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Research Article

ANTIDIABETIC AND ANTIHYPERLIPIDEMIC ACTIVITY OF *LUFFA ACUTANGULA* FRUIT EXTRACTS IN STREPTOZOTOCIN INDUCED NIDDM RATS

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ABSTRACT

In recent years, herbal remedies have evolved with great potential of cure. *Luffa acutangula* is a fruit belonging to family Cucurbitaceae. Many members of this family have been successfully screened for their antidiabetic potential.

Objectives: The present study aimed at investigation of antioxidant, antidiabetic and antihyperlipidemic potential of methanolic and aqueous extracts of *Luffa acutangula* (LA) fruits.

Material and Methods: The extracts were screened initially for *in vitro* antioxidant activity, total phenolic content and α- glucosidase inhibitory activity. Streptozotocin, STZ (65 mg/Kg, *i.p.*) along with nicotinamide (120 mg/kg, *i.p.*) was used to induce non insulin dependent diabetes mellitus (NIDDM) in rats. The effect of different extracts of LA (100, 200 and 400 mg/kg, *p.o.*) in the management of diabetes and related complications were determined. Various biochemical markers of blood and tissue origin were estimated. The observations were statistically analyzed by ANOVA followed by Dunnett's test.

Results: Our study revealed that the methanolic extract at a dose of 100 mg/kg was found to be active ($p < 0.05$) but the antidiabetic activity was increased significantly ($p<0.01$) at a dose of 200 and 400 mg/kg as compared to the aqueous extract (LAW). Also, the methanolic extract had dose dependent pronounced ($p<0.01$) antihyperlipidemic activity over the aqueous extract. **Conclusion:** From this study it was concluded that the LAM and LAW extracts had ant diabetic and antihyperlipidemic activity. The LAM extract was superior to LAW extract in management of diabetes and its associated lipid imbalance.

Keywords: Antioxidant, diabetes, glibenclamide, ridge gourd, STZ.

INTRODUCTION

Since, the main concern of the general public and science is in finding new natural and therapeutically active agents; scientists all over the globe have started screening plants for searching new phytochemicals[1]. Global diabetic population is increasing and hence the search for new antidiabetic drugs is constantly on. Various different culinary herbs have been screened for their biological activities in management of chronic diseases such as diabetes and hypercholesterolemia[2-4].

Luffa acutnagula Linn. var. *amara* Roxberg of Cucurbitaceae family is a perennial climber native to southern and western India. It is commonly known as ridge gourd, sponge gourd or angled luffa, Karviturai in *hindi* and dodake in *marathi*[5] .The plant possesses various medicinal properties such as treatment of jaundice, splenic enlargement and laxative. The plant is also reported to have potent α-glucosidase inhibitory effect. α-glucosidase is an enzyme that is responsible for breakdown of carbohydrates in intestine[6] .

Reactive oxygen species (ROS), including superoxide anion radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), are physiological metabolites. Small amounts of ROS are continuously formed during life as a result of the metabolism of oxygen.[7] Much evidence indicates that exposure to ROS leads to deleterious changes of cell function by a number of alterations, such as lipid peroxidation[8-10] .

Many other plants belonging to Cucurbitaceae family have been successfully screened for antidiabetic study. Cucurbitacin is an important class of compound responsible for the antidiabetic activity of these plants. Available literature revealed that *Luffa acutangula* can be a substantial candidate for antidiabetic study. The aim of present study was to compare the potential of various LA extracts as an antioxidant, antidiabetic and antihyperlipidemic agent.

The different extracts screened for the study was petroleum ether extract (LAPE), methanolic extract (LAM) and aqueous extract (LAW) which, were compared against Glibenclamide. Streptozotocin (STZ) was used to induce diabetes. STZ-induced diabetic rats have a severe oxidative stress condition and they respond to a moderate dose of orally administered antioxidants[11].

MATERIALS AND METHODS

Plant Material

Luffa acutangula fruits were obtained from the local markets of Pune and were authenticated by Botanical Survey of India, Western Circle, Pune with voucher specimen no. LABHP-1.

Preparation of Extracts

Petroleum ether extract (LAPE), methanolic extract (LAM) and aqueous extracts (LAW): The fresh *Luffa acutangula* fruits were grated, dried in shade and coarsely powdered. The powder was successively extracted with petroleum ether followed by methanol in a Soxhlet apparatus[12]. The aqueous extract was prepared by maceration with distilled water for 24 hrs. The extracts were concentrated under reduced pressure and were stored at 8-10 ^{0}C throughout the study. The yield of LAPE, LAM and LAW were 0.97 % w/w, 2.31 % w/w and 7.18 % w/w respectively.

Chemicals and reagents

Gallic acid (GA), L-ascorbic acid; nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), the stable free radical 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), yeast α-glucosidase, 4-nitrophenyl α-D-glucopyranoside (4-NPGP), Streptozotocin (STZ) were obtained from Sigma-Aldrich, USA. Whereas; acarbose, analytical grade petroleum ether (b.p. 40 ^{0}C - 60 ^{0}C), methanol and all other chemicals were obtained from Merck, USA.

Determination of total phenolic content[1]

The total amount of phenolics in the extracts was determined by following Folin–Ciocalteu's reagent method. All the extracts were mixed with 1 ml of Folin–Ciocalteu's reagent and 0.8 ml of (7.5 % w/v) aqueous sodium carbonate solution. The tubes were mixed and allowed to stand for 30 min at room temperature. Concentration of phenolics was determined spectrophotometrically at 765 nm using gallic acid as standard.

Scavenging activity of (DPPH) radical[13-16]

DPPH (1, 1-diphenyl-2-picrylhydrazyl) is a free radical, when dissolved in ethanol it yields a purple colour. The loss of colour

indicates radical scavenging activity and the objective of this assay is to determine the loss of colour, in terms of absorbance, by using plant extracts. About 3 ml, 60 M ethanolic DPPH solution was added to 1 ml of each extracts separately. The test tubes were then incubated at room temperature for 15 min. Absorbance was read at 517 nm using L-ascorbic acid as standard.

The antioxidant activity was calculated as % inhibition of DPPH radical formation:

$$\text{Inhibition (\%)} = \{(A_{(control)} - A_{(extract)}) / A_{(control)}\} X\ 100$$

Where, $A_{(control)}$ and $A_{(extract)}$ were the absorbance of control (L-ascorbic acid) and extract respectively at 517 nm.

Superoxide Anion Radicals Scavenging Activity[14, 16, 17]

Superoxide radicals are generated in phenazine methosulphate (PMS) – NADH systems, by oxidation of NADH and are assayed by the reduction of nitroblue tetrazolium (NBT). In this study, the Superoxide radicals were generated in 3 ml of Tris-HCl buffer (16 mM, pH 8.0) containing 1 ml of NBT (50 µM), 1 ml NADH (78 µM) and various concentration of each extracts (20, 40 and 60 µg). The reaction was started by adding 1 ml of PMS solution (10 µM) to the mixture. The reaction mixture was incubated at 25° C for 5 min; the absorbance was read at 560 nm using a spectrophotometer (Jasco V600 UV-vis spectrophotometer) against blank samples using l-ascorbic acid as a control. Decreased absorbance of the reaction mixture indicated increasing superoxide anion scavenging activity.

The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\text{\% inhibition} = [(A_0 - A_1)/A_0] \times 100$$

Where, A_0 was the absorbance of the control (L- ascorbic acid), and A_1 was the absorbance of extracts.

In vitro α-Glucosidase inhibitory activity[6]

The *in vitro* α-glucosidase inhibitory activity was determined according to Matsui et al. (1996), by measuring the release of 4-nitrophenol from 4-nitrophenyl α-D-glucopyranoside (4-NPGP). The assay media contained 0.1 M sodium phosphate buffer, pH 6.8, 2mM 4-NPGP, 0.1U α-glucosidase (from yeast) and plant extract or control drug in the range of 0.2–2000 µg/ml assay media, in a total volume of 1 ml.The assay was started by addition of 4-NPGP and the change in absorbance at 405 nm was followed with a Jasco V600 UV-vis spectrophotometer.

Animals

Albino mice (20-22 g) and rats (200 ± 20 g), of either sex were obtained from the Yash farms, Pune. The animals were kept in polyethylene cages in the departmental animal house at 26 ± 2 ^{0}C and relative humidity 40-55 % with 12 hrs light and dark cycles. The animals were fed with standard pellet chow diet (Hind liver) and were allowed free access to water. All the experimental protocols for animal care procedures were approved by the Institutional Animal Ethical Committee (IAEC) with a protocol number MCP/IAEC/02/2009.

Acute toxicity studies[16]

Swiss albino mice weighing 20-25 g were taken for the study. The animals were divided into three groups. The animals were fasted overnight but were allowed free access to water prior to the day of study. First group served as control and received 1 % carboxymethyl cellulose (CMC) in distilled water. While the other two groups received 2 g/kg *p.o*. dose of LAM and LAW respectively. The extracts were suspended in 1 % carboxymethyl cellulose (CMC) solution in distilled water as they were not easily soluble in water.

The animals were observed for 5 min every 30 min till 2 hrs and then at 4, 8 and 24 hrs to detect any change in the autonomic or behavioral response and also for tremors, convulsion, salivation, diarrhea, lethargy, sleep and coma and then were further observed daily for 14 days for mortality.

Experimental induction of NIDDM in rats

NIDDM was induced by injecting a single *i.p.* dose of nicotinamide (120 mg/kg) followed by *i.p* administration of STZ (65 mg/kg) in 0.1 M citrate buffer (pH 4.5). [18-19] The fasting blood glucose level was estimated after 72 hrs from STZ administration. Rats exhibiting blood glucose concentration more than 145 mg/dl were considered diabetic and were included in the study.

Experimental design

A total of 54 rats (6 normal + 48 diabetic) were used. The animals were divided into nine groups each containing six. Group 1 was normal untreated rats and received 1 % carboxymethyl cellulose (CMC) solution whereas; group 2 represented STZ-Control diabetic rats which were untreated. Group 3 contained diabetic rats treated with glibenclamide orally (0.25 mg/kg). Group 4 (LAM 100), 5 (LAM 200), and 6 (LAM 400) were diabetic rats treated with oral dose of 100 mg, 200 mg and 400 mg/kg respectively of *Luffa acutangula* methanolic extract (LAM). Group 7 (LAW 100), 8 (LAW 200) and 9 (LAW 400) served as groups of diabetic rats, which were treated with oral dose of 100 mg, 200 mg and 400 mg/kg respectively of *Luffa acutangula* aqueous extract (LAW).

Estimation of Biochemical parameters

Fasting blood glucose levels[20]

The blood glucose levels were estimated on 0th, 7th, 14th and 21st day after induction of NIDDM. The blood was collected from the tip of the tail using glucostix (One touch Ultra, Johnson and Johnson).

Serum insulin and glycosylated hemoglobin (HbA1C)[21-22]

Both serum insulin and glycosylated hemoglobin were estimated on 21st day after induction of NIDDM. Serum insulin and HbA1C were measured according to the method described by Prabhu et al. (2008) and Shirwaikar et al. (2005) respectively.

Serum lipid profile[20]

The lipid profile was done on 21st day after induction of NIDDM. The serum lipid parameters such as total cholesterol, triglycerides, high density lipoproteins, low density lipoproteins and very low density lipoproteins were estimated using commercial kits (Span diagnostics, Mumbai).

Liver glycogen, aspartate transaminase (AST) and alanine tranaminase (ALT)[23]

The tissue enzyme estimations were made on 21st day after induction of NIDDM. Various enzymes such as aspartate transaminase (AST) and alanine tranaminase (ALT); and liver glycogen content were estimated according to Maiti et al. (2004).

Body and liver weight

The rats were weighed on 21st day after induction of NIDDM to determine any gain or loss in weight. The animals were sacrificed on same day and liver weight was recorded.

Oral glucose tolerance test (OGTT)

Oral glucose tolerance test was performed in overnight fasted rats. Glucose (2 g/kg p.o.) was fed 30 min before administration of the extract. Blood was withdrawn from the tail tip at 0, 30, 60, 90 and 120 min after administration of the extracts.

Statistical Analysis

The above estimations were analyzed statistically by applying One-way analysis of variance (ANOVA) followed by Dunnet's test for multiple comparisons. The differences were considered significant when $P < 0.05$.

RESULTS

Phytochemical analysis

After performing the preliminary phytochemical analysis the presence of fixed oils and sterols were found in petroleum ether extract whereas; carbohydrates, saponins, amino acids, phenols and flavonoids were present in methanolic and aqueous extracts. Table 1 illustrates the phytoconstituents of all extracts of LA.

Table 1: Phytochemical analysis of various extracts of *Luffa acutangula*

Tests	Observations		
	LAPE	LAM	LAW
Carbohydrates	-	+	+
Proteins	-	-	+
Amino acids	-	+	+
Fixed oils	+	-	-
Steroids	+	-	-
Volatile oils	-	-	-
Saponin glycosides	-	+	+
Coumarin glycosides	-	-	-
Flavonoids	-	+	+
Alkaloids	-	-	-
Phenols	-	+	+
Vitamin C	-	-	+

LAPE = *L. acutangula* petroleum ether extract, LAM = *L. acutangula* MeOH extract and LAW = *L. acutangula* aqueous extract. (+) = present and (-) = absent.

Total phenolic content

As can be observed in Fig. 1, the total phenolic content of methanolic extract of *Luffa acutangula* was found to be more as compared to petroleum ether extract and aqueous extracts when compared against gallic acid, a standard.

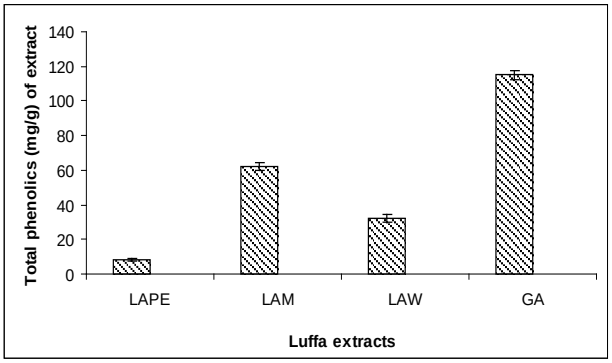

Fig 1: Total phenolic content (mg/g) of extract

LAPE = *L. acutangula* petroleum ether extract, LAM = *L. acutangula* MeOH extract and LAW = *L. acutangula* aqueous extract. Values are expressed as Mean ± SD, (n = 3).

DPPH free radical scavenging activity

Fig 2 reports the FRSA's in the DPPH assay of petroleum ether, methanol and aqueous extracts. Methanolic and aqueous extracts showed a FRSA activity which was comparable to that recorded for the reference (l-ascorbic acid)

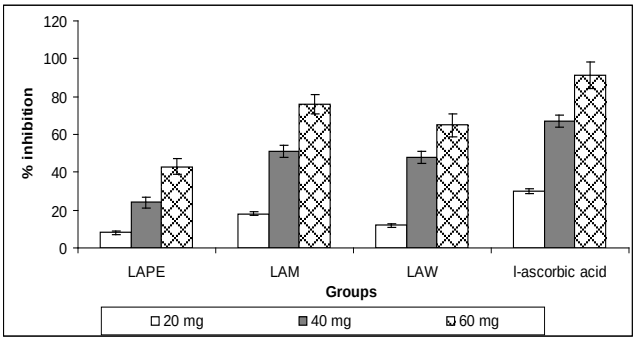

Fig 2: DPPH free radical scavenging assay of various LA extracts.

LAPE = *L. acutangula* petroleum ether extract, LAM = *L. acutangula* MeOH extract and LAW = *L. acutangula* aqueous extract. Values are expressed as Mean ± SEM, (n = 3)

Superoxide anion radical scavenging activity

All the extracts were screened for SOD radical scavenging activity (Fig. 3). In this assay both methanolica nd aqueous extracts showed significant dose dependant antioxidant activity similar to l-ascorbic acid.

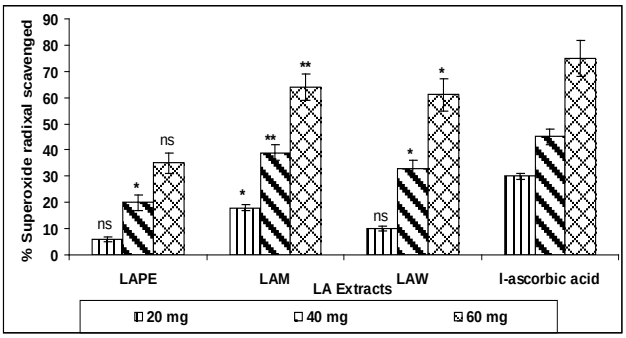

Fig 3: Percentage Superoxide anion radical scavenging activity of various LA extracts.

LAPE = *L. acutangula* petroleum ether extract, LAM = *L. acutangula* MeOH extract and LAW = *L. acutangula* aqueous extract. ANOVA followed by Newmans Kuel test. ** = p < 0.01, * = p < 0.05, ns = not significant (n = 3)

Alpha-glucosidase inhibitory activity

As seen in Fig. 4, alpha-glucosidase enzyme was inhibited prominently by the methanolic extract and aqueous extract of LA as compared to the petroleum ether exytract. Acarbose was used as a reference standard for this activity.

Acute toxicity study

Even after acute oral administration of various extracts at a dose of 2 g/kg *p.o.*, indicated no mortality or any change in the autonomic or behavioral responses upto 14 days. Neither of the animals died nor exhibited any unusual symptoms.

Effect of LA extracts on fasting blood glucose level

The data presented in Fig. 6 clearly indicated that the fasting blood glucose level is significantly lowered by the methanolic extract after completion of 21 days. A dose dependent antidiabetic activity was exhibited by the methanolic extract. The methanolic extract at a dose of 100 mg/kg was found to be active ($p<0.05$) but the activity was increased significantly ($p<0.01$) at a dose of 200 and 400 mg/kg. Aqueous extract at a dose of 100 mg/kg did not proved to be

effective in decreasing the FBG, but significantly (p<0.01) decreased the FBG level at 200 and 400 mg/kg.

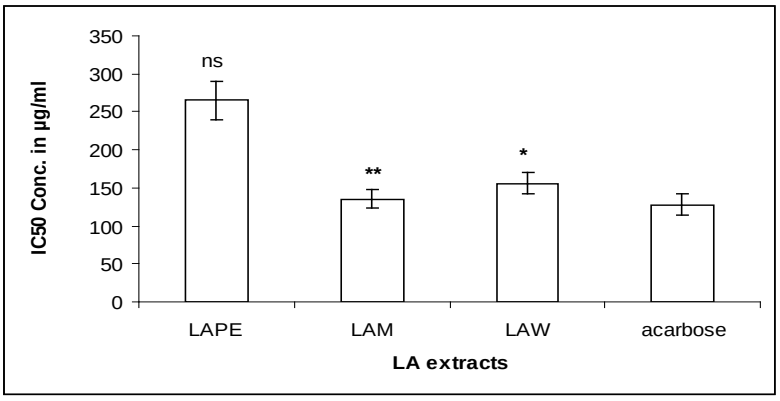

Fig 4: Alpha-glucosidase inhibitory concentration (IC_{50}) of various LA extracts and acarbose over alpha-glucosidase activity.

LAPE = *L. acutangula* petroleum ether extract, LAM = *L. acutangula* MeOH extract and LAW = *L. acutangula* aqueous extract. Values are expressed as mean ± SEM. ** = p < 0.01, * = p < 0.05, ns = not significant (n = 3)

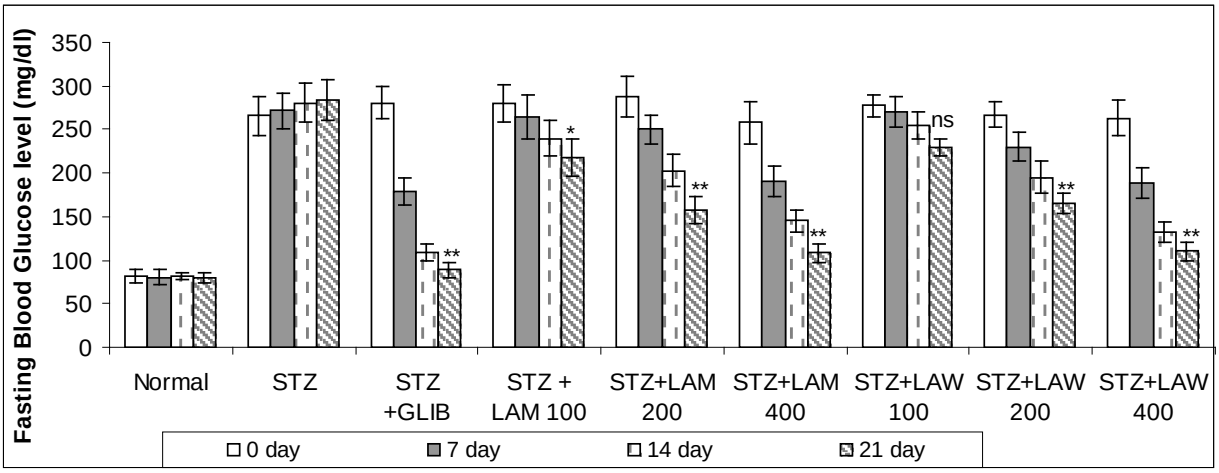

Fig 5: Effect of graded doses of *Luffa acutangula* extracts on fasting blood glucose level in normal and NIDDM rats.

LAM 100, LAM 200 and LAM 400 are oral doses of *L. acutangula* MeOH extract in 100, 200 and 400 mg/kg respectively whereas; LAW 100, LAW 200 and LAW 400 are oral doses of *L. acutangula* aqueous extract in 100, 200 and 400 mg/kg respectively. Values are expressed as mean ± SEM. One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test was applied both in normal and NIDDM rats groups. The difference were considered to be significant when * P < 0.05 compared to respective normal and NIDDM-control groups. (n = 6)

Effect of LA extracts on serum insulin and glycosylated hemoglobin (HbA1c) level

The group receiving a dose of 100 mg/kg of methanolic extract did increase the serum insulin level significantly (p<0.05) but this dose was ineffective in reducing HbA1C. Reverse was seen with the group receiving a dose of 100 mg/kg of aqueous extract. Both the aqueous and methanolic extract at doses of 200 and 400 mg/kg were significant (p<0.01) when compared against the standard. Table 2, summarizes the effect of LA extract on serum insulin and HbA1C.

Table 2: Effect of *Luffa acutangula* extracts on Serum Insulin and glycosylated hemoglobin (HbA1c) level in normal and NIDDM rats.

Groups	Serum Insulin (µU/ml)	HbA1C (mg/dl)
Normal	15.21 ± 0.53 **	0.51 ± 0.03 **
STZ Control	05.03 ± 0.30	0.78 ± 0.03
STZ + Glibenclamide	13.12 ± 0.90 **	0.59 ± 0.04 **
STZ+LAM 100	05.96 ± 0.29 *	0.75 ± 0.05 ns
STZ+LAM 200	08.26 ± 0.19 **	0.67 ± 0.06 **
STZ+LAM 400	10.14 ± 0.41 **	0.61 ± 0.02 **
STZ+LAW 100	05.91 ± 0.37 *	0.77 ± 0.03 ns
STZ+LAW 200	06.02 ± 0.44 *	0.71 ± 0.04 *
STZ+LAW 400	08.41 ± 0.51 **	0.68 ± 0.03 **

LAM 100, LAM 200 and LAM 400 are oral doses of *L. acutangula* MeOH extract in 100, 200 and 400 mg/kg respectively whereas; LAW 100, LAW 200 and LAW 400 are oral doses of *L. acutangula* aqueous extract in 100, 200 and 400 mg/kg respectively. Values are expressed as mean ± SEM. One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test was applied both in normal and NIDDM rats groups. The difference were considered to be significant when * P < 0.05 compared to respective normal and NIDDM-control groups. (n = 6).

Effect of *Luffa acutangula* extracts on lipid profile

Table 3 exhibits the effects of LA extracts on lipid profile of the animals. Overall the methanolic extract showed dose dependent significant ($p<0.01$) activity on various lipids as compared to the aqueous extract. The aqueous extract at a dose of 100 mg/kg was found to be inactive in reducing the triglyceride and LDL-C levels. Both the methanolic and aqueous extract had similar significant ($p<0.01$) effect in lowering VLDl-C.

Effect of *Luffa acutangula* extracts on OGTT

The methanolic extract proved to be more active in controlling the glucose level in OGTT as compared to the aqueous extracts. The methanolic extract at a dose of 400 mg/kg showed exactly similar effect as that of glibenclamide. The other doses of methanolic extract were also significant ($p< 0.01$) in activity. Fig. 6 highlights the effect of LA extracts on OGTT.

Effect of LA extracts on liver Glycogen, Aspartate transaminase (AST) and alanine transaminase (ALT)

The methanolic and aqueous extracts failed in increasing the liver glycogen at a dose of 100 mg/kg. Whereas the methanolic extract proved to be beneficial in increasing the liver glycogen, at a dose level of 200 and 400 mg/kg. Both the LAM and LAW had promising ($p<0.01$) effects on AST and ALT enzyme levels. The effects of LA extracts on liver Glycogen, AST and ALT are expressed in Table 4.

Effect of LA extracts on body and liver weight

Table 5 shows the effects of LA extracts on body and liver weight. The 100 and 200 mg/kg dose of LAM and LAW could not increase the body and liver weight as that of the standard. Whereas; the 400 mg/kg dose of LAW alone significantly ($p<0.01$) increased the body and liver weight upto normal level.

Table 3: Effect of *Luffa acutangula* extracts on lipid profile of normal and NIDDM rats.

Groups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL –C (mg/dl)
Normal	088.1 ± 7.9 **	47.3 ± 3.3 **	42.1 ± 3.1 **	49.6 ± 1.3 **	09.11 ± 0.61 **
STZ Control	132.3 ± 9.1	94.9 ± 4.3	15.3 ± 1.2	98.3 ± 2.4	30.01 ± 1.23
STZ + Glibenclamide	118.2 ± 7.8 *	88.3 ± 3.2 *	18.1 ± 2.5 ns	91.2 ± 5.8 ns	27.20 ± 1.01 *
STZ + LAM 100	122.2 ± 4.3 *	86.0 ± 4.1 *	22.3 ± 3.2 *	87.6 ± 6.3 *	18.87 ± 0.76 **
STZ + LAM 200	104.3 ± 6.6 **	60.3 ± 5.2 **	33.5 ± 1.2 **	59.4 ± 2.9 **	15.33 ± 0.54 **
STZ + LAM 400	097.4 ± 4.5 **	52.3 ± 3.2 **	37.2 ± 1.8 **	55.5 ± 4.4 **	14.01 ± 0.63 **
STZ + LAW 100	120.5 ± 8.3 *	89.0 ± 4.6 ns	29.6 ± 2.6 **	90.1 ± 5.8 ns	19.13 ± 0.32 **
STZ + LAW 200	100.1 ± 4.8 **	85.8 ± 1.3 *	32.5 ± 4.1 **	87.9 ± 3.7 *	16.02 ± 0.44 **
STZ + LAW 400	094.1 ± 9.1 **	78.3 ± 2.6 **	36.8 ± 2.1 **	86.5 ± 6.8 *	14.89 ± 0.26 **

LAM 100, LAM 200 and LAM 400 are oral doses of *L. acutangula* MeOH extract in 100, 200 and 400 mg/kg respectively whereas; LAW 100, LAW 200 and LAW 400 are oral doses of *L. acutangula* aqueous extract in 100, 200 and 400 mg/kg respectively. Values are expressed as mean ± SEM. One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test was applied both in normal and NIDDM rats groups. The difference were considered to be significant when * P < 0.05 compared to respective normal and NIDDM-control groups. (n = 6).

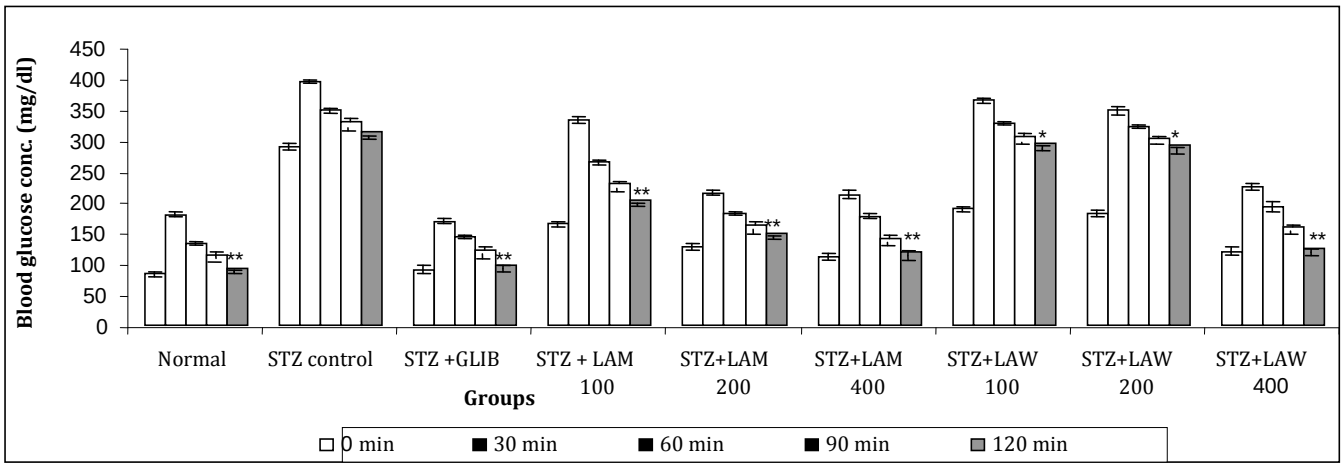

Fig 6: Effect of graded doses of *Luffa acutangula* extracts on OGTT in normal and NIDDM rats.

LAM 100, LAM 200 and LAM 400 are oral doses of *L. acutangula* MeOH extract in 100, 200 and 400 mg/kg respectively whereas; LAW 100, LAW 200 and LAW 400 are oral doses of *L. acutangula* aqueous extract in 100, 200 and 400 mg/kg respectively. Values are expressed as mean ± SEM. One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test was applied both in normal and NIDDM rats groups. The difference were considered to be significant when * P < 0.05 compared to respective normal and NIDDM-control groups. (n = 6.)

Table 4: Effect of *Luffa acutangula* extracts on liver Glycogen, Aspartate transaminase (AST) and alanine transaminase (ALT) in rats.

Groups	Liver Glycogen (µg glucose/ mg liver)	AST (Units / mg tissue)	ALT (Units / mg tissue)
Normal	3.80 ± 0.19 **	040.12 ± 6.66 **	042.13 ± 3.24 **
STZ Control	0.81 ± 0.25	130.28 ± 3.41	140.15 ± 5.54
STZ +Glibenclamide	3.41 ± 0.16 **	108.12 ± 4.21 *	110.22 ± 3.22 *
STZ + LAM 100	2.60 ± 0.36 ns	110.3 ± 4.60 *	121.8 ± 7.44 *
STZ + LAM 200	2.90 ± 0.05 *	065.5 ± 5.65 **	080.3 ± 5.35 **
STZ + LAM 400	3.30 ± 0.19 **	058.3 ± 4.35 **	061.1 ± 4.77 **
STZ + LAW 100	1.70 ± 0.25 ns	114.6 ± 6.66 *	122.5 ± 8.31 *
STZ + LAW 200	2.13 ± 0.16 ns	064.4 ± 3.71 **	118.3 ± 5.54 *
STZ + LAW 400	2.41 ± 0.21*	060.3 ± 4.19 **	100.2 ± 3.22 **

LAM 100, LAM 200 and LAM 400 are oral doses of *L. acutangula* MeOH extract in 100, 200 and 400 mg/kg respectively whereas; LAW 100, LAW 200 and LAW 400 are oral doses of *L. acutangula* aqueous extract in 100, 200 and 400 mg/kg respectively. Values are expressed as mean ± SEM. One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test was applied both in normal and NIDDM rats groups. The difference were considered to be significant when * P < 0.05 compared to respective normal and NIDDM-control groups. (n = 6)

Table 5: Effect of *Luffa acutangula* extracts on body and liver weight of normal and NIDDM rats.

Groups	Body wt(g)	Liver Wt (g/100g of body wt)
Normal	238.4 ± 12.3	8.21 ± 0.51
STZ Control	181.6 ± 10.4	5.23 ± 0.61
STZ + Glibenclamide	234.5 ± 12.8 **	7.88 ± 0.32 **
STZ + LAM 100	212.6 ± 10.1 ns	5.64 ± 0.81 ns
STZ + LAM 200	203.6 ± 08.6 ns	6.32 ± 0.35 ns
STZ + LAM 400	221.3 ± 09.8 ns	7.35 ± 0.54 *
STZ + LAW 100	218.6 ± 11.6 ns	6.23 ± 0.41 ns
STZ + LAW 200	210.6 ± 10.9 ns	7.12 ± 0.39 ns
STZ + LAW 400	234.3 ± 13.4 **	7.92 ± 0.65 **

LAM 100, LAM 200 and LAM 400 are oral doses of *L. acutangula* MeOH extract in 100, 200 and 400 mg/kg respectively whereas; LAW 100, LAW 200 and LAW 400 are oral doses of *L. acutangula* aqueous extract in 100, 200 and 400 mg/kg respectively. Values are expressed as mean ± SEM. One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test was applied both in normal and NIDDM rats groups. The difference were considered to be significant when * P < 0.05 compared to respective normal and NIDDM-control groups. (n = 6).

DISCUSSION

A variety of biologically active, non-nutritive compounds known as phytochemicals, are present in plant foods. These phytoconstituents have beneficial (e.g. antioxidant activity) beyond basic nutritive value. Yet in India, plant foods have received less attention in terms of quantifying their antioxidant activities. In the present study, we examined the antioxidant, antidiabetic and antihyperlipidemic activity of *Luffa acutangula*. Three various extracts such as petroleum ether, methanolic and aqueous extracts were prepared. The comparative effect of these extracts was studied on antioxidant, antidiabetic and antihyperlipidemic in NIDDM rats.

Luffa acutangula belongs to Cucurbitaceae family[5] and most of the members from this family exhibit considerable antidiabetic potential.[24] The preliminary phytochemical analysis of the powder showed the presence of phytoconstituents essential for antioxidant, antidiabetic and antihyperlipidemic acivity. Petroleum ether,

methanol and aqueous these three extracts contained different concentrations and types of phytoconstituents. An effort was made to compare the potential of these extracts for their antidiabetic acitivity.

The phenolic content was determined by Folin–Ciocalteu's reagent method. The methanolic extract exhibited maximum phenolics as compared to the aqueous and petroleum ether extract when compared against gallic acid, a standard. Presence of phenolics makes the extract a strong candidate for antioxidant and thus antidiabetic activity. Many studies in past have justified the role of phenolics in antioxidant activity[25-27].

Using two different systems we found that LA exhibits antioxidant activity. The activity was found to be dose dependent. Initially, we determined the total antioxidant activity by measuring its potential to scavenge 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical.[14] The DPPH is a stable free radical and withdraws an electron to become a stable diamagnetic molecule. [28] DPPH solution in methanol produces purple color which has high absorbance. As the reaction between antioxidant molecule and radical progresses, the antioxidant molecule donates hydrogen that leads in scavenging of free radical. This scavenging causes decrease in absorbance of DPPH solution. Decrease in absorbance is because of discoloration of DPPH solution from purple to yellow. Fig. 2 indicates the effect of LA extracts on scavenging of DPPH free radicals.

During ischemia-reperfusion endogenous reactions like conversion of hypoxanthine to xanthine by flavoenzymes like xanthine oxidase leads to generation of superoxide anion radicals as a byproduct. In vitro, the superoxide anion radicals can be generated from dissolved oxygen by the coupling reaction between phenazine methosulphate (PMS)-NADH-nitroblue tetrazolium (NBT). The decrease of absorbance at 560 nm with LA extracts is the indication of consumption of superoxide anion in the reaction mixture[14] .

Alpha glucosidase (α-D-glucoside glucohydrolase, EC 3.2.1.20), is located in the intestinal brush-borders membranes. It is an important enzyme in catalyzying the ultimate step in digestion of carbohydrates.[29] The LA extracts tested in this study have significantly inhibited the enzyme, Fig. 4. We can assume from this study that one or more phytoconstituents present in the extracts can play an important role in the inhibition of alpha glucosidase enzyme.

The total phenolic content, *in vitro* antioxidant activity and α-Glucosidase inhibitory activity were not significant for the LAPE so, it was ruled out for the animal trials. NIDDM causes a disturbance in uptake and metabolism of glucose. STZ (65 mg/ kg) causes partial destruction of pancreatic β-cells. Many normal β-cells should be present even after administration of a low dose of STZ.[30] Antidiabetic plants act through a variety of mechanism. At present, it is neither possible to prove the exact mechanism of action of LA extracts nor to identify the active principle(s) responsible for antidiabetic activity. However, some hypothetical suggestions can be made. Both the methanolic and aqueous extract of LA had the potential to significantly reduce the elevated blood glucose level in NIDDM rats. The antidiabetic action of LA extracts could be due to potentiation of insulin from the normal pancreatic β-cells. This is evident from the significant rise in serum insulin concentration and fall in fasting blood glucose level. However, glibenclamide had more significant activity.

Administration of methanolic and aqueous extract of LA for 21 days resulted in significant diminution of FBG level of diabetic rat. Whereas, no significant change in FBG level of the STZ control group, further strengthen the antidiabetogenic effect of the extracts.

In present study, a marked increase in serum triglycerides and cholesterol levels were observed, which is in agreement with previous reports[18]. The increased levels of serum triglycerides and cholesterols may lead to cardiac complications. Thus the LA extracts can act as preventive measure against cardiac complications in NIDDM.

Glycosylated hemoglobin level increases remarkably in diabetic rats[31]. Many proteins including hemoglobin are glycated in diabetes[32].The significant reduction in glycosylated hemoglobin in NIDDM rats treated with LA extracts clearly indicated the efficiency of these extracts ion glycaemic control.

Glycogenesis in muscle and liver is mainly regulated by serum insulin level[33].The decrease in hepatic glycogen reported in this study may be due to low level of serum insulin in NIDDM rats, which could have inactivated the glycogen synthesis system. Treatment with LA extracts for 21 days to NIDDM rats has resulted in increase in liver glycogen levels. This highlights the one possible way of antidiabetogenic action of LA extract.

Gluconeogenesis and ketogenesis increases in diabetes and may be due to increased level of transaminases like AST and ALT.[34] Supplementation of methanolic extract (200 and 400 mg/kg) and aqueous extract (400 mg/kg) for 21 days has restored the of AST and ALT enzymes to normal level. This fact further strengthens the antidiabetogenic effect of these extracts. Also, AST and ALT levels act as indicators of hepatic function and restoration of these enzymes to normal levels indicates normal functioning of liver.

Weakness; and loss of body and liver weight are the common symptoms of NIDDM rats.[14] The groups treated with methanolic extract (200 and 400 mg/kg) and aqueous extract (400 mg/kg) showed remarkable gain in body and liver weight as compared to the STZ control rats. Improvement of liver and body weight of the extract treated animals' further support the antidiabetogenic effect of these extracts.

CONCLUSION

Our study suggests that methanolic and aqueous extracts of *Luffa acutangula* fruit may have beneficial effects in treatment of type-II diabetes mellitus. The extracts might be useful as a medicinal food or as a source of natural alpha glucosidase inhibitors for suppressing postprandial hyperglycemia in the management of Type II diabetes. Furthermore, the extracts may have other mechanism of actions as well. However, complete phytochemical and pharmacological research is obligatory to uncover the precise mechanism of these extracts for their antidiabetic effect.

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