacrificed under diethyl ether anesthesia. Blood samples were collected, Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters.

Assessment of kidney function

Biochemical parameters i.e., Estimation of Blood urea, Creatinine and uric acid were conducted according to the reported methods. The kidneys were removed, weighed and morphological changes were observed.

Statistical analysis

Results were expressed as mean ± S.E.M. The statistical difference between the groups in the term of the mean rate of wound healing was calculated in terms of ANOVA mean ± S.E.M. The difference was considered significant if $P< 0.05$.

Effect of Extract on Gentamicin induced nephrotoxicity

In gentamicin treated group of animals the concentration of serum urea and creatinine were considerably increased than the normal animals which indicated severe nephrotoxicity. Treating (group 3 & 4) with extract showed significant decrease ($p<0.001$) in concentration of serum urea and creatinine compared to negative control group Treatment with methanolic extract significantly decreased the uric acid levels in group 3& 4 compared to negative control group (table no 1)

Table 1: Effect of Ficus dalhousiae oral on serum creatinine; blood urea and serum uric acid in Gentamicin induced nephrotoxicity model

Group	Drug treatment	Serum creatinine (mg/dl)	Blood urea (mg/dl)	Uric acid (mg/dl)
1	80mg/kgGentamicin & NS 5ml/kg mlml/kg, i.p, NS	0.681±0.05309	22.622±1.783	4.0233±0.4233
2	80 mg/kg,i.p, gentamicin	1.261±0.03701	118.76±5.981	5.136±0.273
3	80 mg/kg,i.p, gentamicin+200 mg/kg	0.8566±0.0417***	54.932±6.196***	3.933±0.2693**
4	80 mg/kg,i.p, gentamicin+400 mg/kg	0.7441±0.04849***	49.962±4.204***	3.5733±0.1719**
5	80 mg/kg,i.p, gentamicin+Silymarin 25 mg/kg	0.7041±0.03849***	47.762±4.204***	3.2533±0.1719**

Kidney weight:

In gentamicin treated group of animals weight of kidneys were considerablyincreased compared to normal animals and treatment with methanolic extract showed significant decrease in kidney weight(table 2).

Table 2: Effect of Ficus dalhousiae on **kidney weight Gentamicin induced nephrotoxicity model**

Group	Drug treatment	Kidney weight (gm)
1	80 mg/kg Gentamicin, i.p & NS	0.567±0.0136
2	80 mg/kg,i.p, gentamicin	0.712±0.0138
3	80 mg/kg,i.p, gentamicin+200 mg/kg extract	0.6±0.0146***
4	80 mg/kg,i.p, gentamicin+400 mg/kg extract	0.567±0.0099***
5	80 mg/kg,i.p, gentamicin+silymarin 20 mg/kg	0.546±0.0078***

N=6 animals in a group; Values are expressed as Mean ± SEM;

Table 3: Effect of Ficus dalhousiae on **SGPT,SGOT and ALP in Gentamicin induced nephrotoxicity model**

Group	Drug treatment	SGPT levels (U/L)	SGOT levels (U/L)	ALP levels (U/L)
A	80 mg/kg Gentamicin, &, NS,i.p	42.6.8±1.23	45.25±1.36	34.56±1.56
B	80 mg/kg,i.p, gentamicin	123.45±1.45**	136.19±3.48***	92.52±2.77***
C	80 mg/kg,i.p, gentamicin+200 mg/kg	89.38±0.87**	92.45±1.76***	73.74±1.38**
D	80 mg/kg,i.p, gentamicin+400 mg/kg	65.26±2.14***	55.38±1.45***	51.38±1.54**
E	80 mg/kg,i.p, gentamicin+silymarin 20 mg/kg	45.47±1.31***	48.18±1.57***	44.47±1.67***

Effect of Extract on Acetaminophen-induced nephrotoxicity

Biochemical parameters:

In Acetaminophen treated group of animals the concentration of serum urea and creatinine were considerably increased than the positive control animals (group 1) which indicated severe nephrotoxicity. Treating (group 4 & 5) with ethanol extract showed significant decrease in concentration of serum urea and creatinine compared to Negative control group. Treatment with methanolic extract significantly decreased the uric acid levels in group 3 & 4 again compared to negative control group (table 4)

Table 4: Effect of Acetaminophen and Ficus dalhousiae leaves oral *on* serum creatinine; blood urea and serum uric acid in treated rats for 14 days

Group	Drug treatment	Serum creatinine (mg/dl)	Blood urea (mg/dl)	Uric acid (mg/dl)
1	Positive Control	0.29±0.01	21.59±3.73	2.35±0.12
2	Negative Control	0.96±0.04***	118.76±5.981***	8.72±0.21
3	200 mg/kg extract+acetaminophen	0.82±0.01***	59.86±5.1***	6.95±0.17***
4	400mg/kg, extract+Acetaminophen	0.52±0.01***	47.76±3.2***	6.15±0.24***
5	200mg/kg,Acetaminophen+Silymarin 20mg/kg	0.43±0.01***	44.26±4.20***	5.35±0.11***

N=6 animals in a group; Values are expressed as Mean ± SEM;
*: p<0.05, **p<0.01, p<0.001 vs Normal Control. ns indicate no significant.

Kidney weight:

In Acetaminophen treated group of animals weight of kidneys were considerably increased compared to positive control group animals and treating (group 3 & 4) with methanolic extract showed significant decrease (p<0.001) in kidney weight.(tab 5)

Table 5: Effect of Acetaminophen and Ficus dalhousiae oral on **kidney weight in treated rats for 14 days**

Group	Drug treatment	Kidney weight (gm)
1	Positive Control	0.467±0.0136
2	Negative Control	0.812±0.0138***
3	TEST 1	0.66±0.0146***
4	TEST 2	0.587±0.0099***
5	Standard	0.536±0.0078***

N=6 animals in a group; Values are expressed as Mean ± SEM;
*: p<0.05, **p<0.01, p<0.001 vs Normal Control. ns indicate no significant.

Rats treated with Actaminophen developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes like SGPT, SGOT and ALP when compared to positive control. Treatment with Silymarin, methanolic extract had showed good protection against Actaminophen induced toxicity to liver. Test groups indicated a significant reduction in elevated serum enzyme levels compared to negative control animals.(table no 6)

Table 6: Effect of Acetaminophen and Ficus dalhousiae oral on **SGOT,SGPT,ALP in treated rats for 14 days**

Group	Treatment	SGPT levels (U/L)	SGOT levels (U/L)	ALP levels (U/L)
A	Positive control	31.8±1.37	40.87±1.49	28.78±1.62
B	Negative Control	105.87±1.69***	128.91±3.33***	86.02±2.68***
C	TEST 1	51.26±0.91***	50.64±1.35**	47.02±1.95***
D	TEST 2	72.17±2.02***	76.88±1.41***	59.86±1.42***
E	Standard	60.4 9±1.36***	53.07±1.94***	50.47±1.58***

3. DISCUSSION

Drug induced nephrotoxicity is often associated with marked elevation in blood urea, serum creatinine and acute tubular necrosis[6]. So these biochemical parameters have been used to investigate drug induced nephrotoxicity in animals and man. In the present study drug induced nephrotoxicity was established by single daily of the Gentamicin for 10 days and single daily administration intraperitoneal acetaminophen for 14 days. This toxicity was characterized by marked elevation in the circulating levels of blood urea, serum creatinine. However these changes were attributed by concomitant treatment with single daily graded doses of FDLME extract for 10 days. Oral administration of plant extract significantly decreased the urea and creatinine level in both treatment groups. Apart from the direct nephrotoxic effect of Gentamicin and Acetaminophen the acute elevation in the biochemical parameters could also be attributed to increased catabolic state of the rats due to prolonged anorexia[7].

In renal diseases, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance. Elevation of urea and creatinine levels in serum was taken as the index of nephrotoxicity. Creatinine is derived from endogenous sources by tissue creatinine breakdown[8]. Thus serum urea concentration is often considered a more reliable renal function prediction than serum creatinine. Anyhow the level of uric acid is nonsignificantly increased in the toxicant group when compared to control. Oral administration of plant extract significantly decreased the uric acid level in both treatment groups. It was established that Gentamicin and Acetaminophen are actively transported into proximal tubules after glomerular filtration in a small proportion where it causes proximal tubular injury and abnormalities in renal circulation that leads to a reduction of GFR[9].

SGOT is a mitochondrial enzyme released from heart, liver, skeletal muscle and kidney[10]. nephro toxicity elevated the SGOT levels in serum due to the damage to the tissues producing acute necrosis, such as severe viral hepatitis & acute cholestasis. Alcoholic liver damage and cirrhosis can also associate with mild to moderate elevation of transaminase[11]. In the current study treatment of animals with ethanolic extract of leaves of Ficus dalhousie significantly($p<0.05$) decreased the levels of SGOT in serum which is an indicative of nephroprotective activity.In case of toxic kidney, alkaline phosphatase levels are very high, which may be due to defective hepatic excretion or by increased production of ALP by parenchymal or duct cells[12]. In the current study treatment of animals with methanolic extract of Ficus dalhousie significantlydecreased the levels of ALP in serum as an indication of nephroprotective activity.

All the above findings suggests the potential use of methanol extract of Ficus dalhousiae as therapeutically useful nephroprotective agent. Therefore further studies to explain the mechanism of action should be conducted to aid the discovery of new therapeutic agents for the treatment of renal diseases.

4. CONCLUSION

From the documented reports which revealed that, plant extract showed the presence of terpenoids which offers organ protection by virtue of their free radical scavenging activity.Hence, the role of these phytoconstituents as free radical scavengers and consequent nephroprotection cannot be ruled out to describe the nephroprotective effect of the plant.

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