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IN VITRO AND *IN VIVO* ANTIDIABETIC POTENTIALITIES OF *GARUGA PINNATA* ROXB STEM BARK

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ABSTRACT

Diabetes mellitus [DM], referred to common endocrine metabolic disorder characterized by chronic hyperglycemia (high blood glucose) in the context of insulin secretion by pancreas is inadequate, or might be improper response by body's cells to insulin, or both. The main objective of the present study is to evaluate the antidiabetic potentials of *Garuga pinnata* stem bark. The *in vitro* antidiabetic activities are evaluated by using α-amylase and α-glucosidase enzyme inhibition assays. The results revealed that methanol extract exhibited significant percentage of inhibition 70.9, 66.2 % at 40 and 20 mg/ml and followed by aqueous extract 63.3, 56.9% at 40 and 20 mg/ml. The IC_{50} values of methanol extract for α-amylase and α-glucosidase are 52.5 and 51.6 noticed at 10 mg/ml. *In vivo* antidiabetic efficacy of methanol and aqueous extracts of stem bark was evaluated in streptozotocin-induced diabetic rats. The study revealed that methanol extract was found more significant ($P<0.01$) in reduction of blood glucose levels and also in the increase in the animal body weight.

Key Words:- α-amylase, α-glucosidase, *In vitro*, I*n vivo*, Streptozotocin.

INTRODUCTION

Diabetes mellitus [DM], referred to common endocrine metabolic disorder characterized by chronic hyperglycemia (high blood glucose) in the context of insulin secretion by pancreas is inadequate, or might be improper response by body's cells to insulin, or both. Carbohydrates fail's to metabolize due to absolute deficiency of insulin consequently, attribute to many complications, either acute (short term) or chronic (long term). Diabetic patients experience in polyuria i.e., frequent urination, increasingly thirsty i.e., polydipsia and hungry i.e., polyphagia. Increased oxidative stress, glucose autoxidation and mechanism alteration of polyol pathway are the major risk factors considered for developing of diabetic complications viz., microvascular (diabetic nephropathy, diabetic retinopathy, diabetic neuropathy) and macrovascular (diabetic cardiomyopathy) (Patel *et al*., 2011).

At one set of these complications, attention is being paid towards the discovery of safer hypoglycemic agents from medicinal plants has been increased (Sharma and Arora 2006; Morton 1988; Subbalakshimi and Naik, 2001). Contemporary oral anti-diabetic compounds viz., Sulfonylureas, Thiazolidinediones, α-Glucosidase inhibitors etc., are commonly used as single or in the combination for the better glycemic control. However, each of these oral antidiabetic agents are associated with a numerous of hazardous effects (Oze *et al*., 2008; Zhang *et al*., 2005 Kim *et al*., 2006). Hence, to minimize these adverse effects, antidiabetic drug discovery has been

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shifted its focus to plant resources to generate efficient and minimal side effect compounds. These discoveries help for many rural population specifically in the case of poor availability of current day drugs and as well as providing these at low cost in developing countries especially India. Last 25 years, there is a strategic increase in the usage of herbs as an alternative medicine. Under the consideration of the data released by World Health Organisation (WHO), 65-80% of the world population relied on traditional medicines for their primary health care. It is estimated that diabetes mellitus affects approximately 4% of global population and is expected to rise 5.4% in 2025 (Kim *et al*., 2006).

Garuga pinnata belongs to family Burseraceae commonly known as Golika is the deciduous tree grows up to 50 feet or 15 meter in height, with a special characteristic of bark peeling off in flakes. G. pinnata plant composites have been widely used for the traditional treatment of DM (Chopra, *et al*., 1958). G. pinnata exhibits several medicinal properties and is availed in the treatment of chronic diseases, stomach problems, asthma, regular eye-drops and to promote healing of bone fractures etc (Manzur-ul-Kadir, *et al*., 2009).

Kathad *et al*., (2010) reported antioxidant activities of *Garuga pinnata* leaves ethanol extract showed significant inhibition percentage of DPPH, hydroxyl radical, nitric oxide and super oxide anion. Annie Shirwaikar *et al*., 2007 also reported significant antidiabetic potentials of stem bark aqueous extract in streptozotocin-nicotinamide induced diabetic rats. Prapai Wongsikongman *et al*., (2002) reported that the methanolic crude extract of *Garuga pinnata* Roxb possessed promising cytotoxic activity against human tumor drug-resistant sublines. Diarylheptanoids garuganins I–III of *Garuga pinnata* exhibit structural similarities with that of rifamycin SV, a typical ansamycin antibiotic, regarding steric disposition, polarity of essential functional groups and similar functionality of the aliphatic chains suggest the mechanisms of antibacterial action (Gyorgy Mikos Keseru and Mihaly Nogradi 1993; Krishnaswamy BYS, Pattabhi, & Gabe, 1986).

With the context to the above mentioned biomedical applications of *Garuga pinnata*, a systemic study was conducted for the evaluation of *in vitro* and *in vivo* antidiabetic activities of stem bark of this plant.

MATERIAL AND METHODS

Plant material

Stem bark of *G. pinnata* was collected from Rampet village, Warangal district, Andhra Pradesh, India. It has been authenticated by Professor V.S Raju, Department of Botany, Kakatiya University.

Extraction procedure

The stem bark was chopped in to smaller fragments, shade dried and grinded in homogenizer in to coarse powder. The 100 grams of powdered material is extracted with methanol, ethyl acetate, chloroform, acetone, petroleum ether and aqueous which were concentrated under rotavapour at their boiling points.

Chemicals

Streptozotocin -Hi-media- CMS1 7585, alpha amylase-SRL- 28588, alpha glucosidase-SRL-75551, para nitrophenyl beta glucopyranoside-pNPG-Sigma-1377. Whereas, all other chemicals purchased were analytical grade.

In vitro enzyme inhibitory effects of G. *pinnata* extracts.

Alpha-amylase inhibitory activity

Alpha amylase (EC 3.2.1.1) inhibitory activities of crude *Garuga pinnata* extracts was determined according to the method described by Kim et al., (2005) with slight modification. Briefly 0.25 µl of urinary alpha amylase (5 U/ml) was pre-incubated with 0.20 µl of 20 and 40 mg/ml G. *pinnata* various extracts (methanol, ethyl acetate, chloroform, acetone petroleum ether and aqueous) for 15 min at 37^{o}C water bath. The reaction was started by addition of 0.2 µl of 0.5% potato starch dissolved in 20mM phosphate buffer, pH 6.9. The reaction mixture was then incubated at 37^{o}C for 20 minutes and terminated by addition of 2.0 ml of DNS reagent (1% 3,5-dinitrosalicylic acid, 12% sodium potassium tartrate in 0.4 M NaOH). The reaction mixture was heated for 15 min at 100^{o}C. Amylase activity was determined by measuring the absorbance at 540 nm and expressed as percentage of the blank control (without the extract).

Alpha glucosidase inhibitory activity

The effect of the plant extracts on alpha glucosidase activity was determined according to the chromogenic method described by Kim et al., (2005). The substrate solution p-nitrophynyl glucopyranoside (pNPG) was prepared in 20 mM phosphate buffer, pH 6.9. Five units of alpha glucosidase (E.C. 3.2.1.20) were pre-incubated with 20 and 40 mg/ml of the different G. *pinnata* extracts (methanol, ethyl acetate, chloroform, acetone petroleum ether and aqueous) for 15 minutes. Three millimolar (pNPG) as a substrate dissolved in 20mM phosphate buffer, pH 6.9 was then added to start the reaction. The reaction mixture was incubated at 37^{o}C for 20 minutes and stopped by adding 2 ml of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the yellow colored p-nitrophenol released from

pNPG at 400 nm. The results were expressed as percentage of the blank control.

In vivo antidiabetic activities of G. *pinnata* extracts

Animals

Albino rats of Wistar strain, of male sex, weighing 150 – 250 g were purchased from National Institute of Nutrition, Hyderabad, India and housed under standard environmental conditions (temperature: 24 ± 1^{o} C, light / dark cycle: 10/14 h). The rats were fed with standard pellet diet (Amrut laboratory animal feed, Maharashtra, India) and water *ad libitum*. Animals were acclimatized to laboratory conditions at least 1 week before conducting the experiments according to the guide lines of CPCSEA –New Delhi, (Registration No. - 915/ac/05/CPCSEA).

Experimental Design

All animals were grouped into the five groups;

Group I: Consisted of 6 rats which served as normal control and were given only distilled water daily for 28 days.

Group II: Consisted of 6 STZ induced diabetic rats and served as diabetic control and were given distilled water only for 28 days.

Group III: Consisted of 6 STZ induced diabetic rats and were treated orally with glibenclamide, used as a reference drug and was administered orally at 4 mg/kg as a suspension in 1% w/v CMC for 28 days.

Group IV: Consisted of 6 STZ induced diabetic rats and were treated orally with methanol extract of *G. pinnata* stem bark at the dose of 2000 mg/kg body weight daily for 28 days, once a day.

Group V: Consisted of 6 STZ induced diabetic rats and were treated orally with aqueous extract of *G. pinnata* stem bark at the dose of 2000 mg/kg body weight daily for 28 days, once a day.

Induction of diabetes

Streptozotocin was freshly dissolved in 0.1 M citrate buffer (pH = 4.5) at the dose of 50 mg/kg body weight and injected intraperitoneallly within 15 min of dissolution in a vehicle volume of 0.3 mL with 1 mL of tuberculin syringe fitted with 24 gauge needle, whereas normal control group was given citrate buffer only (0.3 mL). Diabetes was confirmed by the determination of fasting glucose concentration on the third day post administration of streptozotocin.

Body weight

Each animal was weighed daily using mouse balance (Adventurer SL, Switzerland), for the period of four weeks which the experiment lasted.

Acute toxicity studies

Wistar rats (n=6) of either sex selected by random sampling techniques were employed in this study. The animal were kept fasting for overnight providing only water. Then the methanol and aqueous stem bark extracts were administered orally at the dose of 2000 mg/kg by intragastric tube and observed for 2 days for the gross behavioral changes and mortality.

Induced diabetic rats were kept for 24 hours (post administration of streptozotocin) on 10% glucose solution bottles in their cages. After seventy-two hours of injection, fasting blood glucose levels was measured by glucometer. The treatment was continued for 28 days. At the end of every week, rats were fasted for 16 hours and the blood biochemical parameters were determined as follows; blood was collected from retro-orbital plexus and plasma was separated by centrifugation. Animal housing, care and application of experimental procedures were in accordance with institutional animal ethic guidelines.

STATISTICAL ANALYSIS

Data obtained from pharmacological experiments are expressed as mean ±SD (Difference between the treatments in this experiment was tested for significance using Paired *t*-test). P value < 0.05 considered as significant.

RESULTS

Inhibitory activities of *Garuga pinnata* stem bark extracts on α-amylase and α-glucosidase

Among the various organic extracts of *Garuga pinnata* stem bark tested, methanol extract exhibited significant inhibition percentage of α-amylase and α-glucosidase enzyme activities compared with that from other extracts Figure 1 & 2. The maximum inhibition percentage of α-amylase and α-glucosidase activities was noticed at a concentration of 40 mg/ml are 70.9, 66.2 % respectively. Next to the methanol extract aqueous extract showed good inhibition activity with 63.3, 56.9% noticed for α-amylase and α-glucosidase respectively. Whereas, all other extracts were also showed moderated inhibition activities at all tested concentrations. The inhibition percentage recorded for methanol and aqueous extracts are significantly comparable with reference standard acarbose 82.0% at 10 mg/ml. The IC_{50} values of methanol extract for α-amylase and α-glucosidase are 52.5 and 51.6 noticed at 10 mg/ml.

Measurement of body weight of animals

At the end of the experiment (period of 28 days) animals of all groups expect group-II (diabetic control) showed considerable weight gain (Table 1). Group IV (Methanol extract at 2000 mg/kg) animals gained their body weight significantly of about 58.9%. Group V (Aqueous extract 2000 mg/kg) animals notably gained their body weight by 31.7%. Whereas, animals of group III (Treated with 4 mg/kg glibenclamide) increasingly gained their body weight by 79.3% and animals of group II lost their body weight by 77.1%. Animals of group I (control) had normally gained their body weight by 34.8%.

Determination of blood Glucose levels

As shown in Table 2, at the first week of treatment, the fasting blood glucose increased immediately after induction of diabetes with streptozotocin in groups IV significantly higher (P<0.001). Animals in group V that were treated with aqueous extract showed normal blood glucose level that is comparable to those of the animals in group IV. As from the 7th day of treatment, drastic reduction of blood glucose levels (P<0.01) were noticed from the diabetic animals. Animals of group III treated with 4 mg/kg glibenclamide significantly (P<0.01) increased their blood glucose levels comparing with that from animals of group IV and V treated with 2000 mg/kg methanol and aqueous extracts respectively.

Table 1. Body weight measurement (SEM± SD) of streptozotocin-induced Wistar rats during four weeks of treatment with *Garuga pinnata* stem bark methanol and aqueous crude extract

Days in number	Day 7	Day14	Day21	Day28
Normal	1.9±0.8*	2.0±0.8*	1.6±0.6*	1.8±.7*
Diabetic control	3.7±1.5*	3.5±1.4*	1.8±0.7*	4.7±1.9*
Diabetic standard	1.6±0.6*	1.8±0.7*	1.6±0.6*	1.5±0.6*
Aqueous extract 2000mg/kg	2.7±1.3*	2.0±0.8*	3.1±1.2*	2.9±1.2*
Methanol extract 2000mg/kg	2.4±1.0*	3.3±1.3*	2.3±0.9*	2.8±1.1*

*p < 0.001of methanol extract compared with that from weight of normal animals and diabetic control.

Table 2. Fasting blood glucose (SEM ± SD) of streptozotocin-induced diabetes in Wistar rats during four weeks of treatment with Garuga pinnata stem bark methanol and aqueous crude extract

Days in number	Day 7	Day14	Day21	Day28
Normal	11±5.8	10±5.3	9.5±4.2	8.7±3.9
Diabetic control	4.4±2.2	4.0±2.0	4.2±2.1	5.0±2.5
Diabetic standard	3.8±1.7	5.1±2.5	4.9±2.4	3.3±1.6
Aqueous extract 2000mg/kg	3.4±1.4	3.7±1.8	7.6±3.8	4.2±2.1
Methanol extract 2000mg/kg	5.3±2.6*	2.5±1.2*	3.6±1.8*	1.7±0.8*

*p < 0.001of methanol extract compared with that from glucose levels of normal animals and diabetic control.

Fig 1. The inhibitory effects of *Garuga pinnata* stem bark various extracts *on* human urinary α-Amylase compared with that from standard acarbose

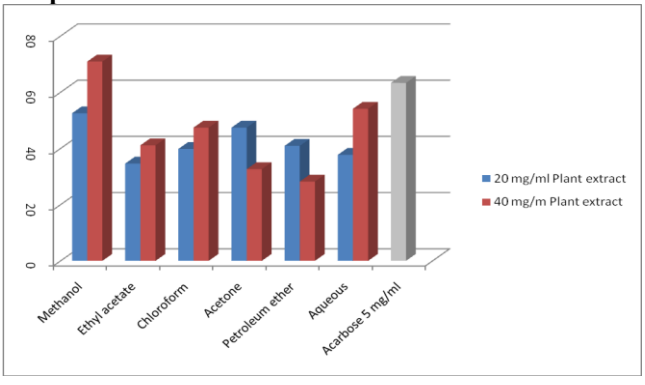
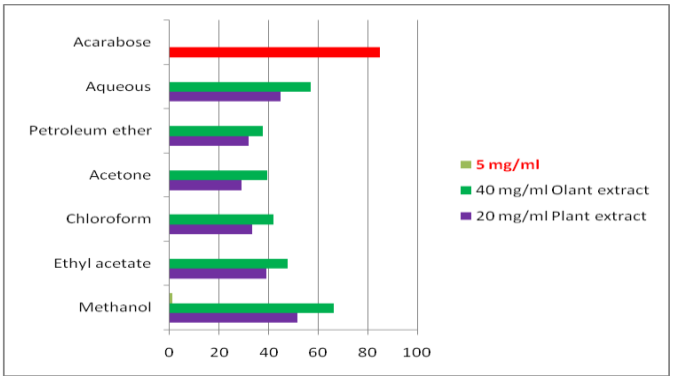


Fig 2. The inhibitory effects of *Garuga pinnata* stem bark various extracts *on* α-glucosidase compared with that from standard acarbose



DISCUSSION

The use of *Garuga pinnata* stem bark, in this study has been demonstrated its *in vitro* and *in vivo* antidiabetic properties, significantly lowering blood glucose in the streptozotocin-induced diabetes to values that are comparable to those of the normal consistently for a period of 4 weeks. *In vitro* antidiabetic effects of *Garuga pinnata* stem bark revealed that methanol and aqueous extracts reported pronounced enzyme inhibitory activities compared with that from other extracts. By these enzyme inhibition assays, it has been understood that, inhibition by stem bark was not specific for both enzymes and share common reaction mechanism (Inohara-Ochiai *et al*., 1977). We strongly believe reason for non-specific manner of inhibition is because of structural similarities of both enzymes (Inohara-Ochiai *et al*., 1977). The inhibition mechanism of these extracts may differ from that of standard acarbose, which competitively inhibits pancreatic α-amylase and intestinal α-glucosidase hydrolase enzymes which delay or inhibit the mechanism of digestion (final stage of carbohydrate mechanism conversion of disaccharide to monosaccharide i.e., glucose) and absorption of carbohydrates in the small intestine. However, this antidiabetic agent has been a problematic because of its associated with gastro intestinal adverse effects, leading to accumulation of undigested starch in the large intestine (Madar 1989).

Synthetic drugs attributed with hazardous effects are replaced with naturally derived drugs. Therefore, an increased focused is maintained on medicinal plants for isolation of naturacetuicals which are safe and don't possess any sort of side effects. Till the date, we have not found any scientific studies of *Garuga pinnata* extracts on the inhibition of digestive enzymes viz., pancreatic *α*-amylase and intestinal α-glucosidase. Literature survey also revealed that there are reports on *in vivo* antidabetic activities of methanol stem bark extract. Thus, these documentary reports would become first report on *in vitro* and *in vivo* antidiabetic activities of this plant. However, Annie Shirwaikar (2007) of studied *in vivo* antioxidant and Anti-hyperglycemic activities of *Garuga pinnata* stem bark aqueous extract in streptozoticin induced rats and proved that aqueous extract of stem bark showed moderate activity on reduction of blood glucose levels.

In the present study we have been hypothesized the antidiabetic efficacy of *Garuga pinnata* stem bark methanol and aqueous extract in streptozotocin induced diabetic rats to establish comparative studies. Methanol and aqueous extracts were proved significant in enzyme inhibitions *in vitro*. Thus, only these two extracts were taken in to consideration to evaluate *in vivo* antidiabetic potentials. Our findings, directly indicate that stem bark methanol extract possess antidiabeitc compounds which resulted in high blood glucose level reduction compared with that from aqueous extract. Initially, glucose levels of animals were found to be increased till the 7^{th} day after streptozotocin administration and thereafter, from 12^{th} day, we noticed a drastic decrease in blood glucose levels of group IV animals treated with methanol extract at 2000 mg/kg body weight. On the other hand animals of group V treated with aqueous stem bark extract at 2000 mg/kg body weight resulted, very lower decrease in blood glucose levels 1.7±0.8 and 4.2±2.1 ($P<0.01$). Reduction in the glucose levels after 7 day onwards may be because of regeneration of β-cells of the pancreas, which were destroyed by streptozotocin.

CONCLUSION

Based on the results documented in present study, it can be concluded that *Garuga pinnata* stem bark methanol and aqueous extracts exhibited *in vitro* inhibitory effect on α-amylase and α-glucosidase. It also exhibited *in vivo* antidiabetic activities.

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