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Antihyperglycemic and antioxidant effects of *Solanum xanthocarpum* leaves (field grown & *in vitro* raised) extracts on alloxan induced diabetic rats

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ABSTRACT

Objective: To investigate antidiabetic efficacy of the extract of field grown and *in vitro* raised leaves of *Solanum xanthocarpum* (*S. xanthocarpum*) against alloxan induced diabetic rats. **Methods:** The antidiabetic activity of the crude methanol extracts of the field grown and in vitro raised leaves of *S. xanthocarpum* at different concentrations (100–200 mg/kg bw) was tested against alloxan induced diabetic rats. The antidiabetic efficacy was validated through various biochemical parameters and the antioxidant effect was also determined. The phytochemical analyses of field grown *S. xanthocarpum* and *in vitro* rasied *S. xanthocarpum* leaves were done by estimating their chlorophyll, carotenoids, total sugar, protein, amino acid and minerals contents. **Results:** The results revealed that the methanol extracts of both the leaves (field grown and *in vitro* raised) of *S. xanthocarpum* was efficient anti hyperglycemic agents at a concentration of 200 mg/kg bw and posses potent antioxidant activity. However, the extracts of *in vitro* rasied *S. xanthocarpum* raised leaves exhibit higher efficacy than the field grown leaves in all tested concentrations. Proximal composition and mineral analysis of *S. xanthocarpum* revealed higher concentration of contents in *in vitro* rasied *S. xanthocarpum* than field grown *S. xanthocarpum*. **Conclusions:** From the results it can be concluded that the leaves extracts of *S. xanthocarpum* can be a potential candidate in treating the hyperglycemic conditions and suits to be an agent to reduce oxidative stress.

1. Introduction

Diabetes mellitus is a major health problem worldwide and among Asians the disease is feared to raise 2–3 folds in recent time[1]. Diabetes is a chronic disease caused by inherited and or/acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. It has already been established that chronic hyperglycemia of diabetes is associated with the long term damage, dysfunction and eventually the failure in organs, especially the eyes, kidney, nerves, heart and blood vessels[2]. The World Health Organization has predicted that the major burden of diabetes will occur in the developing countries and there will be a 42% increase (from 51 to 72 million) in the developed countries and 170% increase (from 84 to 228 million) in the developing countries[3]. The countries with the large number of diabetes patients are, and will be in the year 2025, India, China and United States[4, 5].

Apart from currently available therapeutic options for diabetes like oral hypoglycemic agents and insulin, which have limitations of their own, many herbal medicines have been recommended for the treatment of diabetes[6]. Herbal remedies are apparently effective, produce minimal or no side effects in clinical experience and are of relatively low

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costs as compared to oral synthetic hypoglycemic agents[7].

The use of medicinal plants for the treatment of diabetes mellitus dates back from the Ebers Papyrus of about 1550 B.C. A multitude of herbs species and other plant materials have been described for the treatment of diabetes throughout the world[8]. In the past few decades, secondary metabolite production from plant tissue culture has been identified as a tremendous resource for new drug development and clinical research in the fields of pharmacology and medicine. The *in vitro*-propagated medicinal plants furnish a ready source of uniform, sterile, and compatible plant material for biochemical characterization and identification of active constituents. In addition, compounds from tissue cultures may be more easily purified because of simple extraction procedures and absence of significant amounts of pigments, thus possibly reducing the production and processing costs[9].

Solanum xanthocarpum (*S. xanthocarpum*) is a prickly diffuse bright green perennial herb and distributed throughout India, found mostly in dry places as a weed on road side and waste lands[10]. It is one of the members of the dasamula (ten roots) of the Ayurveda, which is considered to be a noxious weed. Numerous reports are available on the medicinal use of *S. xanthocarpum*, especially in Ayurvedic medicine for asthma[11], diabetes[12] rheumatism, catarrhal fever, cough, chest pain, stone in the bladder, flatulence, toothache, bronchospasm, constipation.

Thus the present study was designed to test and evaluate the efficacy of antihyperglycemic and antioxidant properties of the extracts of field grown and *in vitro* raised leaves of *S. xanthocarpum* in alloxan induced diabetic rats.

2. Materials and methods

2.1. Plant material

The field grown leaves of *S. xanthocarpum* was collected from the habitats near to the bank of Cauvery river, Tamilnadu, India, during the month of August to November, 2009. The plant was identified and authenticated from standard resources. The leaves was brought to the laboratory and thoroughly washed in running tap water to remove debris and dust particles and then rinsed in distilled water, shade dried, coarsely powdered and stored in an air tight container for further use.

2.2. Callus initiation and plant regeneration

Seeds of *S. xanthocarpum* were collected from the plants found around the habitats near to the bank of cauvery river during the month of August to November, 2009. They were washed with tap water, teepol solution, then with 0.1% mercuric chloride for 2 min and germinated using germination medium [MS medium supplemented with 100 mg/L myo-inositol (Himedia), 30 g/L sucrose (Himedia), 7g/L agar (Himedia) and pH[5.8].

Leaf explant from the grown seedlings were aseptically excised and grown in MS medium[13] supplemented with 2, 4-dichlorophenoxy acetic acid (2.0 mg/L) and Kinetin (1.0 mg/L). Callus culture was maintained at 25℃±1℃ under a 16-h photoperiod with a light intensity of 30 μ mol m^{-2} S^{-1} provided by cool-white 40 W fluorescent tubes (Philips). To regenerate plants, calli were transferred in to regeneration medium [MS medium supplemented with 1.0 mg/L 6-benzylaminopurine and 0.5 mg/L Kinetin, 100 mg/L myo-inositol (Himedia), 30 g/L sucrose (Himedia), 7 g/L agar (Himedia)][14]. Leaves obtained from the plantlets were shade dried, pulverized and stored in an air tight container for further use.

2.3. Proximal composition and mineral analysis

Leaves of *S. xanthocarpum* were used to evaluate their proximal composition. Chlorophyll and carotenoids were estimated by the method of Arnon, 1949[15]. Total sugar estimation was done by the method of Dubois *et al*[16]. Total Protein was estimated according to the procedure described by Lowry *et al*[17]. Total amino acid was estimated by the method of Moore and Stein[18]. Estimation of total lipid was carried out by the method of Folch *et al*[19]. Mineral content like phosphorus, potasium, magnesium, manganese, zinc, calcium and sulphur were determined using atomic absorption spectrophotometer as per the method suggested by the Association of Official Analytical Chemists and express in per cent of dry matter[20].

2.4. Preparation of plants extract

100 g of both the field grown and *in vitro* raised leaves powder of *S. xanthocarpum* were taken separately and mixed with 500 mL of methanol and then magnetically stirred in a separate container for overnight at room temperature. The residue was removed by filtration. The filtrate was concentrated under reduced pressure in a rotary evaporator at (60±1)℃ to yield 9.5 g of crude extract (9.5%). The crude extract was used throughout this study without further purification.

2.5. Experimental animals

Adult male Wistar rats weighing around 180-200 g were purchased from Tamil nadu Veterinary and Animal Sciences University, Chennai, India. The animals were kept in polypropylene cages (three in each cage) at an ambient temperature of (25±2)℃ and 55%-65% relative humidity. (12±1) h light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions, and were fed with commercially available rat chow (Hindustan Lever Ltd., Bangalore. India) and had free access to water. The experiments were designed and conducted in accordance with the institutional

guidelines (Reg. No. 1142/ab/07/CPCSEA).

2.6. Acute toxicity studies

The acute oral toxicity study was carried out according to the guidelines set by OECD. Starting dose was selected to be 500 mg/kg bw and finally a dose of 5 000 mg/kg bw was evaluated for toxicity.

2.7. Experimental induction of diabetes

Diabetes was induced in male wister albino rats by intraperitoneal injection of alloxan monohydrate (150 mg/kg) (SD Fine Chem. Limited, Mumbai) in normal saline by the method described by Nagappa *et al*[21]. Alloxan was first weighed individually for each animal according to the weight and then solubilized with 0.2 mL saline (154 mM NaCl) just prior to injection. Two days after alloxan injection, blood was collected from the tip of the tail vein and the blood glucose was measured using Gluco Chek glucose estimation kit [Aspen diagnostic (P) Ltd. Delhi, India]. Fourty eight hours after alloxan injection, rats screened for diabetes having glycosuria and hyperglycemia with blood glucose level of 300-400 mg/dL were taken for the study. All animals were allowed free access to water and pellet diet and maintained at room temperature in polypropylene cages. Treatment with plant extracts was started 48 h after alloxan injection.

2.8. Experimental design

The animals were divided into following groups comprising 6 animals in each group.

Group Ⅰ: Normal rats;

Group Ⅱ: Diabetic (Alloxan induced) control rats;

Group Ⅲ: Diabetic induced rats treated with methanol leaves extract of field grown *S. xanthocarpum* (100 mg/kg bw) for 25 days;

Group Ⅳ: Diabetic induced rats treated with methanol leaves extract of field grown *S. xanthocarpum* (200 mg/kg bw) for 25 days;

Group Ⅴ: Diabetic induced rats treated with methanol leaves extract of in vitro rasied *S. xanthocarpum* (100 mg/kg bw) for 25 days;

Group Ⅵ: Diabetic induced rats treated with methanol leaves extract of in vitro rasied *S. xanthocarpum* (200 mg/kg bw) for 25 days;

Group Ⅶ: Diabetic positive control animals treated with glibenclamide (600 μg/kg bw)[22].

The leaves extract and the drug glibenclamide were given in aqueous solution daily using an intragastric tube for 25 days. Fasting blood glucose was monitored for every week throughout the experiment.

At the end of the experimental period, the animals were deprived of food overnight and then sacrificed by decapitation. Blood was taken from the jugular vein and collected in two tubes, one with potassium oxalate and sodium fluoride solution for plasma and another without anticoagulant for serum separation. The kidney was immediately dissected out, washed in ice-cold saline, patted dry and weighed to measure their antioxidant status.

2.9. Estimation of blood glucose and body weight

The fasting blood glucose level was determined after 25 days of treatment with leaves extracts and drug control. The blood was collected from the tip of the tail vein from the overnight fasted rats and the blood glucose was measured using Gluco Chek glucose estimation kit [Aspen diagnostic (P) Ltd. Delhi, India]. The results were expressed in terms of milligrams per deciliter (mg/dL) of blood. Body weight of all experimental animals was recorded using a digital weighing scale.

2.10. Estimation of insulin

Insulin was estimated using Radio immuno assay (RIA) kit supplied by Linco research Inc, Stat diagnostic, Mumbai, India.

2.11. Estimation of lipid profile

Total cholesterol (TC), high density lipoprotein (HDL-C) cholesterol and triglycerides (TG) were estimated using standard kits purchased from Transasia Bio Medical Limited, Mumbai, India. For the determination of very low density lipoprotein (VLDL-C) and low density lipoprotein (LDL-C) cholesterol Friedewald's formula was used which states: VLDL cholesterol = Triglycerides/5 and LDL cholesterol = Total cholesterol − (VLDL-C + HDL cholesterol)[23].

2.12. Estimation of urea and creatinine

Urea, uric acid and creatinine were estimated using standard reagent kits purchased from Coral clinical systems, Goa, India.

2.13. Estimation of antioxidant enzymes

Thiobarbituric acid reactive substances (TBARS) in kidney were estimated by Fraga *et al* method[24]. The Hydroperoxide was estimated by Jiang *et al* method[25]. The activity of SOD was assayed by the method of Kakkar *et al*[26], Catalase activity was estimated according to Abei's method[27], Glutathione peroxidase was assayed by the method of Paglia and Valentine's[28].

2.14. Statistical analysis

Values are presented as means±SEM. The statistical significance was evaluated by one-way using the statistical software SPSS Version 17 (Origin Lab Corporation, USA). The data were analyzed by one way analysis of variance

(ANOVA) followed by Tukey's test.

3. Results

3.1 Proximal composition and mineral analysis of field grown S. xanthocarpum and in vitro rasied S. xanthocarpum leaves

Phytochemical screening of field grown *S. xanthocarpum* and *in vitro* rasied *S. xanthocarpum* leaves were analyzed on dry weight basis. The result of proximal composition showed a higher value in the contents of *in vitro* rasied *S. xanthocarpum* leaves than the field grown *S. xanthocarpum* leaves (Figure 1). Mineral analysis also showed that the presence of high amount of phosphorus, nitrogen, potassium, magnesium, manganese, zinc, calcium and sulphur content in *in vitro* rasied *S. xanthocarpum* leaves (Figure 2).

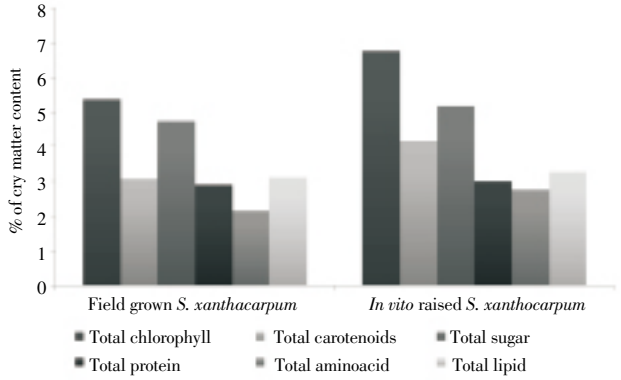

Figure 1. Proximal composition of *S. xanthocarpum* leaves.

3.2. Acute toxicity studies

Acute toxicity study revealed the non-toxic nature of the extracts. There was no lethality or any toxic reactions found at any of the doses selected until the end of the study period.

3.3. Effects of field grown S. xanthocarpum and in vitro rasied S. xanthocarpum leaves extract on body weight, blood glucose and insulin

Table 1 describes the body weight, blood glucose and serum insulin levels in normal and experimental animal groups. In alloxan induced diabetic animals, the body weight was significantly decreased when compared to the levels in normal animals. After administration of the leaf extract, the animals regained the body weight to near normal which is comparable with the control and glibenclamide treated animals.

The level of the insulin in Group Ⅱ alloxan induced diabetic control animal was decreased by 55.09% when compared with Group I animals. In Group Ⅲ, and Ⅳ animals, insulin levels were found to have increased significantly by 72.67% and 80.18%, respectively when compared to the alloxan induced diabetic animals. Similarly a sharp insulin raise was seen in Group Ⅴ and Ⅵ animal by 102.70% and 113.96%, when compared Group Ⅱ alloxan induced diabetic animals. This increase was almost equal to the levels of insulin in glibenclamide administrated Group Ⅶ animals, which was 104.35% when compared to diabetic control.

In alloxan induced Group Ⅱ diabetic animals, the levels of blood glucose were increased significantly by 208.54% compared with normal control group. After administration of methanol leaves extract of field grown *S. xanthocarpum* to Group Ⅲ, and Ⅳ the level of glucose were decreased by 35.45%, and 45.98%, In *in vitro* rasied *S. xanthocarpum* administrated Group Ⅴ, and Ⅵ animals, the levels of glucose were found to have decreased greater extent by 47.13% and 61.40%, where as in glibenclamide treated animals the glucose levels were decreased by 61.81%, compared to diabetic control.

3.4. Effects of field grown S. xanthocarpum and in vitro rasied S. xanthocarpum leaves extract on lipid levels

Table 2 shows the levels of lipids profile such as TC, LDL-C, VLDL-C, HDL-C and TG in each group. Alloxan induction resulted in significant elevation of TC, TG, LDL-C, VLDL-C, and reduction of HDL-C levels as compared to the normal control rats ($P < 0.01$). Field grown *S. xanthocarpum* extract treated groups showed depletion in TC (19.60%, 24.99%), TG (20.30%, 25.98%), LDL-C (28.22%, 38.44%), and VLDL-C (20.34%, 26.02%). Also, there was a significant increase of HDL-C by 6.05% and 19.36% as compared with Group II diabetic rats.

In vitro rasied *S. xanthocarpum* extract treated groups showed fall of TC (24.36%, 29.89%), TG (25.27%, 40.66%), LDL-C (35.44%, 44.77%), and VLDL-C (25.29%, 40.69%), where as decreased HDL-C levels were increased by 12.92%, 34.70%, respectively when compared to the levels in alloxan induced diabetic control Group Ⅱ. As expected in glibenclamide treated Group Ⅶ animals, the increased levels of TC, TG, LDL, VLDL and decreased HDL was reverted to near normal values.

3.5. Effects of field grown S. xanthocarpum and in vitro rasied S. xanthocarpum leaves extract on urea, uric acid and creatinine levels

Table 3 explains the levels of urea, uric acid, and creatinine in normal and experimental animals in each group. In diabetic rats, the all three tested renal markers urea, uric acids and creatinine were significantly increased 95.00%, 29.05% and 141.86% than the normal control Group Ⅰ. Administration of field grown *S. xanthocarpum* leaf extract at dosage of 100 and 200 mg/kg bw, reversed changes to near normal levels, urea (18.00%, 31.00%), uric acid (0.52%, 10.47%), creatinine (8.60%, 18.26%). Similarly administration of *in vitro* rasied *S. xanthocarpum* leaves extract, brought down the levels of urea (28.28%, 40.49%) uric acid (2.61%, 20.90%) creatinine (17.30%, 38.46%) to near normal, in comparison to the diabetic control group. Administration of glibenclamide to Group Ⅶ animals reversed the changes to near normal levels.

3.6. Effects of field grown S. xanthocarpum and in vitro rasied S. xanthocarpum leaves extract on TBARS and hydroperoxides levels

Figure 3 depicts the levels of TBARS and hydroperoxides in kidney homogenates of control and experimental rats. There was significant elevation in TBARS and hydroperoxides by 58.88% and 90.00% in diabetic group when compared to the control group. Administration of field grown *S. xanthocarpum* leaf extract to Group Ⅲ and Group Ⅳ rats leads to significant decrease in the levels of TBARS by 4.85%, 13.46% and hydroperoxides by 9.37%, 25.98%. Similarly administration of *in vitro* rasied *S. xanthocarpum* leaf extract to Group Ⅴ and Group Ⅵ animals leads to a much pronounce decrease in the levels of TBARS by 11.24%, 22.36% and hydroperoxides by 22.69%, 35.69%, than the diabetic control group Ⅱ. Administration of glibenclamide to Group Ⅶ animals decreased the elevated levels to near normal levels.

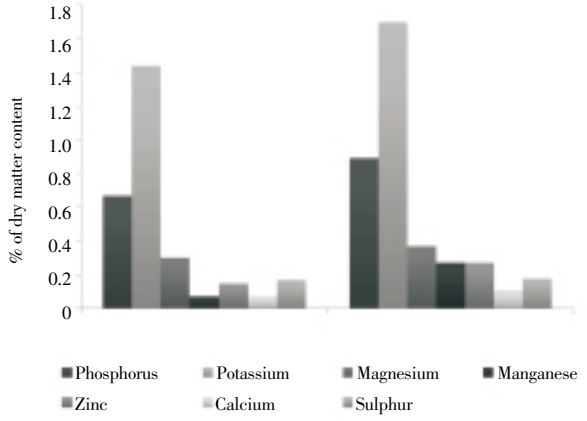

Figure 2. Mineral content of *S. xanthocarpum* leaves.

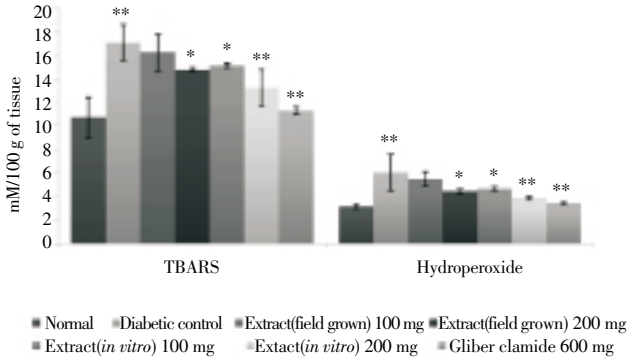

Figure 3. The effect of *S. xanthocarpum* leaves extracton lipid peroxidation and hydroperoxide in kidney.
$^{\triangle\triangle}P<0.001$*vs*. normal control group; **P*<0.01, ***P*<0.001 *vs*. diabetic control group.

3.7. Effects of field grown S. xanthocarpum and in vitro rasied S. xanthocarpum leaves extract on SOD, CAT, GPx in kidney

Table 4 shows the activities of enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). To validate the antioxidant property of the leaf extracts of *S. xanthocarpum*, the activity of SOD, catalase (CAT) and glutathione peroxidase (GPx) of kidney were measured. Reduced levels of antioxidants were observed in diabetic animals. Treatment with field grown *S. xanthocarpum* and *in vitro* rasied *S. xanthocarpum* leaf extract to experimental groups significantly increased the levels when compared with diabetic control rats. Treatment with glibenclamide to Group Ⅶ animals showed a significant change in SOD, CAT, and GPx to near normal values.

4. Discussion

The treatment of diabetes with medicines of plant origin that proved much safer than synthetic drugs is an integral part of many cultures throughout the world and gained importance in recent years. India has a rich history of using various potent herbs and herbal components for treating various diseases including diabetes[29]. The proximal analysis shows *S. xanthocarpum* leaves is a good source of carotenoids, carbohydrates, protein, lipids and minerals. The results indicate that *in vitro* rasied *S. xanthocarpum* leaves contain higher concentration of these constituents than field grown *S. xanthocarpum*. This may be due to the clean and environmental conditions met during the *in vitro* growth.

It is proven that the minerals like potassium, sodium, manganese and iron are playing a vital role in various metabolic activities and responsible for normal growth and function of various organ systems. Potassium is necessary for muscular weakness which is associated with malaria, and also slows down sclerosis of the vascular system. It contributes to fight against bacteria and cleanses the digestive system. Sodium takes part in the metabolism of water, promotes digestion, assimilation, osmosis, cleanses the digestive system, combats stomach acidity and alkalize the blood. Lack of magnesium can be responsible for tetany, tuberculosis, diabetes, cancer and all nervous diseases[30]. Manganese, according to Claude and Paule, 1979 is necessary for the functioning of the pituitary gland, the pineal gland and the brain. It promotes hepato-renal function, combat anaemia and it is also essential for growth. Iron is an energizer but excess can cause fatigue but we hardly have excess if taken from natural source[31].

The result of the mineral composition clearly suggests that *S. xanthocarpum* leaves contain rich source of minerals indicating the usefulness of the leaves to complement the cure of various ailments during treatment with this plant product.

The decrease in body weight with diabetes mellitus has been attributed to the gluconeogenesis *ie*., catabolism of proteins and fats, which is associated with the characteristic loss of body weight due to increased muscle wasting and loss of tissue proteins[32]. Diabetes affects both glucose and lipid metabolism[33]. Administration of extracts to diabetic rats showed a significant decrease in the blood glucose and an increase in the body weight, serum insulin levels.

Table 1

The effect of *S. xanthocarpum* leaves extract on body weight, blood glucose and serum insulin levels of control and experimental group of animals (Mean±SEM, *n*=6).

Groups	Body weight (g)	Blood Glucose(mg %)	Serum insulin (μ U/mL)
I	193.50±6.47	117.00±5.45	14.83±1.13
II	165.83±4.03$^{\triangle\triangle}$	361.00±19.00$^{\triangle\triangle}$	6.66±0.88$^{\triangle\triangle}$
III	169.33±4.78	233.33±21.20	11.50±0.76
IV	184.60±4.73*	195.00±13.35*	12.00±0.28*
V	172.17±4.72**	190.83±12.04*	13.50±1.25**
VI	188.33±5.17**	139.33±6.79**	14.25±0.38***
VII	188.67±5.04**	137.83±6.94**	13.61±0.48**

$^{\triangle\triangle}P$<0.001 *vs*. normal control group; * *P*<0.01, ** *P*<0.001, ***P<0.0001 *vs*. diabetic control group.

Table 2

The effect of *S. xanthocarpum* leaves extract on Lipid profile (mmol/L)(Mean±SEM, *n*=6).

Groups	TC	TG	HDL-C	LDL-C	VLDL-C
I	79.83±3.01	84.16±7.12	30.00±1.52	33.00±0.36	16.83±1.42
II	119.33±2.92$^{\triangle\triangle}$	164.60±4.40$^{\triangle\triangle}$	20.66±1.54$^{\triangle}$	65.73±0.84$^{\triangle}$	32.93±0.88$^{\triangle}$
III	95.83±1.64*	131.17±13.58*	21.91±1.69	47.18±2.62*	26.23±2.71*
IV	89.50±1.25**	121.83±12.37*	24.66±1.35*	40.46±2.59*	24.36±2.47*
V	90.25±1.23*	123.00±12.06*	23.33±1.54	42.20±2.72*	24.60±2.41*
VI	83.66±3.01**	97.66±8.75**	27.83±1.42*	36.30±0.32*	19.53±1.75*
VII	82.33±2.84*	95.00±7.63*	28.16±1.60*	35.16±0.40*	19.00±1.52*

$^{\triangle}P$<0.01,$^{\triangle\triangle}P$<0.001 *vs*. normal control group; * *P*<0.01, ** *P*<0.001 *vs*. diabetic control group.

Table 3

The effect of *S. xanthocarpum* leaves extract on urea, uric acid, creatinine (mg%) (Mean±SEM, *n*=6).

Groups	Urea	Uric acid	Creatinine
I	35.00±1.52	1.48±0.15	0.86±0.10
II	68.33±1.89$^{\triangle}$	1.91±0.11$^{\triangle}$	2.08±0.15$^{\triangle\triangle}$
III	56.00±1.52*	1.90±0.15	1.90±0.15
IV	47.00±1.52*	1.71±0.14*	1.70±0.15*
V	49.00±1.52*	1.86±0.23	1.72±0.16*
VI	40.66±1.76**	1.50±0.45**	1.28±0.15*
VII	36.33±1.50**	1.46±0.14**	1.09±0.09*

$^{\triangle}P$<0.01,$^{\triangle\triangle}P$<0.001 *vs*. normal control group; * *P*<0.01, ** *P*<0.001 *vs*. diabetic control group.

Table 4

The effect of *S. xanthocarpum* leaves extract on SOD, Catalase, GPx (Mean±SEM, *n*=6).

Groups	SOD[a]	CAT[b]	GPx[c]
I	14.50±1.53	35.55±1.54	5.58±0.72
II	8.50±1.53$^{\triangle\triangle}$	19.35±1.49$^{\triangle\triangle}$	2.70±0.15$^{\triangle\triangle}$
III	9.60±1.51	25.00±1.53*	2.85±0.15
IV	10.33±1.50*	27.00±1.53*	3.70±0.65*
V	8.93±1.64*	26.00±1.52*	3.20±0.15*
VI	12.83±1.50**	33.00±1.52**	4.90±0.15**
VII	13.00±1.52**	32.00±1.52**	5.00±0.15**

$^{\triangle\triangle}P$<0.001 *vs*. normal control group; * *P*<0.01, ** *P*<0.001 *vs*. diabetic control group;
[a]: 50% inhibition of epinephrine auto oxidation/min, [b]: μ mol H_2O_2/min/mg protein, [c]: μ mol glutathione/min/mg protein.

Increased levels of serum insulin in diabetic animals treated with plant products is not an uncommon phenomenon as this effect has been reported with some other medicinal plants like *Terminalia chebula* and *Eugenia jambolana*[34]. Hence, the possible mechanism by which *S. xanthocarpum* brings about its hypoglycemic action may be by potentiating the insulin effects of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form. Maximum hypoglycemic effect was observed in *in vitro* rasied *S. xanthocarpum* treated groups.

Diabetes mellitus often involves abnormal lipid metabolism which is metabolic disorder in diabetic complications[35]. Hyperglycemia produced marked increase in serum

triglycerides and total cholesterol[36]; it is well known that the major risk factors of cardiovascular disease are high levels of total cholesterol and LDL cholesterol, whereas increased HDL- cholesterol reduces the risk of cardiovascular disease. Increase in lipids, TG and TC levels in alloxan diabetic rats observed in the present study may be a result of increased breakdown of lipids and mobilization of free fatty acids from the peripheral depots. Regular administration of *S. xanthocarpum* for 25 days normalized lipid profile in diabetic animals. The dose of 200 mg/kg bw of *in vitro* rasied *S. xanthocarpum* not only lowered the TC, TG and LDLC but also enhanced the HDL-cholesterol which is known to play an important role in the transport of cholesterol from peripheral cells to the liver by a pathway termed 'reverse cholesterol transport', and is considered to be a cardio protective lipid. Thus *in vitro* rasied *S. xanthocarpum* has significant impact than field grown *S. xanthocarpum* in improving the imbalance in lipoprotein metabolism.

Diabetes mellitus is one of the major disorders which affect the kidneys; urea, uric acid and creatinine are markers of renal function. In the early phase of diabetic nephropathy, there is a hyper filtration and increase in creatinine clearance resulting in no change in serum creatinine levels but in the later stages the creatinine levels start increasing. Both an increase[37-41] and no change[42] in serum creatinine levels have been reported in diabetic rodents.

In the present study, an increase in urea, uric acid and creatinine levels was observed in diabetic rats and these levels were decreased by oral administration of *S. xanthocarpum* and glibenclamide treated rats when compared to diabetes induced rats. These results are in agreement with other previous studies[43-51] and it can be concluded that field grown *S. xanthocarpum* and *in vitro* rasied *S. xanthocarpum* may protect the protein catabolism in muscle or it ameliorates the renal functions in diabetic rats.

In diabetes, tissue damage is considered to be mediated by free radicals by attacking membranes through peroxidation of unsaturated fatty acids[52]. Lipid peroxidation eventually leads to extensive membrane damage and dysfunction[53]. Decreased lipid peroxidation and improved antioxidant status may be one of the mechanisms by which drug treatment could contribute to the prevention of diabetic complications[54].

The alloxan induced diabetes caused marked alterations in lipid peroxidation and antioxidant levels such as TBARS, hydroperoxide, antioxidant enzymes like SOD, Catalase and GPx in kidney. Administration of plant extract reversed these levels to near normal levels, but higher reduction of enzymes was found in *in vitro* rasied *S. xanthocarpum* treated groups. Thus the antioxidant property of the extract is evident and synergistically it helps in treating diabetes mellitus. The higher potential exhibited by the *in vitro* raised leaves would have been attributed by the quality of growth conditions.

The present investigation clearly concludes that the leaves extracts of *S. xanthocarpum* has a pronounce effect in controlling the hyperglycemic condition and having the ability to combat the complications associated with diabetes mellitus. Even though both the field grown and *in vitro* raised leaves of *S. xanthocarpum* exhibits great magnitude in reverting hyperglycemic condition, the *in vitro* raised leaves extract of *S. xanthocarpum* exhibits remarkable potential to be an ideal candidate to pursue further exploration in developing the choice of an antidiabetic agent.

Conflict of interest statement

We declare that we have no conflict of interest.

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