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Evaluation of antidiabetic activity and antioxidant properties of *Gardenia gummifera* L.f. leaf extract in alloxan induced diabetic models

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Abstract
The present investigation is aimed to evaluate the antidiabetic activity of leaf extracts of *Gardenia gummifera* L. f. studies were carried out in normal and alloxan induced diabetic rats treated with methanolic leaf extracts of *G. gummifera* in 200mg/kg and 400mg/kg doses where Glibenclamide was used as standard drug. The test groups produced a significant increase in High Density Lipoprotein, Super Oxide Dismutase, Catalase, Reduced Glutathione and Glutathione Peroxidase levels; significant decrease in Total Cholesterol, Triglyceride, Low Density Lipoprotein, Very Low Density Lipoprotein, Lipid Peroxidation levels over diabetic control. The above results represent the effect of test drugs in the lipid profile and antioxidant properties of the test animals. Studies on histopathology of liver and kidney tissues of the test animals also provided the further support to the results. Overall result showed dose dependent effect. On conclusion the study provided the scientific evidence for the enthnomedicinal use of *G. gummifera* in the treatment of Diabetes Mellitus.

Keywords: alloxan, antidiabetic activity, antioxidant properties, ethnomedicine, *Gardenia gummifera*, Kalrayan hills

Introduction

Indian traditional system of medicines is one of the important and alternative systems of medicine in which plants have been utilized for the treatment of various diseases since ancient times. The secondary metabolites present in the plants are biologically active compounds and are responsible for the potential disease curing capabilities. This traditional system of medicine is most affordable and easily accessible.

Diabetes mellitus (DM) is often referred as diabetes, which occurs due to a combination of hereditary and environmental causes, resulting in abnormal increase in blood sugar levels (BSL), known as hyperglycemia [1]. It is a metabolic disorder of multiple etiologies such as insulin resistance, relative insulin deficiency and hyperglycemia with disturbances of carbohydrates, fat and protein metabolism [2]. It is also characterized by glycosuria, hyperlipidemia, negative nitrogen balance and sometimes ketonemia [3]. The metabolic deregulations associated with DM causes secondary pathophysiological changes in multiple organ systems, which are associated with oxidative stress and damage to tissues [4].

According to the WHO projections, DM will be the 7th leading cause of death by the year 2030. It is caused by the ineffective production of insulin, a glucose-controlling hormone produced in the pancreas that helps in the transport of glucose from the blood stream into the cells which leads to the increase or decrease in concentration of glucose in the blood. This can cause severe short-term and long term consequences and damage to many of the body systems, particularly the blood vessels and nerves.

As the available treatment options in modern medicine for DM have several limitations such as side effects, expensive, toxicity etc., and plant based remedies are considered to be less toxic and free from side-effects when compared to synthetic medicine [5], there is a need to develop safe and effective substitutes for hypoglycemic antidiabetic drugs for the human wellbeing.

Various medicinal plants have been reported for their anti diabetic actions and there are about 802 plants have been reported for their antidiabetic properties [6]. In the same time, a large number of *in-vivo* studies have been conducted on animals and the antidiabetic properties of many plants have also been demonstrated by various researchers [3, 7].

The traditional healers of *Malayali* tribes of Kalrayan (Kalvarayan) hills of Salem district of Tamil Nadu are using varieties of plants as ethnomedicine. Among them, *Gardenia gummifera* L. f. (Rutaceae) has been used for the treatment of DM. It grows only in the natural forests and not cultivated. It can be seen in specific areas only and not found for a considerable distance indicating its fragmented distribution.

Apart from DM, *G. gummifera* is reported to be used in the treatment of diarrhea, snake bite, cancer [8], skin disease and stomach ulcer [9]. It is also acts as antiseptic, carminative, expectorant, stimulant, vermifuge and repellant [10]. It is also claimed to be useful in the veterinary healthcare for the treatment of dyspepsia, flatulence, for cleaning foul ulcers and keep off flies from sores [11] and in the treatment of wounds [12]. A scrutiny of literature revealed some notable pharmacological activities of this plant such as cytotoxic [13], analgesic, anti-inflammatory, antipyretic and anticonvulsant [14].

The present work was therefore designed to study the antidiabetic activity and antioxidant properties of methanolic leaf extract of *G. gummifera* in Alloxan induced diabetic animals to verify the traditional medicinal claims of tribes of Kalrayan hills.

Materials and Methods

Collection of plant materials

Leaves of *G. gummifera* were collected in fresh from Kalrayan hills, Salem district, Tamil Nadu, India, which lies between 11º 36' and 12º 01' N and 78º 29' and 78º 54' E, at an altitude of 1000ft above MSL. The plant was identified with the help of standard local flora [15] and further authenticated by taxonomists. The voucher specimens were submitted to the Department of Botany, Vinayaka Missions Research Foundation (Deemed to be University), Salem, Tamil Nadu for further references.

Preparation of leaf extract

Collected leaf material was thoroughly washed using running tap water followed by rinsing with distilled water. Afterwards, plant material was severed, shade-dried at room temperature and coarsely powdered. The Soxhlet extraction procedure was carried out using 70% methanol. About 400 ml of the solvent was poured into the round bottom extraction flask and placed on the heating mantle top on which thimble containing 50g of the dried plant powder was placed. The condenser was placed above the thimble and the parts were fixed vertically. The extraction was carried out for 48 hours. The extract was concentrated under reduced pressure using a rotary evaporator and was kept under refrigeration.

Preliminary phytochemical screening

Preliminary phytochemical screening for leaves of *G. gummifera* was carried out to analyse the presence of alkaloids, carbohydrates, flavonoids, glycosides, oils, fats, polyphenols, tannins, terpens, saponins and triterpenoids using standard qualitative assays [16].

Experimental animal

The experiment was carried out using male Wistar albino rats (*Rattus norvegicus* / 6 weeks of age/ measuring 150–200 gm) procured from the Animal house, Nandha college of Pharmacy, Erode, Tamil Nadu, India. All the experimental procedures and protocols used in this study was reviewed by the Institutional Animal Ethics Committee (Regd no: 688/2/C-CPCSEA/2015) and are in accordance with the guidelines of the CPCSEA.

Housing conditions

The animals were housed in polypropylene cages and maintained under standard conditions (25± 2ºC) and relative humidity of 30 – 70% with 12 h dark/ light cycle. All animals were allowed to free access to water *ad libitum* and fed with standard commercial rat chow pellet diet (M/s. Hindustan Lever Ltd, Mumbai, India). Each animal was housed for 45 days.

Chemicals

In our study, Alloxan is used for the induction of diabetes in test animals. Alloxan is a β-cytotoxin which destroys β-cells of islets of langerhans of pancreas resulting in a decrease in endogenous insulin secretion. An insufficient release of insulin causes high blood glucose, namely hyperglycemia, which results in oxidative damage [17].

Alloxan was obtained from Sigma – Aldrich Fine chemicals (St. Louis, MO, USA). Glibenclamide and other chemicals of analytical grade were purchased from local firms (India).

Antidiabetic properties of *Gardenia gummifera* L.f. in animal models

Experimental induction of diabetes

After fasting for 18hrs the animals were administered with single i.p. injection of freshly prepared Alloxan solution (60mg/kg) in cold 0.1M citrate buffer (pH 4.5). After i.p. injection, the animals were allowed to free access to feed and water and provided with 5% glucose solution over night to counter the hypoglycemic shock. After two days of alloxan administration, rats with blood glucose concentration more than 250mg/dl were considered diabetic and were included in the study.

Experimental design

A total of 30 rats, divided into five groups of six each (6 normal; 24 diabetic) were used in the study.

Group I–Served as normal control animals, received 0.5% Carboxy Methyl Cellulose (CMC) solution (1ml/kg PO) for 14 days.

Group II–Alloxan induced diabetic animals (Diabetic control), received 0.5% CMC solution (1ml/kg PO).

Group III–Alloxan induced diabetic animals (Reference control), received the standard drug Glibenclamide 5mg/kg PO.

Group IV–Alloxan induced diabetic animals, received Methanolic extract of *G. gummifera* leaves 200mg/kg orally.

Group V–Alloxan induced diabetic animals received, Methanolic extract of *G. gummifera* leaves 400 mg/kg orally.

Changes in the blood glucose level in control and experimental groups

The test drugs were administered orally once daily for 14 days by suspending in 0.5% CMC (Vehicle material) and Glibenclamide was used as standard drug [3]. All the group of animals received the treatment for 14 days. After 2 hrs of the drug administration, blood samples were collected from tail on 0, 4th, 7th and 14th day to determine the blood glucose level by Glucometer (One-Touch).

Variations in the lipid profile of alloxan induced diabetic rats

On 15th day, blood was collected in a non-heparinized tube by retro orbital sinus puncture, under phenobarbitone anaesthesia. Blood samples were collected in two different tubes (i.e) one with anticoagulant for plasma separation and another tube without anticoagulant for serum separation. Plasma and serum were separated by centrifugation for 10 min at 2000 rpm and were used for various biochemical estimations viz., Total Cholesterol (TC), Triglyceride (TG), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) levels.

Total cholesterol

The principle behind the assay of TC in serum principle is based on enzymatic hydrolysis and oxidation of cholesterol. The indicator compound, quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. The reagents consist of 4-aminoantipyrine (0.03mmol/l), phenol (6 mmol/l), peroxidase (≥0.5 U/ml), cholesterol esterase (> 0.15 U/ml), cholesterol oxidase (> 0.1 U/ml) and Phosphate buffer (80 mmol/L pH 6.8). The serum sample (10 µl) was mixed with 1 ml of reagent, incubated at 37 ºC for 5 min and

absorbance was measured at 500 nm against the reagent blank.

Triglycerols

Serum Triglycerols (TG) were determined by colorimetric method and the assay principle is based on the enzymatic hydrolysis of TG with lipases and the indicator is a quinoneimine formed from hydrogen-peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic activity of peroxidase [18]. The enzyme reagent consists of 4-aminophenazone (0.5 mmol/l), ATP 1.0 m.mol/l), lipases (≥150 U/ml), glycerol-kinase (≥0.4 U/ml), glycerol-3-phosphate oxidase (≥1.5 U/ml) and peroxidase (≥0.5 u/ml). The serum sample (10 µl) was mixed with 1000 µl of enzyme reagent, incubated at 37 ºC for 5 min and absorbance was measured at 500 nm against the reagent blank.

HDL Cholesterol

Serum HDL cholesterol was determined by colorimetric method and the principle behind this assay is based on the quantification of precipitation of low density lipoproteins (LDL and VLDL) and chylomicron fractions by the addition of Phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction, which remains in the supernatant, is determined. The precipitation reagents consists of Phosphotungstic acid (0.55 mmol/l) and Magnesium chloride (25 mmol/l). The serum sample (200 µl) was mixed with 500 µl of precipitation reagent and centrifuged at 4000 rpm for 10 min. The supernatant (100 µl) was incubated at 37 ºC for 5 min and absorbance was measured at 500 nm against the reagent blank.

LDL and VLDL Cholesterol

LDL and VLDL were calculated according to Friedwald formula [19].

LDL = TC – HDL – VLDL

VLDL cholesterol = Triglycerides /5.

Preparation of tissues for histopathology and tissue homogenate for antioxidant study

At the end of the period, the rats were anaesthetized by intramuscular injection of Ketamine (90 mg/kg body weight) and Zylazine (10 mg/kg) and sacrificed by cervical decapitation. Liver and kidney were dissected out and washed with cold saline to clear the blood from the samples. A part of the liver and kidney tissues were immediately kept in ice-cold containers containing 10% formaldehyde for histopathological studies. The weighed quantity of sample tissues were homogenized in Potassium chloride (10mM), Phosphate buffer (1.15%), Ethylene-Diamine Tetra Acetic acid (EDTA; pH 7.4) and were centrifuged at 10,000 rpm for 60 min. The supernatant liquid was used for the assay of the antioxidant markers viz., Lipid Peroxidation (LPO), Super Oxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) and Reduced Glutathione (GSH). Carcasses of the animals were disposed by burial.

Estimation of lipid peroxidation of rat liver and kidney (LPo)

Lipid peroxidation in liver and kidney was estimated calorimetrically by measuring thiobarbituric acid reactive substances (TBARS). 0.1ml of tissue homogenate was treated with 2 ml of TBA-trichloroacetic acid-HCl reagent (0.37% TBA, 0.25 M HCl and 15% TCA, 1:1:1 ratio), placed for 15 min in a water bath and then cooled and centrifuged at 3500rpm for 10 min at room temperature. The absorbance of clear supernatant was measured at 535 nm against reference blank. Values were expressed as Mm/100 g tissue.

Estimation of Superoxide Dismutase (SOD)

The activity of SOD was carried out based on the oxidation of epinephrine adrenochrome transition by enzyme. The post mitochondrial rat suspension (PMS) of rat tissue of 0.5ml was diluted with 0.5ml distilled water. To this, 0.25 ml of chilled ethanol and 0.15ml of chloroform were added. The mixture was shaken for 1 min and centrifuged at 2000rpm for 10 min. The PMS of 0.5ml was added with 1.5ml phosphate buffer (pH 7.2). The reaction initiated by the addition of 0.4ml epinephrine and change in optical density (OD) was measured at 470 nm. SOD activity was expressed as U/mg of protein. Change in OD at 50% inhibition to adrenochrome transition by the enzyme was taken as one enzyme unit.

Estimation of Catalase (CAT)

The reaction mixture of 1.5 ml was taken which contained 1.0 ml of 0.01 M phosphate buffer (pH 7.0), 0.1 ml of tissue homogenate and 0.4 ml of 2M H_2O_2. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (A mixture of 5% Potassium dichromate and glacial acetic acid in 1:3 ratio). Then the absorbance was measured at 530 nm. CAT activity was expressed as µM of H_2O_2 consumed/min/mg protein [20].

Estimation of Glutathione peroxidase (GPx)

The reaction mixture contained 0.2 ml of 0.4 M phosphate buffer (pH 7.0), 0.1ml of 10 mM Sodium azide, 0.2 ml of tissue homogenized in 0.4M phosphate buffer (pH 7.0.), 0.2 ml of reduced glutathione and 0.1 ml of 0.2 mM H_2O_2. These contents were incubated for 10 min at 37ºC and 0.4 ml of 10% TCA was added to stop the reaction and centrifuged at 3200 rpm for 20 min. The supernatant was assayed for glutathione content using Ellman's reagent (19.8 mg 5, 5'-dithiobisnitrobenzoic acid [DTNB] in 100 ml 0.1% Sodium Nitrate). The activities were expressed as µg of GSH consumed/ min/mg protein [21].

Estimation of reduced glutathione (GSH)

The PMS of rat tissue (720 µl) and 5% TCA were mixed to precipitate the protein content of the supernatant. After centrifugation at 10,000 rpm for 5 min, the supernatant was collected. Ellman's reagent [DTNB (5, 5'-dithio-bis-2-nitrobenzoic acid)] was added to it and the absorbance was measured at 412 nm [22].

Statistical Analysis

Number of replicates for each sample was six (n=6). Results were expressed as mean ± SEM. The data were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's 't' test using Graph Pad version 3.

Histopathological studies

Histopathological studies were carried to analyze the changes in the kidney and liver cell homogenates of the test animals. The tissues were dissected out and washed in

running tap water. Tissues were fixed by chemical fixation procedure using 10% aqueous solution of formaldehyde at neutral pH. It inactivates the enzyme reaction otherwise the tissue might be degraded. It is followed by dehydration and clearing process using alcohol (0% to 100%) and xylene. After clearing, the tissues were embedded in paraffin. Then tissue sections of 5-8µm thickness were taken using microtome and stained with Ehrlich Haematoxylin and counter stained with Eosin (H&E) stain. The excess stain was removed by washing the slide in running tap water and air dried. The tissue sections were observed under microscope and photomicrographs were taken.

Results and Discussion

The qualitative phytochemical analysis of the leaves of *G. gummifera* revealed the presence of various phytochemicals such as alkaloids, carbohydrates, flavonoids, glycosides, polyphenols, tannins, saponins and triterpenoids whereas no oils and fats were detected. It correlates with the results of previous findings [13].

Diabetic inducing agents such as Alloxan or Streptozotocin are reported to induce diabetes with generation of free radicals; a significant reduction in antioxidant enzyme levels is indicated as the potential reason for the susceptibility of organs to atrophy in diabetic state [23].

The changes in the BSL in alloxan induced diabetic animal due to the administration of methanolic extract of *G. gummifera* were measured at successive days of 0, 4th, 7th and 14th day and the results were given in the Table 1. In diabetic animal, a gradual increase in BSL was observed due to alloxan administration.

Table 1: Antidiabetic effect of methanolic extract of *Gardenia gummifera* L. f. in alloxan induced diabetic rats.

Groups	Drug Treatment	Blood Sugar Level (mg/dl)					
		Initial	After Alloxan	During Drug Treatment			
				0 Day	4th Day	7th Day	14th Day
I	Normal Control 0.5% CMC	101.67±2.47	100.33±4.40	98.00±3.41	100.00±2.50	98.50±3.07	95.83±2.81
II	Diabetic Control Alloxan (60mg/kg, i.p)	97.33±2.81	267.17±3.58	266.67±3.03	262.33±7.79	267.83±5.26	270.50±5.47
III	Diabetic + Reference Control Glibenclamide (5mg/kg)	96.00±3.79	273.50±5.37	278.50±4.43	194.17±3.09***	129.00±2.57***	99.50±1.77***
IV	Diabetic + *G. gummifera* (200mg/kg)	102.67±4.05	270.1±4.54	274.50±4.92	224.50±5.72**	146.50±4.84***	120.00±4.05***
V	Diabetic + *G. gummifera* (400mg/kg)	100.33±2.12	276.3±6.00	280.33±5.09	210.83±5.14***	134.83±3.61***	100.83±2.57***

Values are in Mean ±SEM (n=5)
*$P<0.05$, ** $P<0.01$ and** *$P<0.001$ Vs Diabetic Control

Diabetic animals treated with methanolic extract of the plant at 200mg/kg showed moderate decrease ($P<0.01$) in BSL on 4th day whereas the same dose on 7th and 14th days and 400mg/kg dose on 4th, 7th and 14th days showed more significant decrease ($P<0.001$) in BSL over diabetic control. Among the two doses, the groups treated with 400mg/kg dose showed more significant decrease than 200 mg/kg dose in all the days. Because the plant extract improved the glucose tolerance in Alloxan induced diabetic rats as compared to the control.

Alloxan induction paves the ways for the decreased utilization of glucose by the tissue and elevation of BSL. Expression of elevated BSL confirmed the development of diabetics in alloxon induced experimental animals [3]. It was evident from the results that untreated diabetic rats have elevated BSL and the test drugs were able to correct the metabolic deviation from the diabetic control significantly. So the extracts are considered as having potential anti-diabetic activity.

Lipid profile of the test animals treated with *G. gummifera* leaf extracts are estimated for the lipid parameters namely TC, TG, HDL-C, LDL-C and VLDL-C on 15th day (Table 2). Diabetic animal group treated with 400mg/kg dose of test drugs showed more significant decrease ($P<0.001$) in TC, TG, VLDL-C and LDL-C and more significant increase ($P<0.001$) in HDL-C over diabetic control. In 200mg/kg dose the results are significantly decreased ($P<0.001$) in TC and LDL, where as in TG and VLDL moderate decrease ($P<0.01$) and in HDL moderate increase ($P<0.01$) were observed over diabetic control.

Table 2. Effect of methanolic extract of *Gardenia gummifera* L. f. on lipid parameters in alloxan induced diabetic rats

Groups	Drug Treatment	Lipid Profile (mg/dl)				
		TC	TG	HDL	VLDL	LDL
I	Normal Control 0.5% CMC	65.30± 2.01	52.08± 1.52	32.32± 1.03	10.42± 0.31	22.57± 2.26
II	Diabetic Control Alloxan (60mg/kg, i.p)	112.62± 1.53	95.39± 1.49	20.48± 1.38	19.07± 0.29	73.07± 0.48
III	Diabetic + Reference Control Glibenclamide (5mg/kg)	87.26± 2.62**	71.42± 1.37**	28.39± 1.22**	14.28± 0.27**	44.58± 1.34***
IV	Diabetic + *G. gummifera* (200mg/kg)	72.22± 1.72***	65.78± 1.65**	27.43± 1.22**	13.16± 0.33**	31.63± 0.92***
V	Diabetic + *G. gummifera* (400mg/kg)	69.53± 1.66***	59.62± 1.46***	30.35± 0.92***	11.92± 0.29***	27.26± 1.93***

The values were expressed as Mean ± SEM (n=6)
*$P<0.05$, **$P<0.01$ & ***$P<0.001$ Vs Diabetic Control

The imbalance in the lipid metabolism plays a key role in aggravating the lipid peroxidation [24]. It is evident from the result that there was a significant increase in HDL-C and significant decrease in TC, TG, LDL-C and VLDL-C. Significant lowering of total cholesterol and rise in HDL cholesterol is a desirable biochemical state for the prevention of hypoglycemia. Several studies showed that an increase in HDL-Cholestrol is associated with a decrease in

LDL-cholestrol [25]. Hence, the test drugs are supposed to have antidiabetic properties. This finding also correlates with the results of antidiabetic effect of *Acacia leucophloea* extracts in diabetic induced rats [26]. Over all result of lipid parameters showed dose dependent effect.

The effect of test drugs on the liver and kidney homogenates of the Alloxan induced diabetic animals were analysed for its antioxidant parameters such as LPO, SOD, CAT, GSH and GPx and the comparative results were provided in the Table 3 & 4.

Table 3: Antioxidant effect of methanolic extract of *Gardenia gummifera* L. f. in liver homogenate of alloxan induced diabetic rats

Groups	Drug Treatment	Liver Homogenate				
		LPO Mm/100g of Tissue	SOD U/mg of Protein	CAT µM of H_2O_2 consumed/ min/mg protein	GSH µg of GSH consumed/min/mg protein	GPx µg of GSH utilized/min/mg protein
I	Normal Control 0.5% CMC	0.23± 0.02	1.46± 0.04	0.97± 0.03	0.69± 0.07	0.98± 0.09
II	Diabetic Control Alloxan (60mg/kg, i.p)	0.56± 0.01	0.88± 0.04	0.54± 0.06	0.35± 0.053	0.49± 0.04
III	Diabetic + Reference Control Glibenclamide (5mg/kg)	0.29± 0.02***	1.31± 0.06***	0.84± 0.04***	0.54± 0.06*	0.89± 0.04***
IV	Diabetic + *G. gummifera* (200mg/kg)	0.26± 0.03***	1.13± 0.07*	0.90± 0.06***	0.48± 0.07*	0.84± 0.06**
V	Diabetic + *G. gummifera* (400mg/kg)	0.25± 0.02***	1.40± 0.10***	0.95± 0.05***	0.59± 0.08*	0.96± 0.06***

The values were expressed as Mean ± SEM (n=6)
*$P<0.05$, **$P<0.01$ & ***$P<0.001$ Vs Diabetic Control

Table 4: Antioxidant effect of methanolic extract of *Gardenia gummifera* L. f. in kidney homogenate of alloxan induced diabetic rats

Groups	Drug Treatment	Kidney Homogenate				
		LPO Mm/ 100 g of Tissue	SOD U/mg of Protein	CAT µM of H_2O_2 consumed/min/mg protein	GSH µg of GSH consumed/min/mg protein	GPx µg of GSH utilized/min/mg protein
I	Normal Control 0.5% CMC	0.26± 0.03	0.75± 0.02	1.24±0.11	0.68±0.09	0.84±0.08
II	Diabetic Control Alloxan (60mg/kg, i.p)	0.56± 0.05	0.37± 0.04	0.67±0.07	0.28±0.07	0.38±0.06
III	Diabetic +Reference Control Glibenclamide (5mg/kg)	0.33± 0.05*	0.71± 0.05***	1.07±0.09*	0.59± 0.09**	0.76± 0.11**
IV	Diabetic + *G. gummifera* (200mg/kg)	0.33± 0.06*	0.64± 0.05**	1.02±0.12*	0.48±0.03*	0.75± 0.06**
V	Diabetic + *G. gummifera* (400mg/kg)	0.29± 0.04**	0.75± 0.04***	1.14± 0.13**	0.63± 0.08***	0.82± 0.13***

The values were expressed as Mean ± SEM (n=6)
*$P<0.05$, **$P<0.001$ & ***$P<0.001$ Vs Diabetic Control

In liver homogenates, more significant increase ($P<0.001$) was observed in SOD and GPx in 400mg/kg dose where as in 200mg/kg dose, less increase in SOD ($P<0.05$) and GPx ($P<0.01$) were observed. In GSH low increase ($P<0.05$), in CAT more significant increase ($P<0.001$) and in LPO more significant decrease ($P<0.001$) were observed in both of the doses. In kidney homogenates, plant extract with 400mg/kg dose showed increase in SOD, GSH and GPx at more significant level ($P<0.001$) and in CAT at moderate level ($P<0.01$), where as in LPO significant decrease at moderate level ($P<0.01$) when compared to diabetic control. In 200mg/kg dose, moderate increase ($P<0.01$) in SOD and GPx, less increase in CAT and GSH ($P<0.05$) and less decrease in LPO ($P<0.05$) were observed. Over all result of antioxidant parameters showed dose dependent effect. From the observations, it is evident that the test extracts not only shown a significant antidiabetic property but also possesses an effective antioxidant activity. The antioxidant activity could be the reason for therapeutic potential in preventing the development of oxidative stress involved diseases. The antioxidant property of the plant is due to the presence of phenolic compounds [27]. Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoids, phenolic acids, tocopherols etc., [28]. These compounds posses an ideal structural chemistry for free radical scavenging activity of the plant extracts [29]. The antioxidative properties of these compounds arise from their high reactivity as hydrogen or electron donors. The phenolic radical has the ability to stabilize and delocalize the unpaid electrons (chain breaking function), chelate metals ions i.e. termination of Fenton reaction [30], inactivate lipid free radical chains and prevent hydroperoxide conversion into reactive oxyoradicals [31]. The more antioxidant property of the extracts was accompanied with high polyphenolic content. Thus the study proves the potential of the plant extract as an antioxidant substance. This antioxidant exphasizes the utility of the plant in the treatment of various disorders for which free radicals are the underlying cause [32]. Recently so many studies revealed that a number of plant products including polyphenolic substances and various plant extracts exert antioxidant activities [33]. Lipid peroxidation is a free radical chain reaction which is triggered by hydroxyl radical and leads to membrane break down and leading to production of more number of free radicals. The flavonoid components of plant extracts are known to be efficient in scavenging the highly reactive hydroxyl radical and superoxide onions. They inhibit the lipid peroxidation by quenching the peroxyl radicals. Hence, these polyphenolic compounds might be responsible for the increase in SOD, CAT and GSH which leads to decrease in lipid peroxidation levels in alloxon induced diabetic animals.

In the present study, a decrease in SOD, CAT, GSH are observed in the liver of diabetic control animal. SOD is ubiquitous cellular enzyme that dismutases super oxide radical to H_2O_2 which is considered as one of the cellular defensive mechanism [34]. Catalase is an enzymetic antioxidant actively involved in red blood cells and liver extensively, spread in all animal tissues. This antioxidant decomposes H_2O_2 and protects the animal tissues from highly reactive hydroxyl free radicals. Depletion of catalase observed in diabetic control group was found to be

indicating the antioxidant property of the selected plant. The most important biomolecule against chemically induced toxicity is GSH which is involved in the elimination of reactive intermediates by the reduction of hydroperoxides [35]. It is evident from the studies carried out in *Sesamum indicum* for its antidiabetic activity that there is a correlation between the antioxidant properties of the plant extract with its antidiabetic activity [36]. So, administration of test drug may produce the antioxidant effect by the increase in antioxidative enzyme levels which in turn responsible for the antidiabetic activity of the selected plant extract.

By the observations it is evident that the test drug showed significant dose dependent effect. This phenomenon is already observed in the results of experiment carried out in diabetic rats with *Strychnos-nux-vomica* extracts [4]. In addition, variations in the lipid profile, antioxidant properties of liver and kidney homogenates of the animals treated with 400mg/kg showed more significant results than the animals treated with 200mg/kg and hence, it is also reflects the dose dependent effect. It correlates with the previous findings carried out by various researchers in different plant extracts [13, 37]. Results of histopathological studies of liver and kidney cells of alloxan induced diabetic animals showed significant healing property of the plant extract. In diabetic control, Alloxan causes severe necrotic changes and thus the liver tissues showed hepatocytes, degeneration of central vein, congestion and cellular necrosis whereas in the reference control rearrangement of normal hepatocytes with mild fatty degeneration and congestion was observed. It is due to the action of Alloxan on the liver tissues which is evident from the studies in *Sida spinosa* [38]. In the liver tissues of animals treated with test drug showed restored hepatic cell rearrangement with well defined normal central veins and lobules in 200 mg/kg dose whereas 400mg/kg dose showed normal architecture with prominent hepatocytes (Fig. 1).

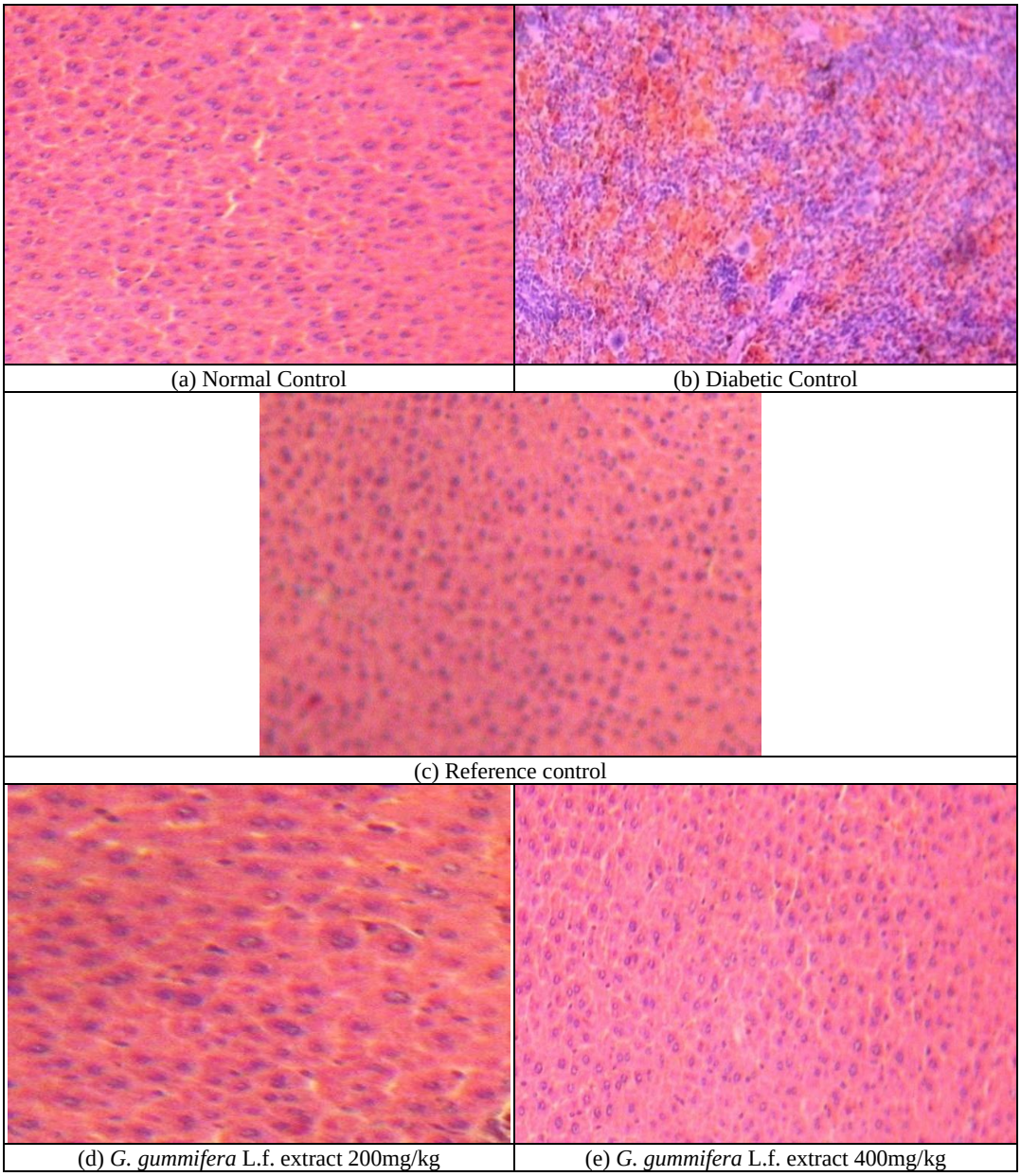

Fig 1: T.S of liver tissues of Alloxan induced diabetic animals treated with methanolic extract of *Gardenia gummifera* L. f.

(a) Normal architecture of hepatocytes with prominent central lobule and vein.

(b) Destructed hepatocytes, degenerated central vein with congested cellular necrosis.

(c) Rearranged normal hepatocytes with mild fatty degeneration and congestion.
(d) Restored hepatic cell rearrangement with well defined normal central veins and lobules.
(e) Normal architecture of tissues with prominent hepatocytes.

The T.S. of kidney cells showed well arranged cells with compact glomerular basement membrane with normal alternating areas of convoluted tubules, glomeruli and straight tubules in normal control animals. In diabetic control animals, congestion of convoluted tubules, disarranged glomeruli with glomerulosclerosis were observed. Mild thickening of glomerular basement with normal cell arrangement were observed in the reference control group. In the animals treated with test drugs showed normal architecture of tubules and compact glomerular basement membrane in kidney tissues in 200mg/kg dose whereas in 400mg/kg dose distinguishable renal capsule, glomerulus and glomerular capsule were observed (Fig. 2). Development of diabetes due to the administration of Alloxan is because of the generation of free radicals; a significant reduction in antioxidant enzyme level is indicated as the potential reason for the susceptibility of organs to atrophy in diabetic states.

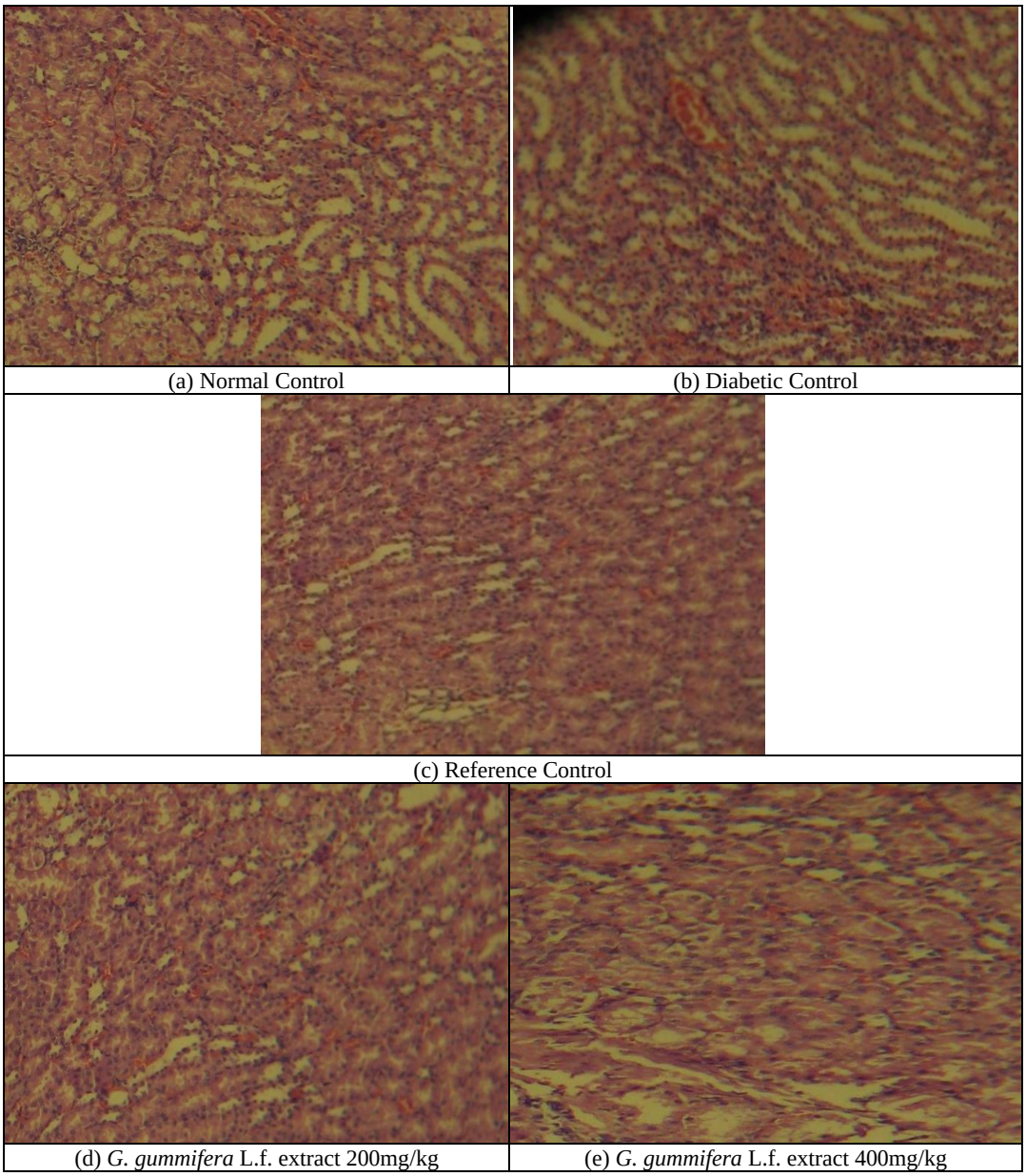

Fig 2: T.S of kidney tissues of Alloxan induced diabetic animals treated with methanolic extract of *Gardenia gummifera* L. f.

a. Normal control – Well arranged cells with compact glomerular basement membrane. Normal alternating areas of convoluted tubules, glomeruli and straight tubules are also noticed.
b. Diabetic control - Congested convoluted tubules, dearranged glomeruli with glomerulosclerosis.
c. Reference control - Mild thickening of glomerular basement with normal cell arrangement.
d. 200mg/kg - Normal architecture of tubules and compact glomerular basement membrane.
e. 400mg/kg - Distinguishable renal corpuscle, glomerulus and also glomerular capsule.

The phytochemicals present in the plants either singly or in combinations with each other could be responsible for the antidiabetic activity of the plant extracts. Recent studies

have shown that phenolic phytochemicals have high antioxidant activity and certain therapeutic effect including antidiabetic activity [39]. Numerous experimental and clinical observations have indicated that hyperglycemia may directly or indirectly contribute to an increased formation of free radicals and consequently to the onset of oxidative stress which has been implicated in diabetic complications. Oxidative stress is a condition of reduction in antioxidant enzymes like SOD, GSH and Catalase levels [40].

From the observations, it is evident that treatment with methanolic extracts of *G. gummifera* improved the glucose tolerance in alloxan induced diabetic rats significantly. The antioxidant property of methonolic extracts has dose dependant effect. Histopathological studies provide the further evidence for the effectiveness of plant extracts for their curative properties. The overall assessment of histopathological studies revealed that the cells of liver and kidney tissues of alloxon induced diabetic animals were recovered from the tissue damage due to the drug administration.

Conclusion

The present study provides the evidence for the scientific validation of ethnomedicinal use of *G. gummifera* for the treatment of diabetes mellitus by the traditional healers of Kalrayan hills of Salem district, Tamil Nadu. In addition to the significant antidiabetic activity, the plant extract also possess effective antioxidant property and produces significant changes in the lipid profile and histopathology of the treated animals. In conclusion leaves of *G. gummifera* could be a source of antioxidant compounds that could be used for the treatment of pathological condition that are associated with disease caused by oxidative stress including DM. However, further investigation on the isolation and purification of the compounds responsible for the antidiabetic property of plant extract is needed.

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