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Phytochemical evaluation and pharmacological screening of *Scrophularia hypercifolia* for hepatoprotective, nephroprotective and antihyperglycemic activity in alloxan induced diabetic rats

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Abstract

Diabetes Mellitus is associated with damage to the liver, kidney and pancreas of patients. The damage varies in proportion and susceptibility among diabetic patients. This study assessed the hepatoprotective ,nephroprotective and antihyperglycemic activities of ethanolic extract of *Scrophularia hypercifolia* in alloxan induced diabetic rats. Extraction of the ethanolic extract from aerial parts of Scrophularia hypercifolia was performed by maceration. Thirty rats were divided into five groups. Group I consisted of normal rats that were given only sterile saline solution and served as control group. Group II consists of normal rats that were given alloxan monohydrate (150mg/kg B.W). Group III consists of alloxan induced diabetic rats that were given daily sterile saline solution, drug extract (200mg/kg), Group IV consists of alloxan induced diabetic rats that were given daily sterile saline solution, drug extract (400mg/kg) & Group V consists of alloxan induced diabetic rats that were given daily sterile saline solution and Metformin (14.2 mg/kg body weight) respectively for 21 days by an intragastric tube with free access to food and water. Several biochemical parameters were assessed. Oral administration of the extract resulted in significant reduction in mean values of blood glucose, cholesterol, triglyceride, LDL-C, urea, uric acid, creatinine accompanied by an increase in the mean values of the total protein, albumin, HDL in diabetic rats. The effects produced by this extract were closely similar to a standard antidiabetic drug, metformin. In conclusion, the present study indicates that the ethanolic extract of *S. Hypercifolia* appears to exhibit nephroprotective, hepatoprotective and antihyperglycemic activities in alloxan induced diabetic rats.Extracts was attributed due to the presence of Flavonoids and Tannins.

Key-Words: Diabetes Mellitus, *Scrophularia hypercifolia*, Hepatoprotective, Nephroprotective, Anti hyperglycemic, Alloxan, Metformin.

INTRODUCTION

Diabetes mellitus (DM), is a group of metabolic diseases in which there is high blood sugar level over a prolonged period [1]. This high blood sugar produces the symptoms of frequent urination, increased thirst, and increased hunger. Untreated, diabetes can cause manycomplications.

Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma. Serious long-term complications include heart disease, stroke, kidney failure, foot ulcers and damage to the eyes[2]. Diabetes is due to either the pancreas not producing enough insulin, or the cells of the body not responding properly to the insulin produced [2]. There are two types of diabetes afflicting mankind. Type 1 diabetes causes damage to the liver, kidney and pancreatic β-cells of patients. This damage varies in proportion and susceptibility from one individual patient to another. The liver is a vital organ in humans, which has a wide range of functions including detoxification of foreign substances in the body as well as serving as the power house for

protein synthesis. Other functions of the liver includes, building complex molecules from simple substances absorbed from the digestive tract, neutralization of toxins, manufacture of bile which aids fat digestion and removal of toxins through the bowels [3] . On the other hand, the kidneys are vital organs that function to keep the blood clean and maintain chemical balance within. They process blood to extract waste products and extra water. These by products become urine to be ultimately excreted from the body. The kidney serves many other important functions, including, filtering out wastes to be excreted in the urine, regulating blood pressure via both urinary excretion of wastes and initiating the renin-angiotensen hormone regulatory system, regulating an acid-base balance via the bicarbonate system and stimulating red blood cell production via the release of the hormone erythropoietin.

The pancreas contains cells that produce juices to break down fats and proteins and a hormone known as insulin to balance blood sugar content in the human body. Any abnormality to these organs may lead to organ dysfunction and threat to human life. Diabetes is one of such causes of damage to these organs leading to organ dysfunction and endocrine related diseases which may be life threatening. Several therapies are available for the treatment of diabetes-induced hepatotoxicity, oxidative stress and nephrotoxicity. These therapies include insulin and various oral antidiabetic agents such as sulfonylurea, biguanides, and α-glucosidase inhibitors, which are used as monotherapies or in combination to achieve better blood sugar regulation. Many of these oral antidiabetic agents have a number of serious adverse effects; thus, managing diabetes without any side effects is still a challenge [4]. Several investigations have shown that herbs and plant materials rich in secondary compounds such as saponins, flavonoids, phenolic and polyphenolic compounds, arginine and glutamic acid possess hypoglycemic, hepatoprotective and nephroprotective effects in animal models [5] .In the absence of reliable liver and kidney-protective drugs in medical practice, herbs have become reliable substitutes and have so far played significant role in the ethnopharmacological management of various liver disorders and the accompanying oxidative stress [6] . In this study, *Scrophularia hypercifolia* was evaluated for its use in the management of diabetes. Genus *Scrophularia* is rich in iridoid glycosides, phenylpropanoids and flavonoids[7]. Traditionally plants belonging to the genus are used for the treatment of wounds [8] , various inflammatory conditions, fever, constipation, swelling [9] , and as remedy for kidney diseases [10] Iridoids from Scrophularia species exert hapatoprotective activity [11] . It is on this premise that this work was designed to evaluate the antihyperglycemic, hepatoprotective and nephroprotective effects of the ethanolic extract of the aerial parts of Scrophularia Hypercifolia in alloxan-induced diabetes using Albino Wistar rats as study model.

MATERIALS AND METHODS

Collection and authentication of plant material

Aerial parts of the plant *Scrophularia hypercifolia*, family Scrophulariaceae were collected. from botanical gardens of Sri Venkateshwara University, Tirupati . The plant was taxonomically identified and authenticated by DR. K. MADHAVA CHETTY Assistant professor of Botany, Department of Pharmacognosy, Sri Venkateshwara University, Tirupathi.

Chemicals

The following chemicals were used during the experiment to analyze and interpret the hepatoprotective, nephroprotective and antihyperglycemic effect of ethanolic extract of *Scrophularia hypercifolia* in alloxan induced diabetic rats. All the chemicals and reagents used for the study were of analytical grade and were obtained from Universal Chemical Corporation (Abids, Hyderabad, India)

Alloxan monohydrate, Metformin, Ethanol.

Preparation of plant extract

The aerial parts were cut into pieces and subjected to shade drying. On complete drying the pieces were powdered and stored in air tight containers at room temperatures. The powder was macerated with ethanol for 7 days and then filtered. The filtrate was evaporated to obtain extract[12]. The extract thus obtained was subjected for evaluation of hepatoprotective, nephroprotective and antihyperglycemic study.

Preliminary phytochemical screening

Standard screening test of the extract was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds,

phenolic compounds, flavonoids, saponins, tannins, glycosides and anthraquinones using standard procedures[13]. The study revealed the presence of Flavonoids, glycosides, phenols, steroids and triterpenoids and saponins.

Acute toxicity studies

Acute toxicity study was performed for the extract according to the acute toxic classic method as per the method of Litchfield & Wilcoxon (1949)[14]. Acute toxicity study was carried out on plant extracts using Male and female Wistar rats. The rats were fasted overnight and the weight of each rat was recorded just before use. The animals were divided into five groups containing 6 animals each, the extract was administered orally in increasing dose up to 2000 mg/kg b.w. After treatment the animals were observed for mortality and toxicity for 72 hours. The biological evaluation was carried out at doses of 200 and 400mg/kg body weight.

Experimental animals

Wistar rats (180-250 gms) of either sex housed in standard conditions of temperature (55±5%) and light (12 hrs light/dark cycles) were used. They were fed with standard pellet diet and water ad libitum. Animals were randomly selected for grouping. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the purpose of control and supervision of experiments on experiments on animals) with clearance no: 004/CEAD/SES/SWCP/14.

Experimental design

Thirty rats were divided into five groups. . Group I consisted of normal rats that were given only sterile saline solution and served as control group. Group II consists of normal rats that were given alloxan monohydrate (150mg/kg B.W). Group III consists of alloxan induced diabetic rats that were given daily sterile saline solution, drug extract (200mg/kg), Group IV consists of alloxan induced diabetic rats that were given daily sterile saline solution, drug extract (400mg/kg) & Group V consists of alloxan induced diabetic rats that were given daily sterile saline solution and Metformin (14.2 mg/kg bw) respectively.

Induction of diabetes

Hyperglycemia was induced by a single i.p. injection of 150 mg/kg of alloxan monohydrate in sterile saline solution. Hyperglycemia was confirmed after 5 days of alloxan injection, and hyperglycemic rats (glucose level > 150mg/dl) were separated and divided into 5 groups of 6 rats each for anti-diabetic studies.

Collection of samples for analysis

Blood samples were collected by cutting the tail vein of rats and blood glucose levels are checked by glucometer.

The normal and diabetic control group rats were given 1 ml normal saline, p.o. Animals in the third and fourth group were treated with ethanolic extract of the aerial parts of of Scrophularia Hypercifolia 200 & 400mg/kg b.w., and that of Fifth group were treated with Metforminat a dose of 14.2mg/kg b.w. for 21 days. Body weight and blood glucose were checked for every three day intervals during the duration of the experiment. The blood glucose levels were determined by tail tipping method using Accu check active glucometer. On twenty first day blood from all the groups were collected by retro-orbital puncture under mild anesthesia, serum was separated quickly for estimating the following parameters to assess the hepatoprotective, naphroprotective and anti-hyperglycemic activity.

- Blood Glucose Levels,
- Total Cholesterol Levels(TC),
- Low Density Lipoprotein Cholesterol(LDLc) Levels,
- High Density Lipoprotein Cholesterol(HDLc) Levels,
- Very Low Density Lipoprotein Cholesterol(VLDLc) Levels,
- Triglyceride Levels & Cholesterol Ratio.
- Total protein
- Albumin
- Urea, uric acid
- Creatinine

Statistical analysis

The results were expressed as mean ± standard deviation. Differences between control and experimental groups were estimated using student's t-test analysis. Within group comparisons were performed by analysis of variance using ANOVA test. Differences were considered significant if P-value was less than 0.05.

RESULTS

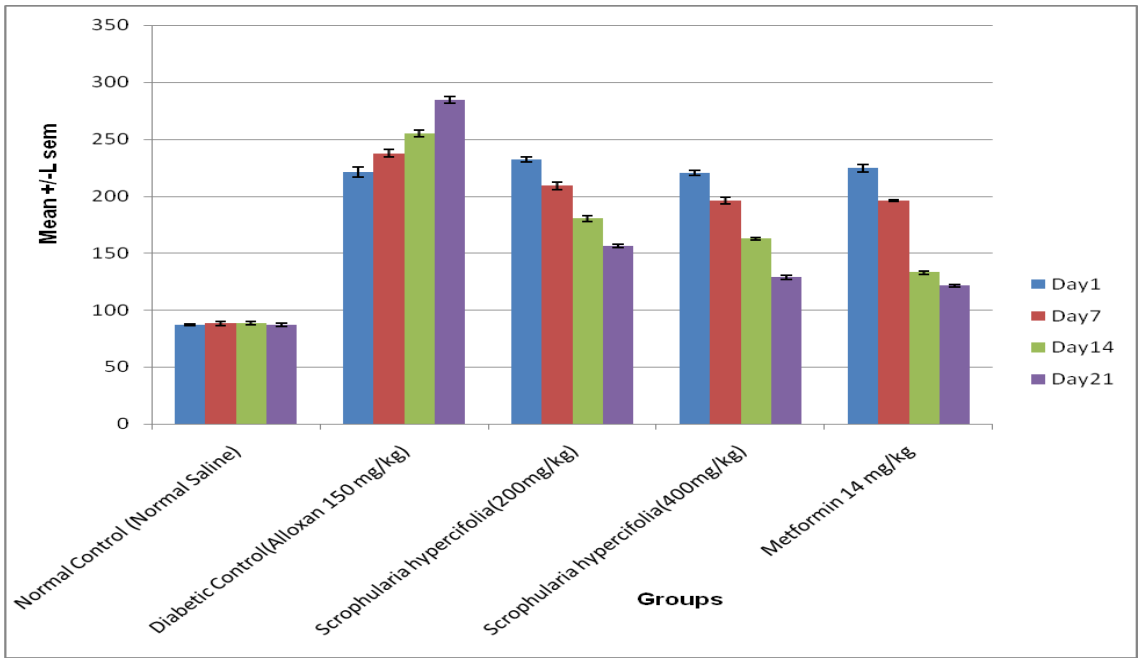
Effect of ethanolic extract of the aerial parts of *Scrophularia hypercifolia* on blood glucose levels (mg/dl) in alloxan induced diabetic rats on 1st, 7th, 14th and 21st day of the treatment.

Table 1: Effect of ethanolic extract of the aerial parts of *Scrophularia hypercifolia* on blood glucose levels

Treatment Groups	1st day	7th day	14th day	21st day
Normal control (Normal Saline)	87.2±0.90	88.3±1.81	88.6±1.1 7	87.3±1.50
Diabetic control Alloxan (150 mg/kg)	221.3±4.61	237.6±3.11	255.3±3.06	284.6±2.75
Scrophularia hypercifolia (200mg/kg)	232.6±2.21	209.0±3.43	180.3±2.39 *	156.3±1.69**
Scrophularia hypercifolia (400mg/kg)	220.3±2.42	196.2±2.68	162.6±1.32 *	129.5±1.8**
Metformin (14.2mg/kg)	224.6±3.13	196±0.82	133.0±1.52**	121.3±1.15**

Values are given as mean ± SEM for group of six animals in each group. Diabetic control rats were compared with normal rats. Diabetic + Scrophularia hypercifolia 200mg/kg body weight, Diabetic + Scrophularia hypercifolia 400mg/kg body weight and Diabetic + Metformin treated rats were compared with Diabetic control rats. ** P<0.05, ** P<0.01, **P<0.001 was considered significant comparing to Diabetic control group. ANOVA followed by Dunnett's t-test **P<0.05, ** P<0.01, **P<0.001 was considered significant comparing to diabetic control group. Hence the administration of ethanolic extract of the aerial parts of Scrophularia hypercifolia for 21 days decreased the elevated blood glucose levels. The above result suggests that Scrophularia hypercifolia at a dose of 400mg/kg.b.w was more effective than 200 mg/kg.b.w and was almost comparable with the effect produced by standard drug Metformin treated group. The results were represented by means of graph 1 by using ANOVA followed by Dunnett's t-test.

Fig 1: Blood Glucose Levels (Mg/Dl) On 1st, 7th, 14th& 21st Day Of Treatment



Effect of ethanolic extract of the aerial parts of *Scrophularia hypercifolia* on serum lipid profile in alloxan (150mg/kg.b.w) induced diabetic rats after 21 days of treatment:Values are given as mean ± SEM for group of six animals in each group.

Table 2: Effect of ethanolic extract of the aerial parts of *Scrophularia hypercifolia* on serum lipid profile

Groups	Serum Low Density Lipoprotein(LDL) (mg/dl)	Serum Very Low Density Lipoprotein(VLDL) (mg/dl)	Serum High Density Lipoproteins(HDL) (mg/dl)
Normal control	33.64±2.19***	16.84±0.80***	32.42±0.42***
Diabetic control	80.21 ± 2.11	27.20 ± 4.18	18.93 ± 3.21
Scrophularia hypercifolia (200 mg/kg.b.w)	39.25±0.89***	21.89±0.52**	30.19±0.19**
Scrophularia hypercifolia (400 mg/kg.b.w)	35.17±0.13***	19.2±0.39***	31.93±0.65***
Metformin treated	33.77±0.27***	16.92±0.62***	32.61±0.33***

Diabetic control rats were compared with normal rats.
Diabetic + Scrophularia hypercifolia 200mg/kg b.w, Diabetic + Scrophularia hypercifolia 400mg/kg b.w and Diabetic + Metformin treated rats were compared with Diabetic control rats.
** P<0.05, ** P<0.01, **P<0.001 was considered significant comparing to Diabetic control group.

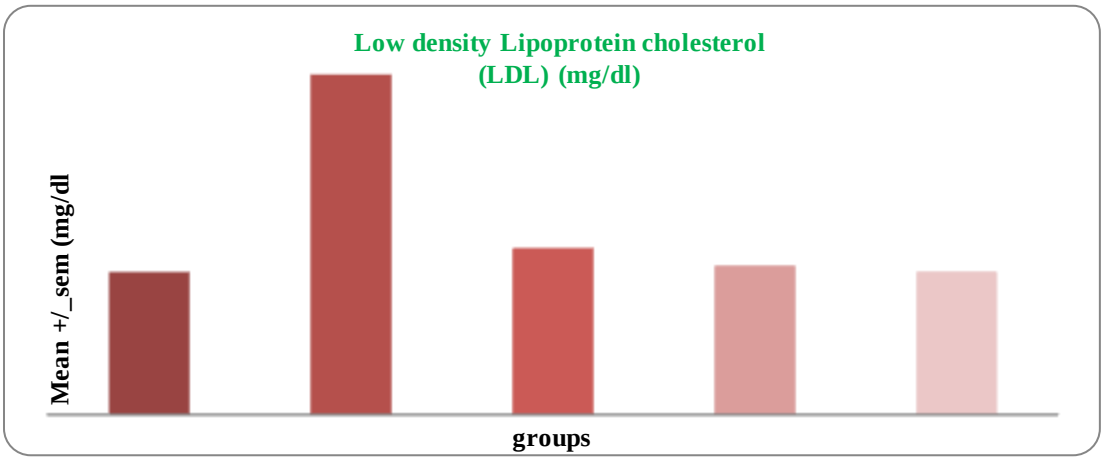


Fig 2: Effect of drug on LDL

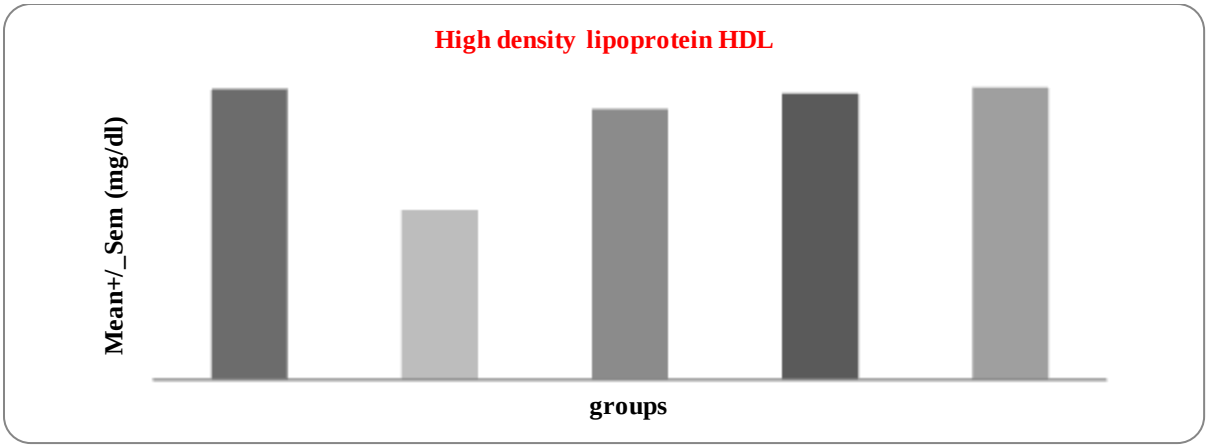


Fig 3: Effect of the drug on high density lipoprotein

Table 3:

Effect of ethanolic extract of the aerial parts of *Scrophularia hypercifolia* on serum total cholesterol, triglycerides & cholesterol ratio in alloxan (150mg/kg.b.w) induced diabetic rats after 21 days of treatment:

Groups	total cholesterol (mg/dl)	total triglycerides (mg/dl)	cholesterol ratio = tc/hdl
Normal control	81.97±0.17***	83.72±0.43***	2.52±0.038
Diabetic control	126.95± 4.89	131.68 ± 1.39	6.70±0.48
Scrophularia hypercifolia (200 mg/kg.b.w)	94.20±0.44**	111.48±0.49*	3.12±0.081**
Scrophularia hypercifolia (400 mg/kg.b.w)	86.4±0.48***	96.00 ± 0.57**	2.70±0.0611**
Metformin treated	82.20±0.48***	84.21±0.71***	2.52±0.112**

Values are given as mean ± SEM for group of six animals in each group.
Diabetic control rats were compared with normal rats.
Diabetic + Scrophularia hypercifolia 200mg/kg b.w, Diabetic + Scrophularia hypercifolia 400mg/kg b.w and Diabetic + Metformin treated rats were compared with Diabetic control rats.
** P<0.05, ** P<0.01, **P<0.001 was considered significant comparing to Diabetic control group.

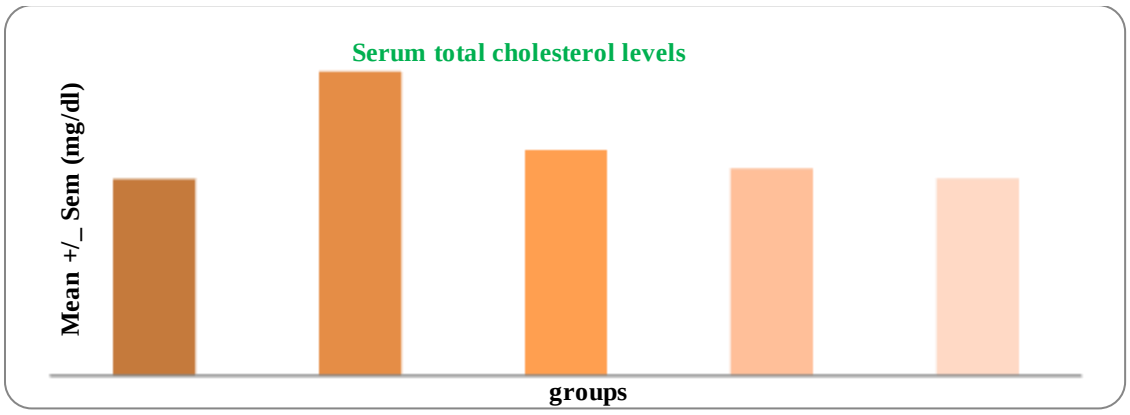


Fig 4: Effect of the drug on serum total cholesterol levels

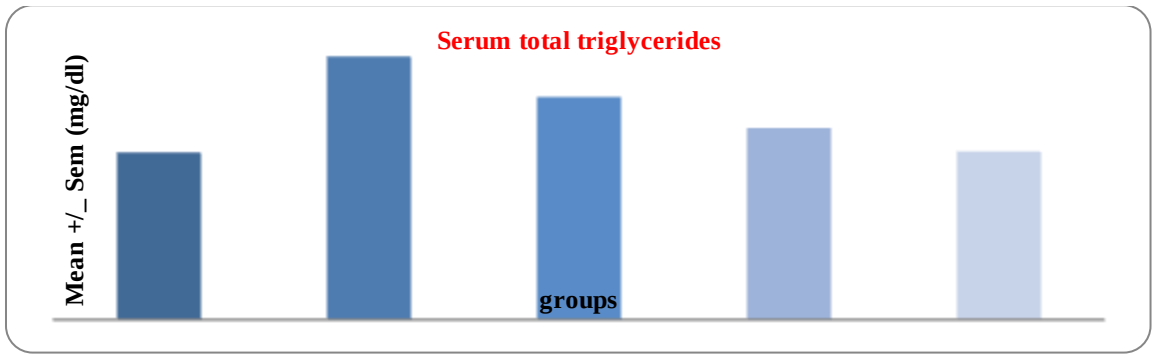


Fig 5: Effect of the drug on serum total triglycerides

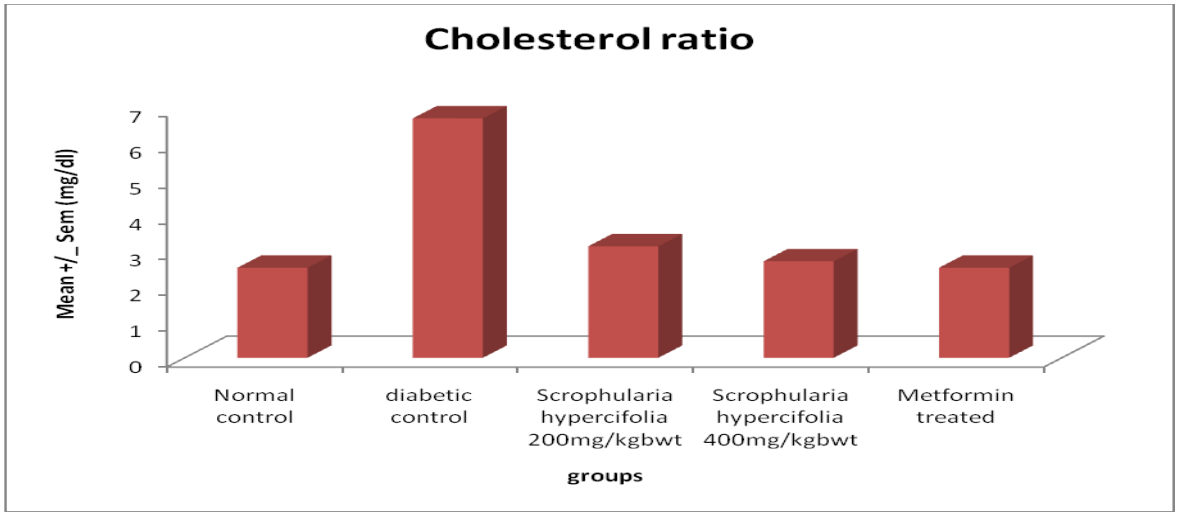


Fig 6: Effect of the drug on cholesterol ratio

Table 4: Effect of *Scrophularia hypercifolia* on total protein and albumin, urea, uric acid and creatinine levels

Effect of ethanolic extract of the aerial parts of *Scrophularia hypercifolia* on total protein and albumin, urea, uric acid and creatinine levels in alloxan (150mg/kg.b.w) induced diabetic rats after 21 days of treatment

Groups	Total protein	Albumin	Urea	Uric acid	Creatinine
Normal control	8.1±0.9	3.4±0.6	26.8±3.1	1.6±0.5	1.0±0.1
Diabetic control	5.3±0.4*	1.9±0.2*	37.2±6.5*	2.6±0.8*	2.8±0.3*
Scrophularia hypercifolia (200 mg/kg.b.w)	5.9±0.5**	2.3±0.8**	34.5±4.8**	2.1±0.2**	1.9±0.3**
Scrophularia hypercifolia (400 mg/kg.b.w)	6.5±0.2**	2.9±0.3**	30.2±4.5**	1.7±0.2**	1.5±0.5**
Metformin treated	7.5±0.9**	3.0±0.2**	29.9±5.6**	1.6±0.3**	1.3±0.2**

Values are given as mean ± SEM for group of six animals in each group.

*: Statistically significant when compared to control group (I) at $P < 0.05$;

**: Statistically significant when compared to untreated diabetic group (II) at $P < 0.05$

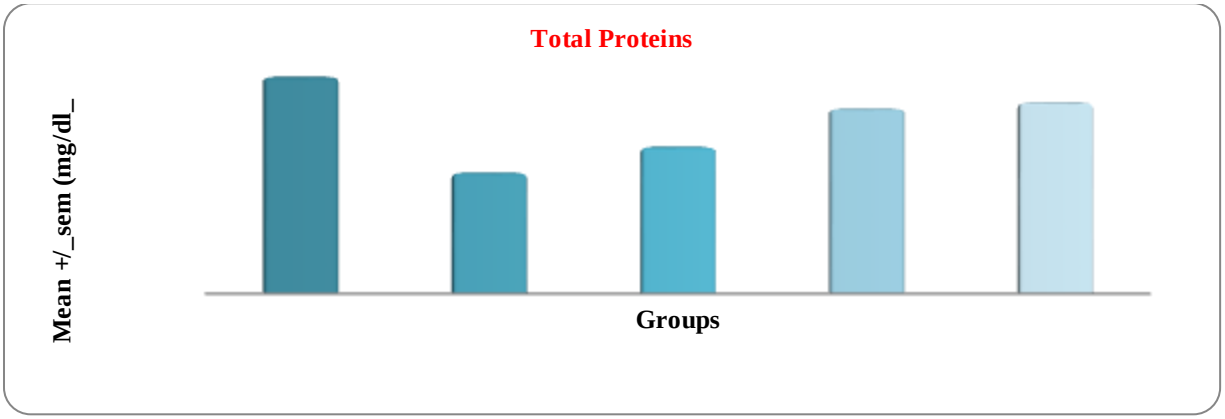


Fig 7: Effect of the drug on Serum total proteins

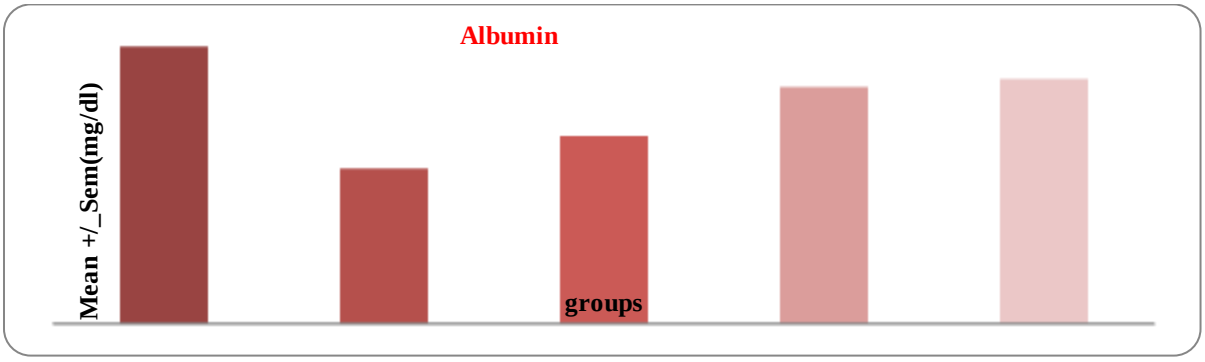


Fig 8: Effect of the drug on Serum Albumin

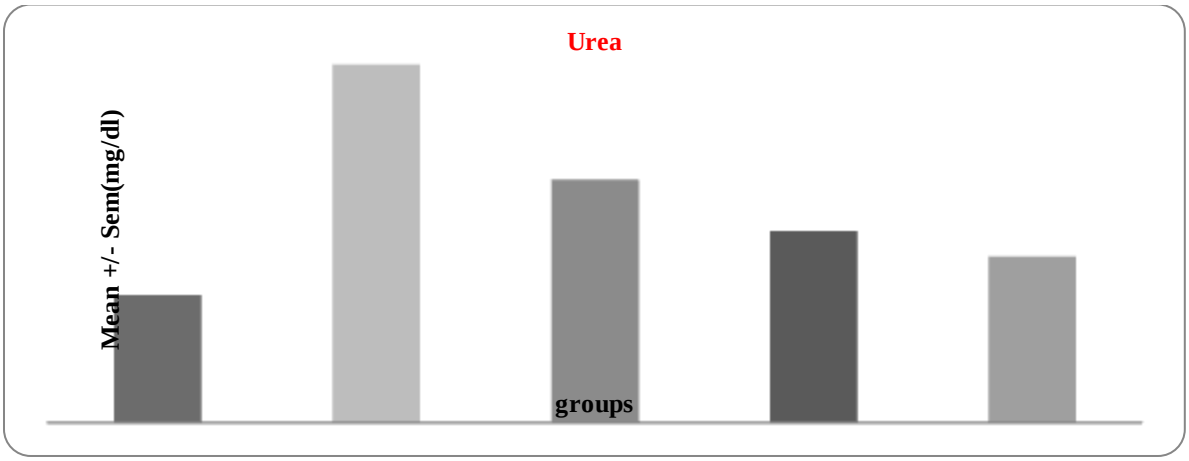


Fig 9: Effect of the drug on serum urea

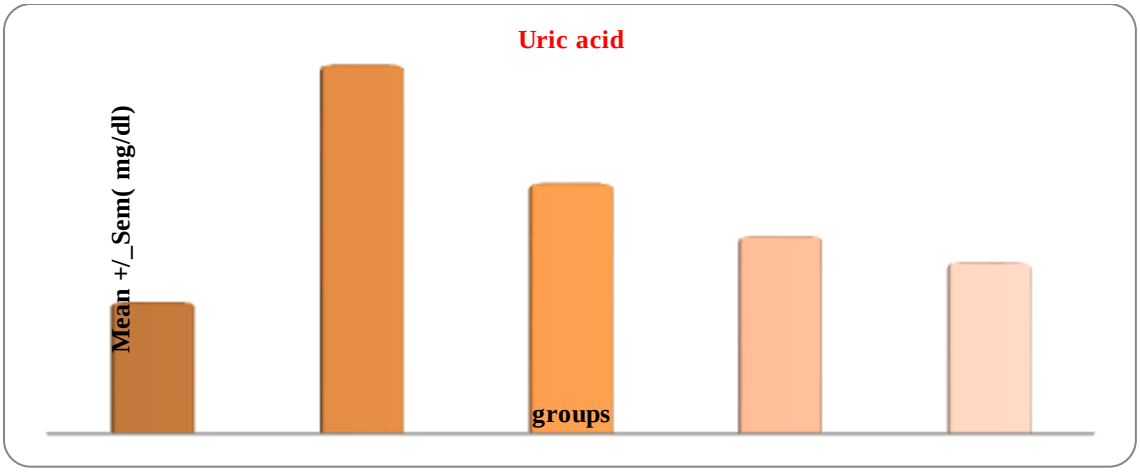


Fig10: Effect of the drug on serum uric acid

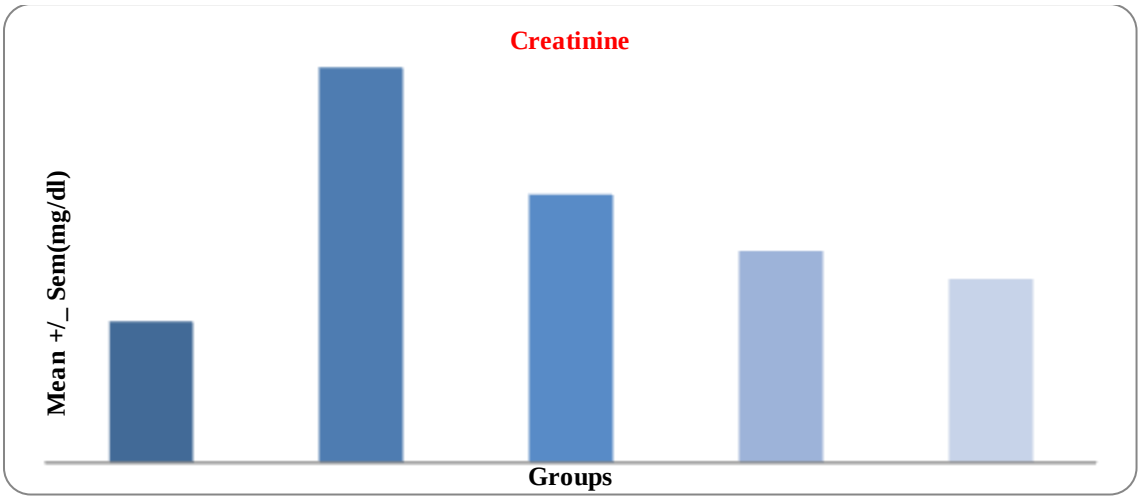


Fig 11: Effect of the drug on serum creatinine

DISCUSSIONS

In the evaluation of antihyperglycemic activity, study groups 2, 3, 4 and 5 induced with diabetes using alloxan developed serum hyperglycemia. This condition was either due to pancreatic β-cells necrosis mediated by alloxan which enhances ATP dephosphorylation resulting in the generation of free radicals into the blood circulation or simply due to decrease in serum insulin or an integration of both processes. Diabetes induction and the generation of free radicals such as superoxide, hydrogen peroxide and hydroxyl radicals, caused the destruction of hepatic, pancreatic and kidney cells and tissues. This study demonstrated that continuous oral administration of the ethanolic extract of *S.hypercifolia* for 21 days significantly decreased blood glucose levels in the diabetic rats by 43% compared with the decreases of 49% with the metformin, a well known antidiabetic drug. In addition to marked hyperglycemia, results revealed that the alloxan diabetic rats developed notable hyperlipidaemia. Diabetes induced hyperlipidaemia was observed in diabetic experimental animal models.This is very important, since elevated concentrations of cholesterol, triglyceride and LDL-C are important risk factors in the development of diabetic complications. The study revealed that this extract normalized serum lipids (cholesterol, triglyceride, HDL-C, LDL-C) closely to the level of the control or normal rats. The findings are similar with a recent study by Bavarva and Narasimhacharya (2010) which reported that leaves of *Leucas cephalotes* lowered both plasma and hepatic lipid profiles (total lipid, triglycerides and cholesterol) and LDL-C while elevating the HDL-C levels[15]. They suggest that these improvements in lipid profiles are most likely due to its insulin-like actions of the leaf

extract of *Leucas cephalotes*. Similarly, a previous study done by Lopes-Virella *et al*. (1983) also reported that diabetic patients taking insulin injections exhibited both high lipoprotein lipase activity and low level of plasma triglyceride concentrations[16] . Thus, it can be concluded that the enhancement of insulin secretion or level is accompanied by enhancement of glucose utilization as well as a reduction of lipid level in diabetic rats. It is possible to suggest that the mechanism(s) of antihyperlipidemic effect of S.hypercifolia might be similar to some of those suggested for anti-diabetic plants exhibiting antihyperlipidemic activity, such as activation of lipoprotein lipase, insulin-mediated lipolytic activity or inhibition of lipogenic enzymes or hormone-sensitive lipase. The study also showed a significant decrease in serum total protein and albumin in untreated diabetic rats, whereas total protein and albumin significantly increased after the administration of this extract. Liver functioning can also be estimated by total protein and albumin levels in blood. Similar results were obtained when the metformin were administered orally in alloxan diabetic. These results suggest that this extract can improve some biochemical parameters that are related to liver functions. Hyperglycemia has also been recently reported in the initiation and development of various types of diabetic complications. Nephropathy is one of these serious microvascular complications that has been observed in diabetic individuals. Blood urea and creatinine concentrations were increased among uncontrolled diabetic rats and this increase could be a result of impaired renal function due to an increased blood glucose level. It was reported that diabetic individuals had lower serum albumin concentrations as well as higher serum uric acid and urea levels than nondiabetic individuals. Thus, the reduction in urea and creatinine levels probably can be explained by a reduction in blood glucose level. Further, DM is also considered as a risk factor for cardiovascular disease, and elevated serum uric acid has been linked to cardiovascular disease, especially if accompanied with high triglyceride and low HDL. Moreover, high levels of serum uric acid, urea and creatine may act as a marker of kidney problems. Thus, it is possible to suggest that this extract might play an important role in reducing risk of kidney problems as well as cardiovascular diseases by lowering serum urea, uric acid, creatinine as well as improving lipid profile. The study of the literature indicated that free radicals are one of the main contributors to development of Diabetes Mellitus as well as its complications[17] [18][19] . It is also worth mentioning that alloxan can induce rapid death of βcells of pancreas, resulting in partial or complete loss of insulin production and leading to the development of hyperglycemia and its complications in experimental animals; and this action of alloxan was mediated by formation of free radicals [20] . Moreover, a wide range of studies have strongly supported the notion that antioxidant compounds derived from plant extracts might play a vital role in the treatment of DM and prevent or delay its complications [18][20] . Thus, it is possible to suggest that these therapeutic values of the drug extract could be attributed to the main antioxidant constituents of this drug extract of *Scrophularia Hypercifolia*.

The preliminary phytochemical screening of the aerial parts shows the presence of phenylpropanoids, flavonoids, iridoids, phytosterols like β sitosterol, triterpenoid, saponins, sugars and terpenes and various other constituents.

Thus the ethanolic extract of aerial parts of Scrophularia Hypercifolia at a dose of 200mg/kg.b.w and 400mg/kg.b.w possess a glucose lowering effect, lipid lowering effect, decrease in uric acid, urea, creatinine and a significant increase in protein, albumin in Alloxan induced diabetic rats. It was also found to be highly effective in managing complications associated with diabetes mellitus. In conclusion, this study revealed that oral administration of the ethanolic extract of *S.hypercifolia* exhibit nephroprotective and hepatoprotective activities via enhances insulin production in the alloxan-induced diabetic rats. Thus, oral use of this extract might positively affect the functional capacities of various rat tissues, particularly kidney and liver against toxic action of alloxan compound (dose of 150 mg/Kg BW). Hence, the therapeutic potential of this plant material should also be seen in combination with other medicinal agents and further biochemical and pharmacological investigations are needed to isolate and identify active ingredients in the extract using other models.

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