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IN VIVO ANTI-ARTHRITIC AND ANTI-DIABETIC ACTIVITY OF DIFFERENT EXTRACTS OF *CISSUS QUADRANGULARIS* LINN

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ABSTRACT

Systematic evaluation of plants used in the traditional medicines may provide more promising information regarding their therapeutic value and could be beneficial in addressing the increasing demand for newer agents to fight infections and diseases. With this view, the plant *Cissus quadrangularis* was selected for our research. As the continuation of our previous effort, the present study was focused on the *in vivo* evaluation of anti-arthritic and anti-diabetic activity of methanol extract of *C. quadrangularis.* Initially, acute toxicity of the extracts was evaluated and the effective dose (ED_{50}) was fixed. Anti-arthritic activity of the extract was evaluated by Complete Freund's adjuvant (CFA) induced arthritic animal models. Anti-diabetic activity of the extract was evaluated by streptozotocin-induced diabetes mellitus animal models. Two doses of test extract, low dose and high dose (200 and 400mg.kg^{-1}) were subjected to the evaluation. In these evaluations, methanol extract in the dose of 400mg.kg^{-1} showed a significant result. Further studies of this extract in future may yield significant results that will be useful for the development of new chemotherapeutic agents.

KEYWORDS: *Cissus quadrangularis* extracts, in vivo anti-arthritic activity, anti-diabetic activity.

INTRODUCTION

Globally, herbal remedies have been extensively utilized to relieve various ailments and disorders since ancient times. Herb-based treatment approaches in various traditional healing practices have consistently motivated and directed researchers to seek new therapeutic options.[1-3] Recent reports have indicated that around two-thirds of the medicines approved worldwide are derived from plants.[4]

The organic compounds, also known as secondary metabolites, found in plants are accountable for their specific physiological functions. Alkaloids, glycosides, flavonoids, essential oils, saponins, resins, phenols are few among them found in various parts of plants, such as the root, stem, flower, fruit, seed, and exudates. These compounds can be utilized in the treatment of various chronic and infectious diseases.[5,6]

A comprehensive assessment is required for the herbal remedies utilized in the traditional healing system, which may provide more promising information regarding their therapeutic value and could be beneficial in addressing the increasing demand for new agents to fight infections and diseases.[7-9]

With this view, *Cissus quadrangularis*, a plant with ethno medicinal significance was chosen for our research. In our previous effort, anti-inflammatory and anti-diabetic activity of different extracts of *C. quadrangularis* Linn was evaluated *in vitro* successfully.[10] As continuation, the present study focussed on the *in vivo* evaluation of anti-arthritic and anti-diabetic activity the selected extract of *C. quadrangularis*, an effort to offer a direction for future investigations.

MATERIALS AND METHODS

Plant extract

In our previous effort, the aerial parts of *Cissus quadrangularis* was collected from Melpalai, a small village located in the Devicode Panchayath of Melpuram Block in Kaniyakumari District of Tamil Nadu State. After proper identification and authentication, drying, powdering and extraction of collected plant material was done by soxhlet extraction, using the solvents of increasing polarity viz., petroleum ether, chloroform, ethyl acetate and methanol. The dried extracts thus obtained was subjected to preliminary phytochemical, *in vitro* anti-inflammatory and anti-diabetic activity evaluation. In this study, the methanol extract showed the presence of majority of phytochemicals and significant *in vitro* activity.[10] Based on these results, the methanol extract was selected for the present study.

Animals and approval of the study

Healthy male Wistar albino rats having 6-8weeks age weighing 160-180gm obtained from the Central animal house, Cape Bio Lab & Research Centre, Marthandam, Kanyakumari, Tamil Nadu, India, was used for the experiments.The study was conducted as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The institutional animal ethical committee approved the study (CBLRC/IAEC/02/02-2020).

Acute toxicity study

The LD_{50} of the selected extract was determined as per the guidelines of OECD 423 and in reference with the standard procedure in published literature.[11,12] Required animals were randomly selected, marked facilitating identification, and kept in the standard environmental conditions like ambient temperature (25±1°C), relative humidity (55±5%) and 12h light/dark cycle for five days prior to dosing for adaptation with laboratory conditions. The animals were fed with standard pellet diet and water *ad libitum*. Following the overnight fasting with free access to water, the test extracts were administered in a single dose by gavage using stomach tube. 50mg.kg^{-1} was selected as initial dose and administered to three animals and after 24h, the animals were observed for the number of death. The same procedure was repeated with next doses 300mg.kg^{-1} and 2000mg.kg^{-1}.

***In vivo* anti-arthritic activity**

The selected extract was subjected to *in vivo* evaluation in reference with standard procedure[3,13] with slight modification.

Treatment protocol

The selected Wistar albino rats were divided into five groups of six each. Group 1 served as the normal control received diet and water only; Group 2 served as arthritic control received Complete Freund's adjuvant (CFA) only for the induction of arthritis. Group 3 was taken as standard control received prednisolone (10mg.kg^{-1}). Group 4 was experiment group received 200mg.kg^{-1} of selected extract of *C. quadrangularis.* Group 5 was treated with 400mg.kg^{-1} of the same extract.

Treatment and evaluation

Arthritis was induced in all the selected animals except group 1 by a single intra-dermal injection (0.1 ml) of CFA containing 1.0mg dry heat-killed *M. tuberculosis*/ml sterile paraffin oil into a foot pad of the left hind paw of male rats. A glass syringe (1ml) with the locking hubs and a 26G needle was used for injection. The rats were anesthetized with ether inhalation prior to and during adjuvant injection. The standard drug and the test extracts were administered orally from the 3rd day of CFA administration and given daily for 21 days. The swelling paws were examined periodically (up to 21 days) in each paw from the ankle using Digital Plethysmometer. Results were expressed as change in paw volume (in ml) by using following formula

$$\text{Increased Volume of edema} = \text{Final paw volume} - \text{Initial paw volume}$$

At the end, blood samples were collected by sublingual route, serum and plasma were separated by centrifugation at 2500rpm for 10min. and stored at –20°C for the investigation of different hematological and biochemical parameters. Different biochemical parameters like Alkaline phosphatase(ALP) marker for bone destruction, Acid Phosphatase (ACP) the lysosomal enzyme activity, Serum glutamate oxalo acetate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT) were estimated by using ALP, ACP, SGOT and SGPT kit in Auto analyser. For the estimation of Total WBC count blood samples were added with WBC diluting fluid and by the help of Neubauer's chamber total numbers of WBC was calculated by using formula

$$\text{Total WBC count} = \text{Total no. of cells} \times \text{Volume correction factor} \times 20$$

***In vivo* anti-diabetic activity**

The study was designed in reference with the standard procedure in published literature. [12,14,15]

Treatment protocol

The selected Wistar albino rats were divided into five groups of six each. Group 1 served as the normal control received diet and water only; Group 2 served as diabetic control received Streptozotocin (STZ) only for the induction of diabetes. Group 3 was taken as standard control received 0.5mg.kg^{-1} of standard drug Glibenclamide. Group 4 was experiment group received 200mg.kg^{-1} of selected extract–1 of *C. quadrangularis*. Group 5 was treated with 400mg.kg^{-1} of selected extract–1.

Treatment and evaluation

All the animals in the five groups except Group 1 kept free from the diet for 12h prior to inducing diabetes. Diabetes mellitus was induced in all except Group 1 by administering a single dose of Streptozotocin [STZ] (60mg.kg^{-1} i.p). Group 1 (normal control) animals were injected with saline only. 72h after the administration of STZ, the blood glucose level of the treated animals was measured by using electronic glucometer. Animals found with blood sugar level ≥ 180mg/k/dl were considered as diabetic and used for the experiments as per the protocol. The standard drug and the test extracts were administered orally from the 3rd day of Streptozotocin administration and given daily for 28 days. The blood glucose level of the animals was estimated once a week by using electronic glucometer.

At the end of fourth week of treatment, all the rats were sacrificed by cervical dislocation and the blood was collected from each rat by direct cardiac puncture. Serum and plasma were separated by centrifugation at 2500rpm for 10 min. and stored at –20°C for the investigation of different haematological and biochemical parameters. Organs such as the liver and pancreas were removed immediately from the sacrificed animals and kept in 10%

formalin solution for histopathological evaluation.

Hematological parameters such as hemoglobin and glycosylated haemoglobin were analyzed by using the equipment, COBAS MICROS OT 18, Roche. Biochemical parameters such as total cholesterol, triglycerides, High-density lipoprotein (HDL), Low-density lipoprotein (LDL), Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), and Alkaline phosphatase (ALP) were analyzed by using the equipment, COBAS MIRA PLUS – S, Roche.

RESULTS AND DISCUSSION

In vivo anti-arthritic evaluation

Based on the results obtained from the *in vitro* evaluation,[10] the methanol extract was selected for the *in vivo* anti-arthritic evaluation. In the acute toxicity evaluation, initially animal death was not observed after the administration of first two doses of the extract. But, two out of three animals died within the 24h of administration of third dose 2000mg.kg^{-1}. So, the third dose was concluded as LD_{50} and from this the ED_{50} was fixed as 200mg.kg^{-1} for the selected methanol extract of *C. quadrangularis*.

Collectively, results of *in vivo* evaluation strongly supported the anti-arthritic activity of methanol extract of *C. quadrangularis* particularly, the high dose (400mg/kg) of the extract revealed a significant activity comparing with the standard control drug (prednisolone). Results of *in vivo* anti-arthritic evaluation is shown in Table 1-3 and Figure 1-3.

Table 1: Effect of methanol extract of *C. quadrangularis* on change in the paw volume of the experimental animals.

Days	Normal control	Arthritic control	Standard control (Prednisolone)	Test extract (200mg/kg)	Test extract (400mg/kg)
00	0.19±0.02	0.60± 0.05	0.57 ± 0.04	0.59±0.02	0.56 ± 0.02
03	0.21±0.04	0.75 ± 0.01	0.65 ± 0.05	0.68±0.03	0.66 ± 0.04
05	0.20±0.01	0.84 ± 0.02	0.64 ± 0.07	0.72±0.01	0.60 ± 0.03
07	0.22±0.03	0.95 ± 0.04	0.60 ± 0.03	0.68±0.02	0.61 ± 0.05**
09	0.24±0.02	1.04 ± 0.05	0.59 ± 0.03**	0.64±0.04**	0.59 ± 0.03**
11	0.20±0.01	1.07 ± 0.06	0.54 ± 0.05**	0.60±0.01**	0.57 ± 0.02**
13	0.19±0.02	1.10 ± 0.03	0.41 ± 0.04**	0.56±0.03**	0.44 ± 0.02**
15	0.22±0.01	1.13 ± 0.05	0.38 ± 0.05**	0.51±0.02**	0.40 ± 0.05**
17	0.20±0.02	1.16 ± 0.07	0.34 ± 0.06**	0.47±0.01**	0.37 ± 0.04**
19	0.21±0.01	1.19 ± 0.04	0.24 ± 0.07**	0.38±0.04**	0.31 ± 0.05**
21	0.19±0.02	1.21 ± 0.05	0.20 ± 0.02**	0.29±0.02**	0.23 ± 0.04**

All values are expressed as mean ± SEM for 6 determinants; ***P*<0.01, compared to arthritic control

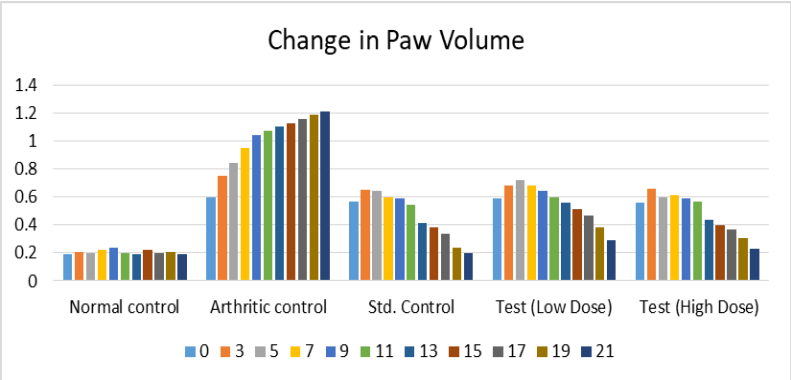

Figure 1: Effect of methanol extract on changes in the paw volume of the experimental animals.

Table 2: Effect of methanol extract of *C. quadrangularis* on biochemical parameters of experimental animals.

Groups	**SGOT** (U/L)	**SGPT** (U/L)	**ALP** (U/L)	**ACP** (U/L)
Normal control	35.40±0.93	35.70±0.34	71.60±1.70	5.80±0.03
Arthritic control	108.2±2.04*	164.20±0.50*	439.3±2.35*	23.51±1.24*
Standard control	47.51±1.31**	42.60±2.35**	152.1±1.08**	6.24±0.53**
Test extract (Low dose)	64.22±2.01**	66.40±1.31**	219.2±0.58**	14.34±0.40**
Test extract (High dose)	49.60±0.48**	54.71±0.81**	178.5±0.22**	7.34±0.24**

All values are expressed as mean ± SEM for 6 determinants; **P*<0.05, compared to normal and ***P*<0.01, compared to arthritic control.

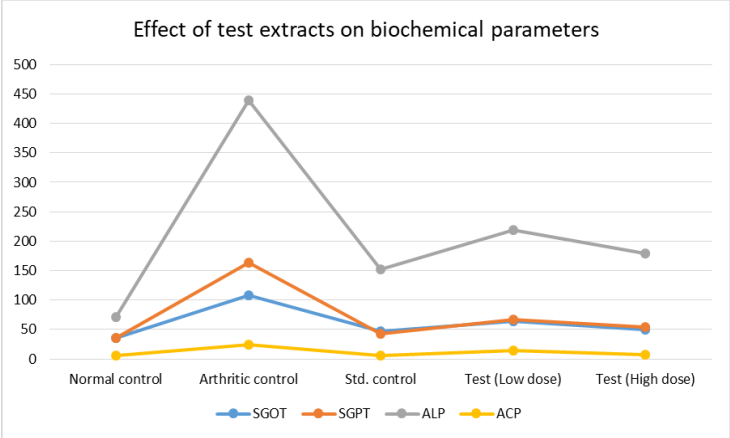

Figure 2: Effect of methanol extract on the biochemical parameters of experimental animals.

Table 3: Effect of methanol extract of *C. quadrangularis* on hematological parameter of experimental animals.

Groups	**WBC** (Cells/ml×10^3)
Normal control	10.21±1.40
Arthritic control	14.49±2.24[a**]
Standard control	11.24±1.69[b**]
Test extract (Low dose)	12.54±1.64[b**]
Test extract (High dose)	12.14±1.49[b**]

All values are expressed as mean ± SEM for 6 determinants; [a]** – Values are significantly different from normal control at $P < 0.01$; [b]** – Values are significantly different from arthritic control at $P < 0.01$

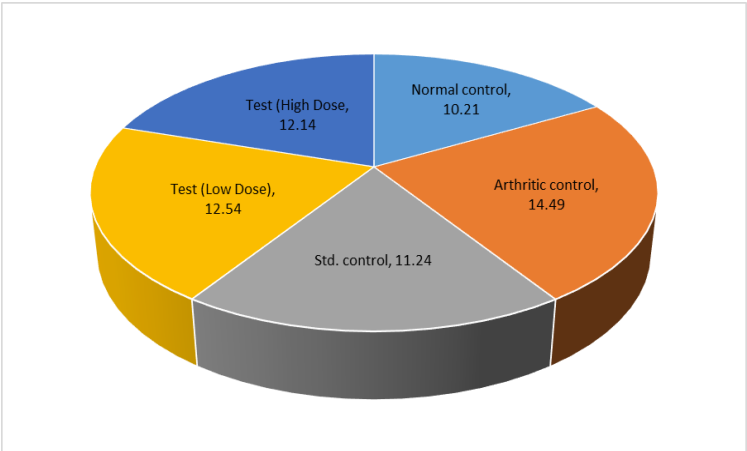

Figure 3: Effect of methanol extract on the WBC level of experimental animals.

***In vivo* anti-diabetic evaluation**

Based on the results of *in vitro* evaluation,[10] the methanol extract was selected for the *in vivo* anti-diabetic evaluation on diabetes-induced Wistar albino rats. Two doses of extract, 200 and 400mg.kg^{-1} were subjected to the evaluation. In this study, blood glucose level, various hematological and biochemical parameters, histopathology of the liver and pancreas of experimental animals were analyzed. The results of blood glucose level analysis are shown in Table 4 and Figure 4.

Table 4: Effect of selected extracts on blood glucose level of experimental animals.

Animal Group	**Blood glucose level (mg/dl)**				
	Day of observation				
	0	**7**	**14**	**21**	**28**
G_1	101.34±6.82	114.29±1.79	110.16±1.14	112.00±1.55	106.82±1.61
G_2	247.00±6.01	261.45±2.60	275.12±2.07	294.65±1.23	320.40±1.64
G_3	345.70±10.90***	119.30±1.23***	175.63±3.38***	114.15±1.16***	113.15±1.92***
G_4	351.13±5.76***	139.15±1.11***	171.23±2.14***	138.15±1.63***	127.65±1.75***
G_5	322.30±6.25***	137.10±2.80***	136.33±2.07***	127.65±1.72***	122.82±1.86***

G_1–Normal control; G_2–Diabetic control; G_3–Standard control; G_4–Group (Methanol extract 200mg.kg^{-1}); G_5–Group

(Methanol extract 400mg.kg^{-1}); All values are expressed as mean ± SEM for 6 animals in each group; ***p<0.001significance between normal control Vs diabetic control and treated groups.

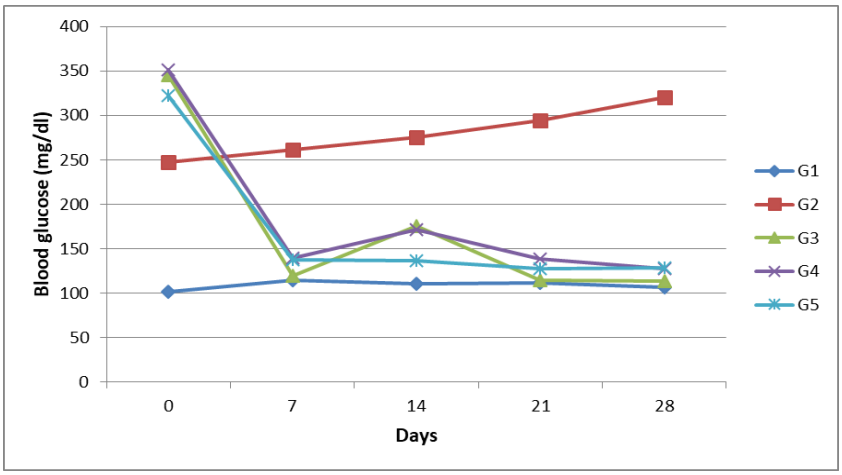

Figure 4: Effect of test extracts and standard on blood glucose level of experimental animals.

The results showed that there were no significant changes in the glucose level of normal control animals in the observation days. But it was observed that the glucose level was increased gradually without any changes in the diabetic control animals. The standard control animals received Glibenclamide showed a marked reduction in the blood glucose level at the 28th-day observation. It was found that on the 28th day of observation, both the doses of tested extract (animals of Group 4&5) showed a significant reduction in the blood glucose level of experimental animals, importantly, the high dose (400mg.kg^{-1}) of the methanol extract (animals of Group 5) showed more significant results. Results of analysis of hematological parameters such as hemoglobin and glycosylated hemoglobin and the level of liver enzymes such as SGOT, SGPT and ALP are presented in Table 5.

Table 5: Effect of selected extracts on haemoglobin and liver enzymes of experimental animals

Animal Group	Haemoglobin	Glycosylated haemoglobin	SGOT	SGPT	ALP
G_1	12.65±1.50	4.75±0.36	25.98±3.17	51.49±8.25	130.82±7.05
G_2	10.85±0.21	12.65±0.77	59.67±6.70	63.59±5.81	203.20±9.35
G_3	12.76±0.37**	5.35±0.38**	34.84±2.09	56.70±3.68	137.64±5.11
G_4	11.77±0.45^{ns}	6.63±0.42**	41.32±2.35	60.59±2.41	145.30±2.15
G_5	12.72±0.25**	6.12±0.65**	38.12±3.60**	59.49±2.56**	141.07±2.04**
G_6	12.92±0.47**	5.67±0.35**	37.24±1.24***	61.75±3.50***	142.40±3.16***
G_7	13.47±0.40***	3.67±0.41**	35.22±2.33***	58.49±2.41***	138.22±2.09***

G_1–Normal control; G_2–Diabetic control; G_3–Standard control; G_4–Group (Methanol extract 200mg.kg^{-1}); G_5–Group (Methanol extract 400mg.kg^{-1}); All values are expressed as mean ± SEM for 6 animals in each group; ns–not significance; **p<0.01, ***p<0.001significance between normal control Vs diabetic control and treated groups

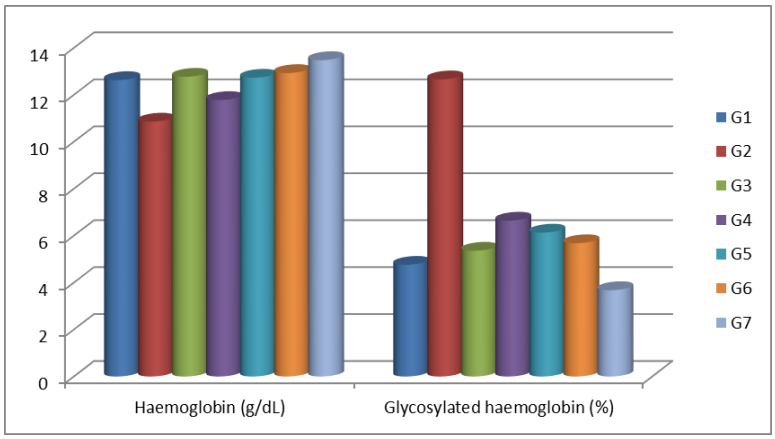

Figure 5: Effect of test extracts and standard on the hematological parameters of experimental animals.

In case of hemoglobin level, the diabetic control group animals (Group 2) showed a marked reduction comparing with normal control group animals (Group 1). But its level get restored in Group 3 animals received the standard drug Glibenclamide. Similarly, the tested extract in low and high dose (200mg.kg^{-1} and 400mg.kg^{-1}) showed a significant rise in its level in experimental animals, particularly, the Group 5 animals received the methanol extract in the dose of 400mg.kg^{-1}. Analysis of changes in the level of glycosylated hemoglobin revealed a significant rise in its level in diabetic control (Group 2) animals compared with normal control (Group 1) animals. Also, it was observed that there were no significant changes in the glycosylated hemoglobin level of treatment group animals (Group 3 to 5) comparing with normal control animals. Importantly, the animals received methanol extract 400mg.kg^{-1} (Group 5) showed a very significant score compared with other treatment groups (Figure 5).

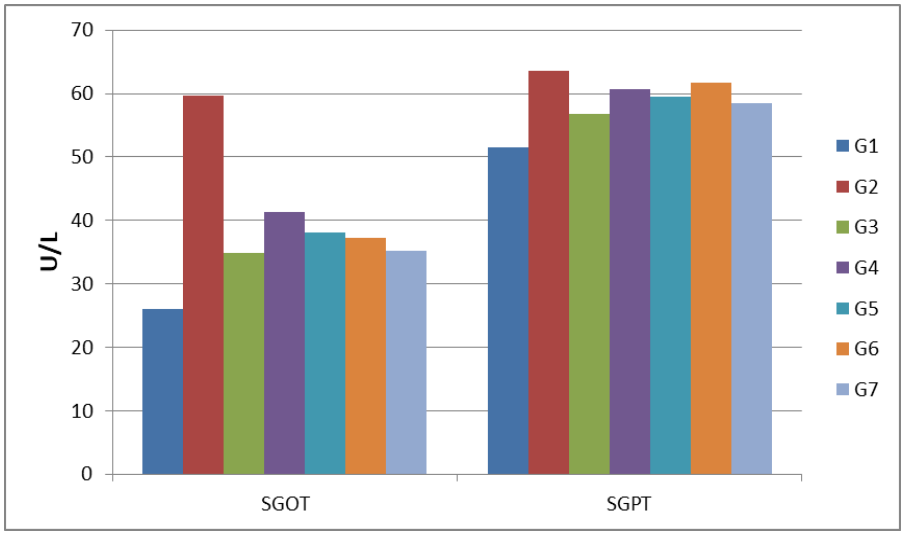

Figure 6: Effect of test extracts and standard on the enzyme system of experimental animals.

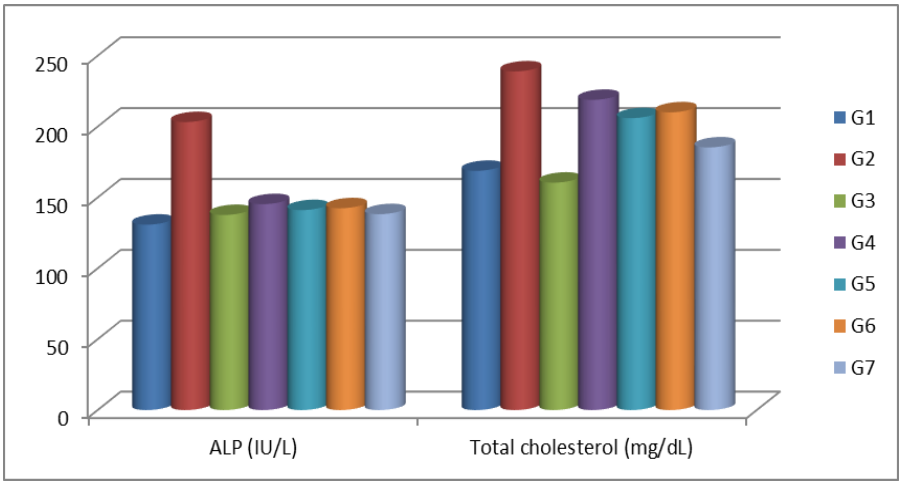

Figure 7: Effect of test extracts and standard on the enzyme ALP and total cholesterol in experimental animals.

From the results, it was found that the level of enzymes such as SGOT, SGPT, and ALP in the diabetic control animals was elevated compared with normal control animals. Treatment of animals with standard drug Glibenclamide (Group 3) showed reversal of their level similar to that of normal control animals (Figure 6 & 7). These results clearly indicated the positive effect of tested extract on the level of these enzymes. Both the doses of methanol extract showed a significant reversal analyzed enzymes level in the experiment animals (Group 4&5) towards normal in comparison with standard control animals, particularly, the high dose of methanol extract showed more significant results. Analysis of changes in the level of total cholesterol, triglycerides, HDL, LDL, and VLDL in the serum of experimental animals showed significant results (Table 6). All these lipid profiles markedly differed abnormally in diabetic control animals comparing with normal control one. But in the treatment group animals, these scores were reversed to normal. Comparing with normal and standard control, the methanol extract-treated animals showed a significant effect than ethyl acetate extract-treated animals (Figure 8 & 9).

Table 6: Effect of selected extracts on lipid profile of experimental animals.

Animal group	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
G_1	168.80±4.55	69.94±3.47	55.54±1.51	50.63±4.10	13.98±0.67
G_2	238.95±4.64	187.57±5.89	22.39±1.31	132.16±0.88	67.50±0.47
G_3	160.44±4.18***	91.21±10.67***	50.46±1.79***	69.90±0.73***	28.63±1.67***
G_4	218.87±4.85 ^{ns}	135.80±13.73 ^{ns}	32.39±1.10^{ns}	97.31±1.43^{ns}	55.72±1.80^{ns}
G_5	205.97±5.55**	109.90±16.59**	36.41±3.51**	89.84±1.94**	42.17±4.61**
G_6	210.06±5.74**	104.37±7.03**	33.42±1.89**	93.89±1.41**	48.35±1.41**
G_7	185.31±2.78***	97.31±9.74***	46.22±1.10***	78.20±1.37***	34.87±1.93**

G_1–Normal control; G_2–Diabetic control; G_3–Standard control; G_4–Group (Methanol extract 200mg.kg^{-1}); G_5–Group (Methanol extract 400mg.kg^{-1}); All values are expressed as mean ± SEM for 6 animals in each group; ns–not significance; **p<0.01, ***p<0.001significance between normal control Vs diabetic control and treated groups.

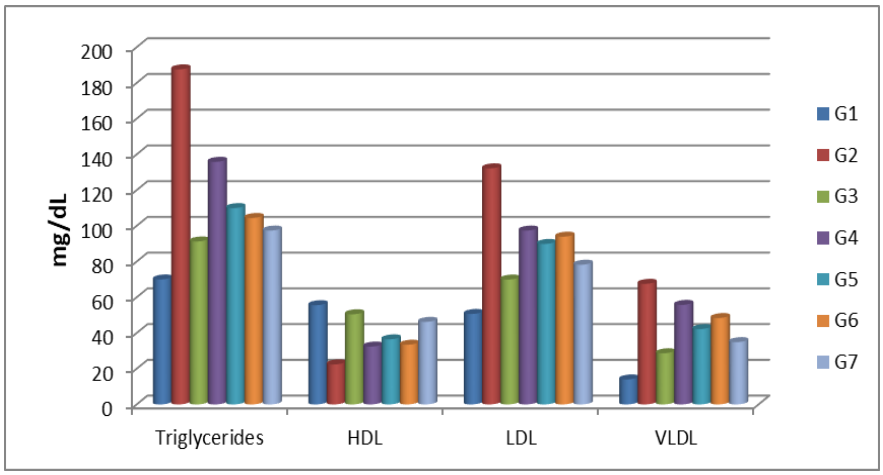

Figure 8: Effect of test extracts and standard on different biochemical parameters of experimental animals.

In the histopathology analysis, normal hepatocyte morphology was found in the liver of normal control animals. In diabetic control, the hepatic congestion at sinusoids and the portal vessel, peri-centre globular micro-steatosis, Kupffer cell proliferation, hepatocyte necrosis and mononuclear infiltrate were identified. But, the standard control showed mild hepatic congestion at sinusoids and the portal vessel, peri-centre globular micro-steatosis. The low dose and the high dose of test extract-treated groups sections exhibited moderate hepatic congestion at sinusoids and the portal vessel, pe-ricentre globular micro-steatosis, less Kupffer cell proliferation, mild hepatocyte diffuse necrosis and mononuclear infiltrate (Figure 9). The small pores system in the liver sinusoidal endothelial cells is the site where the transfer of nutrients between blood and liver take place. In diabetic control, the liver sinusoidal was identified as congested because of the accumulation of high glucose concentration in blood. Section of the entire test extracts treated group exhibited moderate hepatic congestion due to the reduction in the blood glucose level. In diabetic control, it was identified that small droplets of lipids are retained as pericentre globular micro steatosis which may due to hyperglycemia that impaired the normal process of synthesis and elimination of triglycerides. In standard control and the test extract treated group section showed a minimal pericentre globular micro steatosis that pointed out that the impairment in the triglycerides synthesis and elimination was balanced.

The Kupffer cells are the scavenger cells of the liver. In hyperglycemia, the liver cells become inflamed and more Kupffer cells were proliferated to phagocyte the inflammatory cells. It was found that in the diabetic control, the Kupffer cell proliferation was increased because the diabetic condition leads to elevated level of lipopolysaccharides whereas the standard control and test extract treated group showed the Kupffer cell proliferation is moderate which clearly indicated that the treatment increases the insulin sensitivity thereby decreasing the level of lipopolysaccharides leads to reduction in the kupffer cell proliferation and the impairment of β- cell function gets improved.

Histopathological analysis of the pancreas revealed a normal histological architecture in the normal control group animals. In diabetic control, the pancreatic β cell degeneration was found which may due to the administration of STZ. But, it was found that the extract treated groups significantly regenerate the pancreatic β cells. All these results strongly supported the anti-diabetic nature of the tested extract (Figure 10).

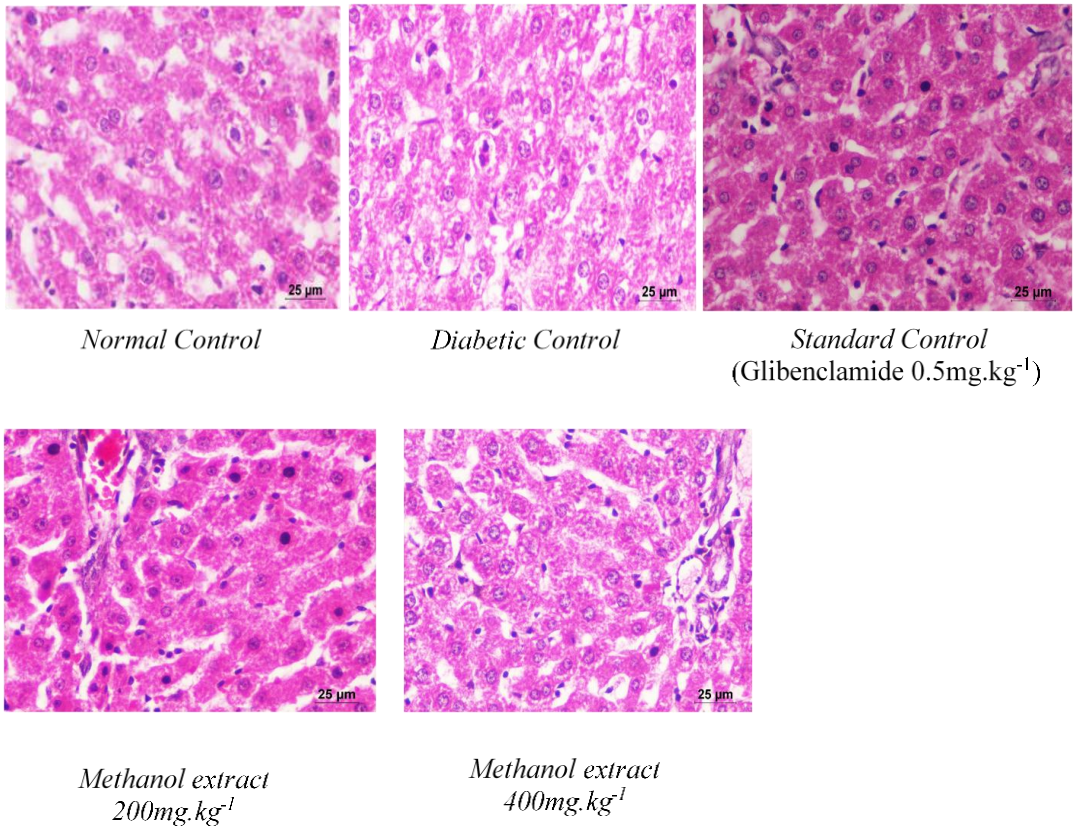

Figure 9: Effect of standard and selected extract in the histopathology of liver.

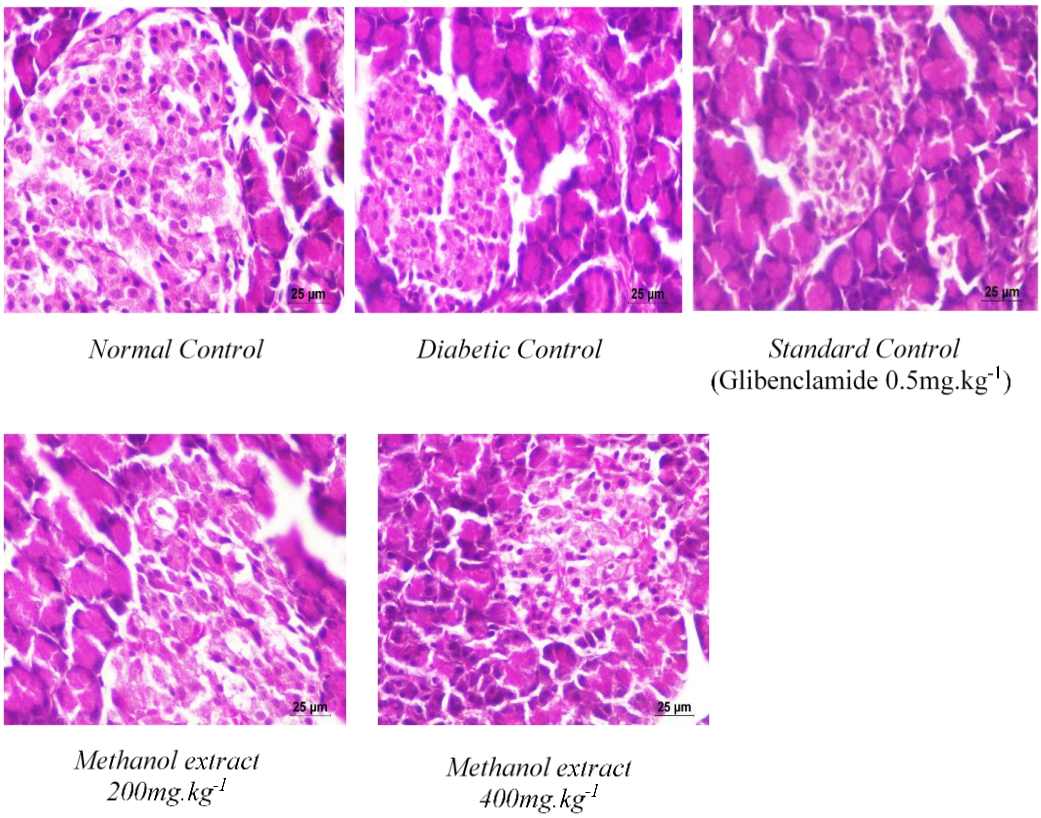

Figure 10: Effect of standard and selected extract in the histopathology of pancreas.

CONCLUSION

As the part of our ongoing investigation, in the present study, the anti-arthritic and anti-diabetic activity of methanol extract of *C. quadrangularis* was evaluated by *in vivo* methods. The results strongly supported the anti-arthritic and anti-diabetic activity of methanol extract of *C. quadrangularis* particularly, the high dose (400mg/kg) of the extract revealed a significant activity. Not only in the present study, but also in our previous study, the methanol extract has established its significance in different area viz., yield of the extracts, phytochemical screening and *in vitro* evaluation of anti-inflammatory and anti-diabetic activity. Polarity of the solvent may be the reason for this. In the future, further studies of this extract may yield significant results that will be useful for the development of new chemotherapeutic agents.

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