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Research Article

PRELIMINARY PHYTOCHEMICAL AND PHARMACOLOGICAL ACTIVITIES OF *LUFFA CYLINDRICA* L. FRUIT

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ABSTRACT

Starting from ancient period and traditional systems of medicine many medicinal plants are play a vital role in the treatment of several diseases. Medicinal plants possess valuable bioactive compounds that protects human from various complications. Therefore, there is a necessity to explore their uses and to conduct pharmacological studies to find out their therapeutic properties. Hence, the present study aims to open new avenues for the improvement of medicinal uses of fruit of *Luffa cylindrica* for the selected area for diabetes.

Keywords: Analgesic, Antipyretic, Anti-diabetic, *Luffa cylindrica*

INTRODUCTION

Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments. Nature has bestowed our country with an enormous wealth of medicinal plants, therefore India has often been referred to as the Medicinal Garden of the world. A medicinal herb as potential source of therapeutic aids has attained a significant role in health system all over the world for both humans and animals not only in the diseased condition but also as potential material for maintaining proper health[1].

Luffa cylindrica Linn (Cucurbitaceae) is large climber with a stout, 5 angled pubescent stem tendrils usually 2-3 branched. Leaves are large, 10-20 cm long and 9-21 cm wide. Orbicular to reniform orbicular in outline, base cordate, 5-7 lobed, lobes broadly triangular, acute, margins irregularly, shallowly dentate, hispid, scab rid above, finely pubescent-hispid beneath, petioles 2-8 cm long. The male and female flowers deep, bright yellow and male flowers borne in racemes on peduncles 6-17 cm long. It is usually aggregated near apex, stamens usually 15-30 cm long (occasionally up to 2.5 m) and 6-10 cm in diameter, smooth with 10 dark green longitudinal lines, fibrous within, seeds 1 to 2 cm long and 0.8 cm wide including the narrow, smooth, marginal wing, broadly ellipsoid, longitudinally compressed, rough on surface[2]**.** The reported pharmacological activities include antiplasmodial and antibacterial activity[3]. Hepatoprotective activity of fruit extracts of *Luffa cylindrica* has already been reported[4]. In the present study, the preliminary phytochemical and various pharmacological activities (analgesic, antipyretic and anti-diabetic) activities of aqueous (AELC) and ethanol (EELC) extracts of *Luffa cylindrica* fruit has been investigated in a scientific manner in rats.

MATERIALS AND METHODS

Plant material

The fruit of *Luffa cylindrica* was collected from the Chhatarpur, Madhya pradesh in the month of October 2010 and authenticated at Safia College of Science, Bhopal. The voucher specimen (264/boi/safia/11) was deposited in Department of Pharmacognosy, Technocrats Institute of Technology-Pharmacy, Bhopal, Madhya Pradesh.

Preparation of extract

The collected, cleaned powder of fruit of *Luffa cylindrica* was used for the extraction process. The powder (500 g) material was evenly packed in the soxhlet apparatus and extracted with ethanol by hot continuous extraction process for about 26 h, while the aqueous extract was prepared by cold maceration method. These extracts were concentrated by vacuum distillation to reduce the volume 1/10. The concentrated extracts were placed in desiccator to remove the excessive moisture[5].

Phytochemical screening

The preliminary phytochemical screening of AELC and EELC were subjected to determine the presence of various phytoconstituents[6].

Animals

Swiss albino mice (20-25 g) and Wistar rats (125-200 g) of either sex and of approximate 9-12 week old were used in the present studies and procured from National Central Laboratory for Animal Science, Hyderabad. These animals were maintained in clean polypropylene cages with 12 h light and dark cycle at a temperature of 27-29^{0}C and a humidity of 62 to 65%. The food and water *ad libitum* and animals were acclimatized to laboratory condition for one week before starting the experiment. The experimental protocol approved by Institutional animal ethics committee (Reg. no. TIT/IAEC/831/P'cog/2012/13).

Acute toxicity study

Acute toxicity study was performed according to the acute toxic classic method as per OECD 420 guidelines. Swiss albino mice were used for acute toxicity study. The animal were kept fasting for overnight providing water *ad libitum* and AELC and EELC were administered orally 2000 mg/kg and observed the mortality of the animals. Then the dose administered was assigned as toxic dose level.

Analgesic activity

The peripheral analgesic activity was evaluated by acetic acid induced writhing method[7]. The percentage of inhibition of abdominal constrictions for the extract treated groups was compared with control group.

Antipyretic Activity

The antipyretic activity was evaluated by Brewer's yeast induced pyrexia in rats[7]. The rectal temperature of the rats was recorded at 0, 60, 120, 180 and 240 min. by insertion of a clinical thermometer to a depth of 2 cm into rectum.

Anti-diabetic activity

Oral glucose tolerance test
Rats were fasted for 14 h and were divided into 7 groups (n=6).

Group I: Normal control rats administered (0.9%, w/v) normal saline.

Group II: Control rats were administered glucose 2 g/kg, p.o.

Group III: Rats were administered with glibeclamide (2.5 mg/kg, p.o.) and treated glucose 2 g/kg, p.o.

Group IV & V: Rats were administered with AELC (100 & 200 mg/kg) and treated with glucose 2 g/kg, p.o.

Group VI & VII: Rats were administered with EELC (100 & 200 mg/kg) and treated with glucose 2 g/kg, p.o.

At 10 min after the administration of treatment 2 g/kg of glucose was administered to each rat. The blood glucose level was measured before the treatment and at 30, 60, 90 and 120 min after the treatment. Blood samples were collected from the tail vein and determined by electronic glucometer (Accu-check) [8].

Induction of non insulin dependent diabetes mellitus (NIDDM)

NIDDM was induced in overnight fasted adult male Wistar albino rats weighing 150-200 gm by a single intraperitoenal injection of 120 mg/kg alloxan monohydrate (Loba Chemie). Hyperglycemia was confirmed by the elevated glucose levels determined at 72 h. Animal with blood glucose level more than 150 mg/dl were considered as diabetic. Rats found with permanent NIDDM were used for anti-diabetic study[8]. The rats were divided into 7 groups (n=6). The extracts were administered for 15 days.

Group I: Normal control rats administered (0.9%, w/v) normal saline.

Group II: Control rats administered (0.9%, w/v) normal saline and treated with alloxan (120 mg/kg, i.p.)

Group III: Rats were administered with glibeclamide (2.5 mg/kg, p.o.) and treated with alloxan (120 mg/kg, i.p.)

Group IV & V: Rats were administered with AELC (100 & 200 mg/kg) and treated with alloxan (120 mg/kg, i.p.)

Group VI & VII: Rats were administered with EELC (100 & 200 mg/kg) and treated with alloxan (120 mg/kg, i.p.)

Fasting blood glucose was estimated on 0, 5, 10 and 15th day by commercially available glucometer (Accu-check). At the end of the experiment on 15th day, Rats were sacrificed by deep anaesthetized. Blood was collected by cardiac puncture and allowed to clot for 30 min at room temperature. The serum was separated by centrifugation at 3000 rpm at 20°C for 15 minutes. The serum samples were subjected to biochemical parameters examination like HDL, LDL, VLDL, triglyceride and cholesterol levels were estimated by using standard kits (Span diagnostics Ltd)[9].

RESULTS

The phytochemical study shows that presence of alkaloid, tannin, steroid and flavonoids (Table 1). The percentage yield of aqueous extract (14.3% w/w) was more than the other extracts and ethanol extract was found to be 9.8 % w/w (Table 2). The AELC and EELC showed significant analgesic activity and EELC 200 mg/kg was showed higher percentage of inhibition of writhes (72.56%) then the other doses (Table 3). The AELC and EELC showed significant (*P < 0.05) antipyretic activity, when compared to control group (Table 4). The result showed that there was an increase in the blood glucose levels of rats in group control that received saline from 97.42 ± 1.82 at zero min to 188.88 ± 1.29 at the 60 min representing 51.58% rise. The AELC and EELC (100 & 200 mg/kg) as well the standard drug caused a time dependent and significant (p < 0.01) reduction of the blood glucose levels of the alloxan induced diabetic rats, when compared to the control group (Table 5). The decreased fasting blood glucose levels was found to be rats at 5th, 10th and 15th day, when compared to the control group (Table 6). AELC and EELC (100 & 200 mg/kg) to deceased the levels of LDL, HDL, VLDL, triglycerides and cholesterol, when compared to the control group (Table 7).

Table 1: Preliminary phytochemical screening of various extracts of fruit of *Luffa cylindrica*

Phytoch-emicals test	Pet. ether	Chloroform	Ethyl acetate	Ethanol	Water
Flavonoids	+	-	+	+	+
Glycoside	-	+	-	-	-
Tannin	-	-	+	+	+
Alkaloid	-	-	-	+	+
Saponin	-	-	-	-	+
Steroid	+	+	+	+	+

+= Positive; - = Negative

Table 2: Percentage yield of various extracts of fruit of *Luffa cylindrica* .

S. No	Solvent	Extract (%w/w)
1	Pet. ether	1.8
2	Chloroform	3.2
3	Ethyl acetate	4.3
4	Ethanol	9.8
5	Water	14.3

Table 3: Analgesic activity (writhing method) of aqueous and ethanol extracts of fruit of *Luffa cylindrica*

Group/Treatment	Wriths in 15 min (Mean ± SEM)	% Inhibition of wriths
Group I : Saline	16.4±0.61	-
Group II :Diclofenac sodium (10 mg/kg)	14.58±0.12	11.09
Group III: AELC (100 mg/kg)	13.2±0.42*	19.51
Group IV: AELC (200 mg/kg)	10.7±0.55**	34.75
Group V: EELC (100 mg/kg)	9.52±1.15**	41.95
Group VI: EELC (200 mg/kg)	4.5±0.23**	72.56

The data obtained were analyzed by one-way ANOVA followed posthoc Dunnet's *t*-test. Each value represents the mean ± S.E.M., n= 6.*P < 0.05, ** P < 0.01, compared with control group

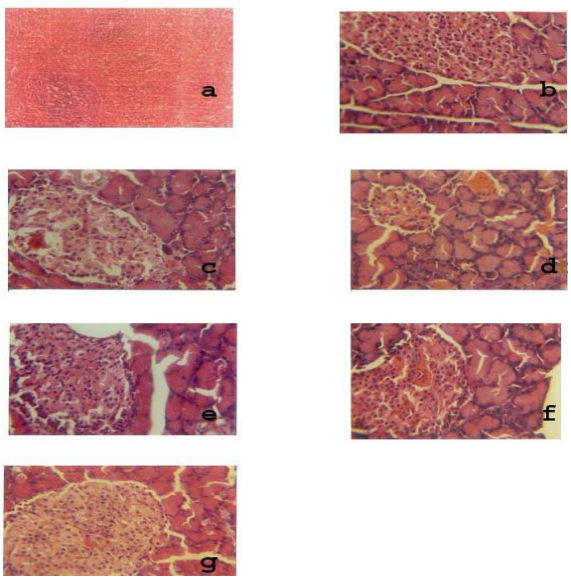

Fig 1: Photomicrographs of histopathological changes in rats pancreas [a- Normal; b- Standard; c- Control; d & e- AELC (100 & 200 mg/kg); f & g- EELC (100 & 200 mg/kg).

Table 4*:* Antipyretic activity of aqueous and ethanol extracts of fruit of *Luffa cylindrical.*

Group /Treatment	Body temperature (mean ± SEM)					
	0 min	After 18 h of yeast	60 min	120 min	180 min	240 min
Group I: Saline	37.6 ± 0.21	40.17 ± 0.32	41.37 ± 0.11	41.62 ± 0.18	41.45 ± 0.32	40.72 ± 0.22
Group II: Paracetamol 100 mg/kg	37.2 ± 0.62	38.45 ± 0.56 *	38.52 ± 0.43 *	38.25 ± 0.52 *	38.53 ± 0.19 *	37.26 ± 0.01 *
Group III: AELC (100 mg/kg)	37.5 ± 0.41	40.27 ± 0.21	39.27 ± 0.47	39.43 ± 0.37	39.21 ± 0.14	39.61 ± 0.63
Group IV: AELC (200 mg/kg)	37.6 ± 0.83	40.15 ± 0.31	40.33 ± 0.62	39.72 ± 0.28	39.25 ± 0.13	39.82 ± 0.90
Group V: EELC (100 mg/kg)	37.4 ± 0.91	40.47 ± 0.21	40.33 ± 0.42	39.57 ± 0.67	39.11 ± 0.74	38.71 ± 0.62 *
Group VI: EELC (200 mg/kg)	37.7 ± 0.54	40.45 ± 0.51	39.87 ± 0.47	39.12 ± 0.32	38.71 ± 0.33 *	38.42 ± 0.30 *

The data obtained were analyzed by one-way ANOVA followed posthoc Dunnet's *t*-test. Each value represents the mean ± S.E.M., n= 6. *P < 0.05, ** P < 0.01, compared with control group.

Table 5: Effect of aqueous and ethanol extracts of fruit of *Luffa cylindrica* on blood glucose level on 0, 30, 60, 90 and 120 min in oral glucose tolerance test.

Groups	Blood glucose (mg/dl)				
	0 min	30 min	60 min	90 min	120 min
Group I: Normal	90.42±3.46	97.52±1.93	100.32±1.26	98.32±1.42	94.72±2.02
Group II: Control	97.42±1.82	179.16±1.02	188.88±1.29	174.57±1.63	170.07±2.03
Group III: Glibenclamide	89.12±1.52	131.32±0.45**	127.19±1.93**	117.17±1.47**	100.12±1.07**
Group IV: AELC (100 mg/kg)	95.36±1.25	156.34±1.89	152.85±1.43	148.27±2.27	143.07±1.67
Group V: AELC (200 mg/kg)	96.72±2.65	151.12±1.72*	146.35±1.43*	141.42±2.67*	130.02±1.69*
Group VI: EELC (100 mg/kg)	98.36±1.45	143.32±2.22*	137.75±0.43*	134.43±1.29*	123.83±2.09*
Group VII: EELC (200 mg/kg)	102.12±0.52	141.46±1.94**	133.65±2.23**	130.87±1.30**	112.07±2.39**

The data obtained were analyzed by one-way ANOVA followed posthoc Dunnet's *t*-test . Each value represents the mean ± S.E.M., n= 6. *P < 0.05, ** P < 0.01, compared with control group.

Table 6: Effect of aqueous and ethanol extracts of fruit of *Luffa cylindrica* on fasting blood glucose level on 0, 5, 10 and 15th day in alloxan induced diabetes in rats.

Groups/Treatment	Fasting blood glucose (mg/dl)			
	0th day	5th day	10th day	15th day
Group I: Normal	105.12±2.40	102.02±1.43	102.12±1.20	98.32±1.42
Group II: Control	105.32±1.52	199.12±1.72	195.81±1.09	194.53±1.43
Group III: Glibenclamide	107.42±2.59	129.32±0.52**	122.15±2.43**	118.87±1.37**
Group IV: AELC (100 mg/kg)	109.32±1.75	189.32±1.52	182.15±2.43	178.87±1.37
Group V: AELC (200 mg/kg)	106.32±1.65	175.72±1.12*	172.15±2.43*	165.82±1.67*
Group VI: EELC (100 mg/kg)	110.37±1.56	169.32±1.62*	165.15±2.43*	158.47±1.27*
Group VII: EELC (200 mg/kg)	112.12±1.42	153.41±1.64**	142.15±2.43**	138.87±2.36**

The data obtained were analyzed by one-way ANOVA followed posthoc Dunnet's *t*-test. Each value represents the mean ± S.E.M., n= 6. *P < 0.05, ** P < 0.01, compared with control group.

Table 7: Effect of aqueous and ethanol extracts of fruit of *Luffa cylindrica* on serum biochemical parameters in alloxan induced diabetes in rats.

Groups/Treatment	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)
Group I: Normal	93.23 ± 3.1	37.00 ± 1.5	19.20 ± 0.8	92.9±1.56	66.89 ± 0.31
Group II: Control	149.16 ± 1.2	90.00 ± 1.4	34.40 ± 0.2	139.2±2.94	126.33 ± 0.10
Group III: Glibenclamide	103.73 ± 2.72	51.50 ± 1.9**	21.30 ± 0.9**	102.0±1.33**	75.60 ± 0.28**
Group IV: AELC (100 mg/kg)	135.16 ± 1.40	80.01 ± 2.90*	28.00 ± 0.5*	136.3±1.2	108.87 ± 0.13*
Group V: AELC (200mg/kg)	128.45 ± 0.19*	69.10 ± 1.85*	26.12 ± 0.90*	127.6±2.5	105.43 ± 0.35*
Group VI: EELC (100mg/kg)	119.33 ±0.29**	64.10 ± 0.85**	24.12 ± 0.90*	120.9±1.6*	100.50 ± 0.17**
Group VII: EELC (200mg/kg)	114.43 ±0.22**	56.10 ± 2.85**	23.12 ± 0.90**	111.9±2.8 *	95.40 ± 0.34**

The data obtained were analyzed by one-way ANOVA followed posthoc Dunnet's *t*-test. Each value represents the mean ± S.E.M., n= 6. *P < 0.05, ** P < 0.01, compared with control group.

Histopathology study

Normal rats showed the islet is normal, architectures with moderate cytoplasm and small round to oval nuclei (Fig 1a). Control rats islet shows different morphology shape and the surrounding acinar tissue is dissolved (Fig 1b). A standard rat shows the islet depletion of cells with moderate cytoplasm and small round to oval nuclei (Fig 1c). AELC (100 & 200 mg/kg) shows the architecture is normal and the acinar cells shows moderate cytoplasm, round to oval nuclei. There is no evidence of inflammation (Fig 1d&e). EELC (100 & 200 mg/kg) shows the islets are normal architectures are preserved. The acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei (Fig 1f&g).

DISCUSSION

The aim of the presence study is to find out possible pharmacological activities (analgesic, antipyretic and antidiabetic) of AELC and EELC. In analgesic studies the both AELC and EELC showed significant analgesic activity at all tested dose level by acetic acid induced writhing methods. The result showed that the AELC and EELC possess a significant antipyretic effect in yeast induced elevation of body temperature in rats. Most of the NASID showed analgesic and the antipyretic activities by inhibiting the prostaglandin synthesis[7]. Hence, the analgesic and antipyretic effects of AELC and EELC may be occurs by inhibiting the prostaglandin

synthesis due to the activities are similar to the paracetamol and diclofenac sodium.

Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from streptozotocin. Alloxan causes a massive reduction in insulin release by the destruction of b-cells of the Islets of langerhans, thereby inducing hyperglycemia[10, 11]. The possible hypoglycemic mechanism of AELC and EELC may be through potentiation of pancreatic secretion of insulin from cell of islets or due to enhanced transport of blood glucose to the peripheral tissues. Insulin deficiency leads to various metabolic alterations in the animal's viz. increased blood glucose, increased cholesterol, increased levels of alkaline phosphate and transaminases[12]. The daily administration of AELC and EELC (100 & 200 mg/kg) for 15 days, resulted in changes in the blood glucose levels and biochemical parameters of alloxan induced diabetic rats. The previous study was reported that tannin and flavonoids possesses analgesic, antipyretic and anti-diabetic activities[7, 12] and same result was found in preliminary phytochemical screening of AELC and EELC. The pharmacological activities (analgesic, antipyretic and anti-diabetic) of AELC and EELC could be due to the presence of above mentioned phytochemicals.

CONCLUSION

Antioxident, analgesic and antipyretic activities of the aqueous and ethanol extract of fruit of *Luffa cylindrica* was studied and showed adequate results. The dose levels of 100 and 200 mg/kg were selected for present studies in which 200 mg/kg showed more significant analgesic, antipyretic activity than 100 mg/kg. In our studies the conclusively damage of pancreas in alloxan treated diabetic control rats and regeneration of beta cells by glibenclamide was observed. The comparable regeneration was also shown by ethanolic and aqueous extracts of fruit of *Luffa cylindrica*. Antihyperglycemic and antihyperlipidemic activites of ethanol and aqueous extracts of fruit of *Luffa cylindrica* may be attributed in the presence of various phytochemical such as alkaloid, steroid, triterpinoid, flavonoid and glycoside. Photomicrographical data in our studies reinforce healing of pancreas by *Luffa cylindrica* fruit extracts as a plausible mechanism of their antidiabetic activity. This study has to some extent validated the medicinal potential of the fruit extracts of *Luffa cylindrica.*

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