

Gc-MS Study of Two Column Fractions from Methanol Extracts of *Loranthus Micranthus* and Their *In Vivo* Antidiabetic Activity on Alloxan Induced Diabetic Rats

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Abstract

The methanol extracts of *Loranthus micranthus* fractions were collected from column chromatography. From TLC, the fraction 17 and 20 were selected based on the clear distinguishable of bands and few phytochemicals. Same fractions when subjected to GC-MS, fraction-17 yielded 13 and fraction-20 yielded 11 prominent compounds. Diabetic rats that were treated with the fraction 17 and 20 showed significantly increase in body weight (16.52 and 12.43 % respectively). Hypoglycemic effect and antihyperlipidemic characters (decrease in the previously elevated levels of serum cholesterol and serum triglycerides) were observed. The two fractions treated rats have also showed the regeneration of pancreatic connective tissue, acini, blood vessels, blood capillaries, pancreatic ducts and their walls, islets capsule and cells in accordance to proper cytoarchitecture. The *in vivo* antidiabetic property may be due to the presence of single or all phytochemicals in the fraction. Greater activity was observed in fraction-17. However, further experimental studies are still needed for identification of the functionally relevant phytochemical(s) in management of diabetes in rats.

Keywords: *Loranthus micranthus*; *In vivo*; Antidiabetic; Antihyperlipidemic; GC-MS; Histopathology

Introduction

International Diabetes Federation (IDF) recent estimate indicates that 8.3% of adults – 382 million people worldwide and 65.1 million people in India suffer from diabetes, and the number of people with the disease is set to rise beyond 592 million in less than the next 25 years. Yet, with 175 million of cases currently un-diagnosed, a vast number of people with diabetes are progressing unawares towards impeding complications. Moreover, with 80 % of the total number affected individuals living in low- and middle-income countries, where the epidemic is gathering pace at alarming rates [1]. It may almost become a serious public health problem, particularly in developed countries as a major threat to global development [2,3]. This shows the necessities and the importance of more alternate and effective antidiabetic drugs and their systematic studies to manage diabetes.

Animals have been extensively used as study models to carry out diabetes research *in vivo*. The effect of “foreign” compounds including plant extracts on the blood constituents of an animal can be used to determine the extent of severity of the particular disorders [4]. Flavonoids, glycosides of triterpenes, steroids and alkaloids of different plant origin showed a promising anti-diabetic activity, as demonstrated in diabetic animal models [5-7]. In animals, it can be induced by partial pancreatectomy or by the administration of diabetogenic drugs such as alloxan, streptozotocin, ditizona and anti-insulin serum. These agents selectively destroy the Langerhans islets β -cells. Alloxan and streptozotocin are widely used to induce experimental diabetes in animals. The cytotoxic action of both these diabetogenic agents is mediated by reactive oxygen species. According to the administered dose of these agents, syndromes similar to either type 1, type 2 diabetes mellitus or glucose intolerance can be induced [8,9].

Loranthus micranthus is a hemiparasitic shrub commonly known as African mistletoe. It grows on woody plants for nourishment and synthesizes its own chlorophyll. *Kola acuminata*, *Kola nitida*, *Mangifera indica*, *Azadirachta indica*, *Jatropha curcas* and *Persia*

sp. were the common host plants of *L. micranthus* [10]. Herbs are the choice over synthetic drugs from past centuries having multiple therapeutic functions and fewer side effects. In this context it was an effort to attend towards antidiabetic studies of *L. micranthus* in an analytical and systematic ways. *L. micranthus* has been widely used in ethnomedicine for various purposes, including antihypertensive, anticancer, antispasmodic, and antidiabetic, in treatment of epilepsy, headache, infertility, menopausal syndrome and rheumatism [11,12] and many of medicinal activities are proven by recent researches. It decreases the blood glucose level and controls the loss of body weight in diabetes mellitus [13].

Presently the study was aimed to carry out GS-MS and *in vivo* antidiabetic activities of *Loranthus micranthus* on alloxan induced diabetic rats. Based on our earlier studies of qualitative, quantitative and some biological activities, column fractions from methanol extracts of *L. micranthus* were selected for *in vivo* antidiabetic studies on rats. Body weight, blood glucose level, lipid profile and histopathological changes of rats were considered in the study.

Materials and Methods

Collection and processing of plant

The fresh leaves of *Loranthus micranthus* growing on the host plant

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Azadirachta indica were collected in the month of April, 2009 during the flowering period at DC Bungalow, Tumkur, Karnataka, India and identified using authenticated herbarium from the Department of Studies in Botany, University of Mysore, Mysore and Government Ayurvedic College, Mysore. The plant material was washed with distilled water, shade-air dried ($26 \pm 2^\circ\text{C}$) and pulverized to a coarse powder in a mechanical grinder, passed through a 40 mesh sieve and stored in air tight container for further work.

Preparation of crude extracts

25 g/100 ml of powdered *Loranthus micranthus* was kept for solvent extraction in rotary shaker at 37°C , 72 rpm for 48 h. The methanol was used with increasing order of their polarity. The solvent extract was centrifuged at 6000 rpm for 10 min and then filtered with Whatman No. 1 filter paper and evaporated at a constant temperature of 62°C in hot air oven until a very concentrated extract was obtained.

Column chromatography

Column chromatography is a type of adsorption chromatography technique. Here stationary phase is a silica gel packed in a vertical column. Cotton wool was plugged at the bottom of knob to hold stationary phase to allow only the solvent and sample. Silica was activated at 110°C for 20 min to remove moisture content. Silica slurry was prepared by starting eluent and packed sufficiently in the column. The column was eluted with an initial effluent to remove further impurities. The extract sample to be separated was placed on the top of packed stationary phase without disturbing the silica bed. The gradient elution was carried out using solvents as an increase in their polarity. The solvents used are hexane, hexane plus toluene (2:1, 1:1, 1:2), toluene, toluene plus ethyl acetate (2:1, 1:1, 1:2), ethyl acetate, ethyl acetate plus methanol (2:1, 1:1, 1:2) and methanol in different ratios. 25 eluted fractions were periodically collected at regular volumes of 5 ml each. Repeatedly the column chromatography was carried out to reach the affordable quantity of fractions for GC-MS analysis and animal studies.

GC – MS analysis

Based upon the distinguishable phytochemical results of column chromatography and thin layer chromatography, column fractions 17, 20 and 21 were selected for GC-MS analysis of methanolic fractions of *Loranthus micranthus*. GC-MS was carried out with Agilent 7890-A having an MS detector 5975-C, ionization for MS is electron impact ionization. Mass analyzer was Quadrupole. The peaks were analyzed using data analysis software NIST-2008. An experiment was carried in column HP- 5 ms, dimensions- 30 m L \times 0.25 mm ID \times 0.25 μm film thickness. The initial temperature ramp was maintained at 40°C , hold time -2 min. At the end the temperature ramp was 310°C and hold time was 10 min. The rate of temperature ramp was $10^\circ\text{C}/\text{min}$. The experiment was programmed with total run time 34 min, helium was used as a carrier gas at a constant flow rate of 1.0 ml/min, split less flow 1ml/min. Injection volume was 1 μl with scan mass range 30 m/z – 600 m/z having positive polarity (+ve).

Selection of animal species

Healthy young male albino wistar rats of 8-10 week old, weighing between 150 g to 200 g were selected for *in vivo* antidiabetic studies. Rats were collected from the animal house of Sree Siddaganga College of Pharmacy, Tumkur and research was carried out in Pharmacology Department of Sree Siddaganga College of Pharmacy. Rats were housed in animal room at $25 \pm 2^\circ\text{C}$ temperatures and maintained with free

access to standard food and pure water *ad libitum*. The animal room was regulated by a 12 h light and 12 h dark schedule.

Toxicity studies (LD₅₀)

Based upon the results of GC-MS analysis and *in vitro* antidiabetic activity, column fraction-17 (CF-17) and 20 (CF-20) were selected for animal studies. Suspensions of dried, concentrated column fractions were prepared by dissolving in 0.9%v/v cold normal saline solution for treatment.

Toxicity studies of column fractions-17 and 20 were carried out in accordance with the modified method Lorke (1983) [14]. Maximum dose, up to 1200 mg/kg body weight was treated through oral route of administration. The animals were grouped into 6 groups involving 5 animals in each group. Group-1: 17th fraction- 400 mg/kg body weight, Group-2: 17th fraction- 800 mg/kg body weight, Group-3: 17th fraction- 1200 mg/kg body weight and Group-4: 20th fraction- 400 mg/kg body weight, Group-5: 20th fraction- 800 mg/kg body weight, Group-6: 20th fraction- 1200 mg/kg body weight. The rats were observed for clinical signs and symptoms of toxicity like behavioural changes and mortality within 24 h. All the procedures were performed in accordance with the Institutional Animal Ethics Committee (IAEC). Lethal dose - 50 (LD₅₀) was then calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose.

Experimental induction of diabetes

The animals were fasted for 16 - 18 h with free access to water prior to the administration of alloxan. Alloxan monohydrate is cyclic urea analogue have unique property of producing chronic experimental diabetes by a specific cytotoxic action on β -cells of the islets of Langerhans by a single diabetogenic dose. Diabetes was induced in nearly 70 rats by intraperitoneal (i.p.) injection of alloxan monohydrate at a dose of 120 mg/kg body weight, dissolved in 0.9%v/v cold normal saline solution [15]. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia [16]. After an observational period of about 72 h, rats with fasting plasma glucose levels above 300 mg/dl were considered diabetic.

Experimental protocol

After an observational period of 72 h, rats with fasting plasma glucose levels above 300 mg/dl were considered as diabetic and were assigned into five groups of ten rats in each group.

Animal fasted overnight were randomly divided into 5 groups:

- | | |
|-----------------|--|
| Group 1: NC: | Normal control, treated with only normal saline (2.0 ml) orally. |
| Group 2: DC: | Diabetic control |
| Group 3: STD: | Diabetic rats treated with reference drug Glibenclamide, at a dose of 0.5 mg/kg body weight. |
| Group 4: CF-17: | Diabetic rats treated with 17 th -fraction of <i>Loranthus micranthus</i> extract, at a dose of 400 mg/kg body weight orally. |
| Group 5: CF-20: | Diabetic rats treated with 20 th -fraction of <i>Loranthus micranthus</i> extract, at a dose of 400 mg/kg body weight orally. |

The fasting blood glucose levels were estimated by glucose oxidase-peroxidase reactive strips (Dextrostix, Bayer Diagnostics) with Accucheck glucometer. Blood samples were collected by cutting the tip of the tail at an interval of 1, 7, and 14 days. The blood results were reported as mg/dl. Blood glucose levels were expressed in mg/dl as mean \pm SEM. On day 14, rat's body weight, serum cholesterol and serum triglycerides were analysed. During the experiment all the rats had free access to

standard rat chow and water at all times. Body weight and glycaemic change were calculated according to the formula;

Change in body weight (bw) (%) = [bw on (1st, 7th, and 14th day) – Initial bw ×100] / Initial bw % Glycaemic change = [Glucose concentration (7th, and 14th day) – Fasting blood glucose 1st day ×100]/ Fasting blood glucose.

The data were statistically analyzed using ANOVA with multiple comparisons versus control group. Values of p < 0.05 or less were taken as significant.

Lipid profile

At the end of 14 day of experimental period, blood was collected in Eppendorf tubes through retro-orbital plexus. Plasma was separated and serum was taken by centrifugation at 4°C using REMI-24 model centrifuge. Lipid profile viz., serum cholesterol and serum triglycerides were measured on automated analyser Olympus AU 400.

Histopathology

It is been important to study the determination of effect of two column fractions on pancreatic cytoarchitecture. At the end of 14th day, all the animals were sacrificed by lethal chloroform vapor anaesthesia and pancreas was excised and rinsed in ice cold normal saline. A portion of the tissue was fixed in 10% formalin, cut into 5 µm thick sections, and stained using hematoxylin-eosin stain and histopathological observations were made.

Results

GC – MS analysis and identification of phytoconstituents

Interpretation of GC-MS mass-spectra were carried out using the database of National Institute Standard and Technology- 2008 (NIST-2008) having more than 62,000 patterns. The spectrums of the unknown components were compared with the spectrum of known components of NIST library and the parameters viz., retention time, molecular weight, structure of the components, total ionic chromatograms and ionization chromatograms were ascertained in naming the particular compound.

Three fractions of phytochemicals were analyzed by GC-MS. The literature survey indicates that some of the compounds which are present in three fractions are already exhibiting antidiabetic activity by possessing different (alternative) mechanisms and when they were isolated from other plants.

In GC-MS studies fraction-17 showed 13 detectable peaks and the compounds identified were, 1,2,3-Propanetriol diacetate, 1-Tetradecene, 2,6-bis(1,1-dimethylethyl)- 2, 5- Cyclohexadiene- 1,4-dione, 2,4- bis (1,1- dimethylethyl) - Phenol, 1-{2- [3- (2-Acetyloxiran -2- yl) -1, 1- dimethylpropyl] cycloprop -2- enyl} ethanone, Hexadecanoic acid methyl ester, 1, 2- Benzenedicarboxylic acid butyl octyl ester, 9-Octadecenoic acid (Z) - methyl ester, Octadecanoic acid methyl ester, Eicosanoic acid methyl ester, 4,8,12,16 – Tetramethylheptadecan -4- olide, 2,6,10,15,19,23-hexamethyl- (all-E)-2,6,10,14,18,22-Tetracosahexaene (Squalene).

In fraction-20 there were about 11 detectable peaks and the compounds identified were, Nitrobenzene, 1-Tetradecene, 2,4-bis(1,1-dimethylethyl)-Phenol, Dodecanoic acid methyl ester, Ar-tumerone (Terpene), Methyl tetradecanoate, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol), Benzenepropanoic acid- 3,5-bis(1,1-

dimethylethyl)-4-hydroxy- methyl ester, trans-13-Octadecenoic acid methyl ester, Methyl 16-methyl-heptadecanoate.

Toxicity studies (LD₅₀)

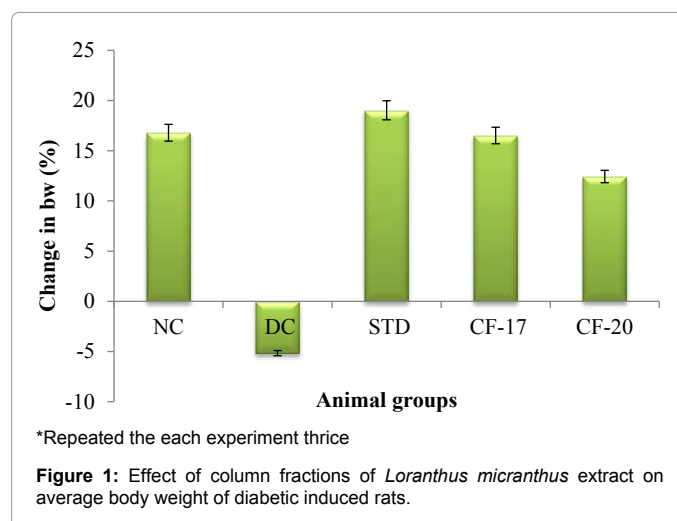
Toxicity Studies of CF-17 and 20 of *Loranthus micranthus* were determined in rats through oral route of administration. There were no toxic features of *Loranthus micranthus* found in rats upto 1200 mg/kg body weight. Clinical signs and symptoms of toxicity like behavioural changes including locomotor activity and sensitivity to touch and mortality within 24 h and also recorded after 48 h after administration. Column fractions did not cause death and any abnormal behavioural changes in rats upto 1200 mg/kg body weight i.e., 100% survival rates were seen and *L. micranthus* was considered non toxic at such concentrations.

Effect on body weight

After the treatment of 14 day schedule it was noticed that, slight increase in the body weight of normal control rats. It may be due to their normal growth. Diabetic control rats showed 5.16 % reduction in their body weight. Diabetic rats treated with standard drug Glibenclamide showed increased in the body weight by 19.03%. Diabetic rats treated with *Loranthus micranthus* fractions were also showed significant increase in body weight by 16.52 % and 12.43 % for CF-17 and 20 respectively (Figure 1 and Table 1).

Hypoglycaemic effect of column fractions of *Loranthus micranthus* extract

After the 14 day treatment schedule, the result clearly indicates that, *Loranthus micranthus* extracts have significant hypoglycaemic effect. The decrease in blood glucose level was observed by 32.05% on 7th day and 58.40% on 14th day for CF-17 at 400 mg/kg body weight. For CF-20 it was observed 18.23% on 7th day and 40.60% on 14th day at 400 mg/kg concentration. The decreased blood glucose levels were found efficient when compared to standard drug Glibenclamide (STD) which shown blood glucose levels by 34.34% on 7th day and 64.18% on 14th day treatment. The increased blood glucose levels by 9.42% on 7th and 18.20% on 14th day were found only in diabetic control group (DC). The blood glucose on the final day of treatment were 93.43 ± 3.86 mg/dl (NC), 376.14 ± 12.28 mg/dl (DC), 112.04 ± 07.15 mg/dl (STD), 133.70 ± 11.10 mg/dl (CF-17), 180.28 ± 6.61 mg/dl (CF-20) (Figure 2 and Table 2).

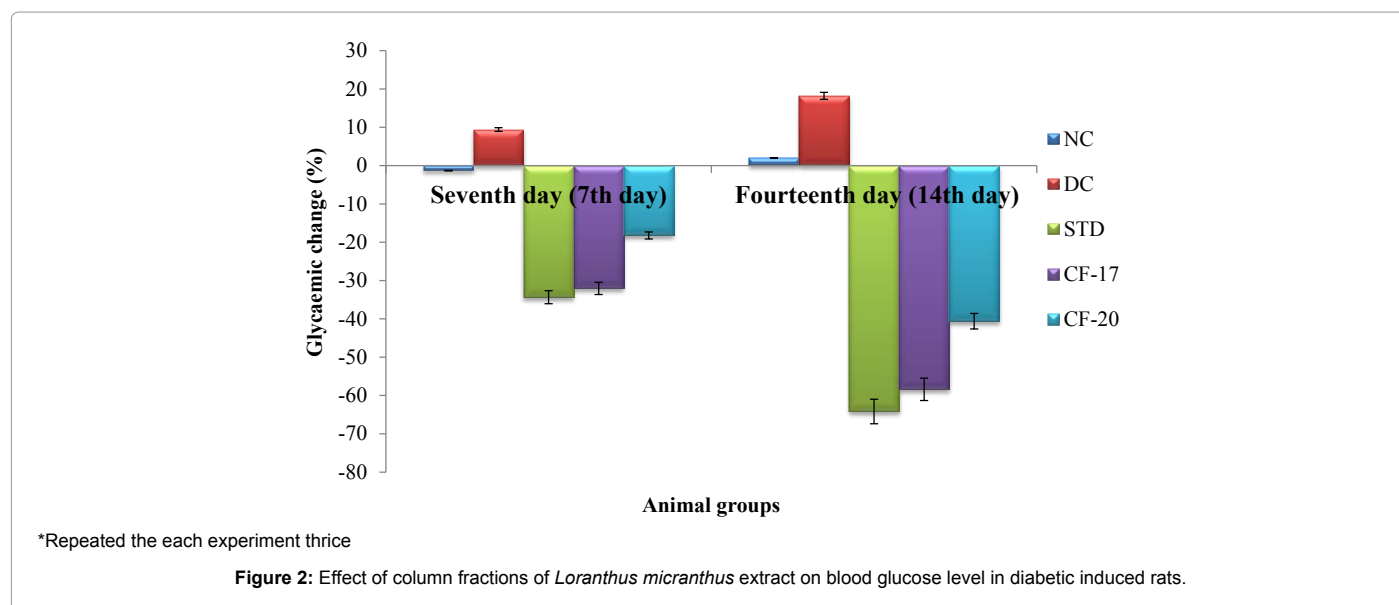


Group	Dose (mg/kg bw)	Average body weight (g)			
		Day 1	Day 7	Day 14	Change in bw (%)
NC	Normal saline	180.34 ± 4.41	196.14 ± 3.65	210.63 ± 5.31	16.79
DC	120.0 mg/kg bw	186.34 ± 6.52	181.56 ± 3.82	176.71 ± 4.69	-5.16
STD	0.5 mg/kg bw	183.43 ± 5.54	213.13 ± 3.61	218.34 ± 5.02	19.03
CF-17	400 mg/kg bw	175.65 ± 4.09	191.39 ± 5.04	204.67 ± 3.19	16.52
CF-20	400 mg/kg bw	178.08 ± 6.26	189.78 ± 4.19	200.23 ± 5.78	12.43

*Repeated the each experiment thrice

Note: NC: Normal control, DC: Diabetic control, STD: Standard drug, CF-17: Column fraction-17, CF-20: Column fraction-20 and bw: Body weight

Table 1: Effect of column fractions of *Loranthus micranthus* extract on average body weight of diabetic induced rats.



*Repeated the each experiment thrice

Figure 2: Effect of column fractions of *Loranthus micranthus* extract on blood glucose level in diabetic induced rats.

Group	Dose (mg/kg bw)	Blood glucose concentration (mg/dl)				
		Day 1	Day 7	Glycaemic change (%)	Day 14	Glycaemic change (%)
NC	Normal saline	91.60 ± 4.51	90.40 ± 5.20	-1.31	93.43 ± 3.86	1.99
DC	120.0 mg/kg bw	318.20 ± 10.50	348.20 ± 14.50	9.42	376.14 ± 12.28	18.20
STD	0.5 mg/kg bw	312.8 ± 12.45	205.36 ± 08.12	-34.34	112.04 ± 07.15	-64.18
CF-17	400 mg/kg bw	321.40 ± 8.50	218.36 ± 7.50	-32.05	133.70 ± 11.10	-58.40
CF-20	400 mg/kg bw	303.54 ± 11.20	248.20 ± 9.50	-18.23	180.28 ± 6.61	-40.60

*Repeated the each experiment thrice

Note: NC: Normal control, DC: Diabetic control, STD: Standard drug, CF-17: Column fraction-17, CF-20: Column fraction-20 and bw: Body weight

Table 2: Effect of column fractions of *Loranthus micranthus* extract on blood glucose level in diabetic induced rats.

Effect of column fractions of *Loranthus micranthus* extract on serum lipid profile

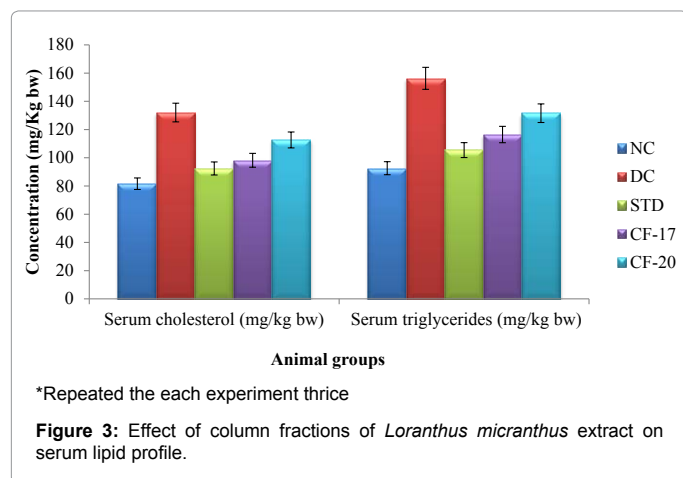
At the end of 14 day of experimental period, hyperlipidemic parameters like serum cholesterol and serum triglycerides were analyzed. The elevated levels of serum triglycerides and serum cholesterol by 132.06 ± 0.95 and 156.31 ± 2.42 mg/kg bw of diabetic control group were found in comparison to the normal control. But in diabetic induced animal groups it was shown decreased levels of serum triglycerides and serum cholesterol when treated with *Loranthus micranthus* fractions. 98.24 ± 1.03 mg/kg bw and 116.48 ± 3.52 mg/kg bw for CF-17, 112.63 ± 1.67 mg/kg bw and 131.61 ± 2.69 mg/kg bw for CF-20 in comparison to the diabetic control group. It was noticed that, CF-17 of *Loranthus micranthus* showed nearly efficient activity compared to standard drug Glibenclamide 92.40 ± 1.08 and 105.46 ± 3.02 (STD) (Figure 3 and Table 3).

Histopathology

Histopathology reports of column fractions CF-17 and CF-20 of *L. micranthus* on alloxan induced diabetic rats were shown significant effects. The results of all five groups were exhibited well differentiable cytoarchitecture (Figure 4). The cellular changes and abnormalities of each group are mentioned below.

Normal control (NC)

There was normal distribution of islets cells, acini, blood capillaries, and pancreatic ducts were observed in normal control group. Pancreatic lobes, inter lobular duct, interlobular septa were prominent. The flow of acinar cells, blood vessels and islets of Langerhans were seems to be good in number and texture. 2-3 types of distinguishable islets cells were observed along with RBC cells. The walls of pancreatic ducts, blood vessels and Langerhans cells were organised and connected



Group	Dose (mg/kg bw)	Serum cholesterol (mg/kg bw)	Serum triglycerides (mg/kg bw)
NC	Normal saline	81.64 ± 1.42	92.61 ± 2.62
DC	120.0 mg/kg bw	132.06 ± 0.95	156.31 ± 2.42
STD	0.5 mg/kg bw	92.40 ± 1.08	105.46 ± 3.02
CF-17	400 mg/kg bw	98.24 ± 1.03	116.48 ± 3.52
CF-20	400 mg/kg bw	112.63 ± 1.67	131.61 ± 2.69

*Repeated the each experiment thrice, diabetic control was compared with the normal control and extract treated groups were compared with the diabetic control. **Note:** NC: Normal control, DC: Diabetic control, STD: Standard drug, CF-17: Column fraction-17, CF-20: Column fraction-20 and bw: Body weight

Table 3: Effect of column fractions of *Loranthus micranthus* extract on serum lipid profile.

with connective tissue. Inflammatory blood vessels and inflammatory pancreatic cells were not seen (Figure 5).

Diabetic control (DC)

There were noted abnormalities in diabetic control group. Marked inflammatory blood vessels and even rupture of blood capillaries were seen. Depletion of pancreatic lobes, acini, islets of Langerhans and exocrine wall were observed. Notedly decreased number of islets of Langerhans, cells of islets and pancreatic ducts were observed. Connective tissue and blood capillaries were depleted. Some depositions on islets cells resembling amyloid formation and shrinkage of islets cells were observed. More RBC cells were interdispersed in endocrine and even in exocrine part of pancreas (Figure 6).

Glibenclamide standard (STD)

Regeneration of pancreatic ducts, acinar cells and connective cells were seen. Interlobular septa, walls of blood vessels and pancreatic ducts were seems to be normal when compared NC. Islets of Langerhans and its cells, acinar cells, pancreatic ducts were evenly distributed. Compared to normal control the texture and numbers of pancreatic cells of glibenclamide treated animals were nearly normal (Figure 7).

CF-17 of *Loranthus micranthus*

Column Fraction-17 exhibited significant effects on alloxan induced diabetic rats by preventing damage of pancreatic cells by oxidative stress, since alloxan induces diabetes through oxidative stress and damage of pancreatic cells. The pancreatic connective tissue, acini, blood vessels, blood capillaries, pancreatic ducts and their walls, islets capsule, islets cells were well organised in accordance with their

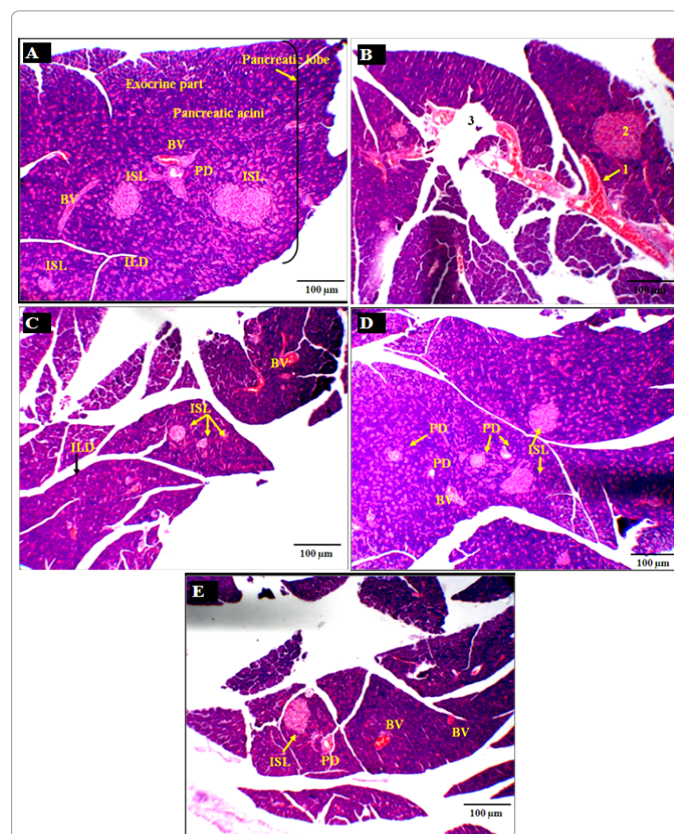
number, texture and dispersion in the pancreatic lobes. Islets cells were distinguished individually and noted the presence of 2-3 types of islets cells along with RBC cells. The architecture of pancreatic histology of CF-17 treated diabetic induced rats is almost similar compared to normal control group (Figure 8).

CF-20 of *Loranthus micranthus*

Column Fraction-20 exhibited nearly comparable results compared to reference drug. Pancreatic architecture was normal. CF-20 exhibited prominent effects in regeneration of pancreatic connective tissue, acini, blood vessels, blood capillaries, pancreatic ducts and their walls, islets capsule. Acinar cells and islets cells were well dispersed. Pancreatic tissue was organised in accordance with their number, texture. Pancreatic ducts and islets cells are evenly scattered. The sizes of islets cells seems to be normal (Figure 9).

Discussion

Out of 25 column fractions of methanolic extract, fractions 17 and 20 were chosen for GC-MS studies based on a clear separation of phytochemicals in TLC (Channabasava et al., 2014). In fraction 17, 13 prominent compounds were identified and out of which 5 compounds (1,2,3-propanetriol, diacetate, Hexadecanoic acid, octadecenoic acid and eicosanoic acid) have already proved as antidiabetic activity by possessing insulin secretion, insulin stimulation, α -glucosidase



Note: BV-Blood vessel, PD-Pancreatic duct, ISL-Islets of Langerhans, ILD-Interlobular duct.

Figure 4: A photomicrograph of over view of different rat pancreatic tissue. A) Normal control (NC), B) Diabetic control having inflammatory blood vessel, acinar and islets cell damage (DC), C) Glibenclamide, standard reference drug (STD), D) CF-17 of *Loranthus micranthus* extract and E) CF-20 of *Loranthus micranthus* extract.

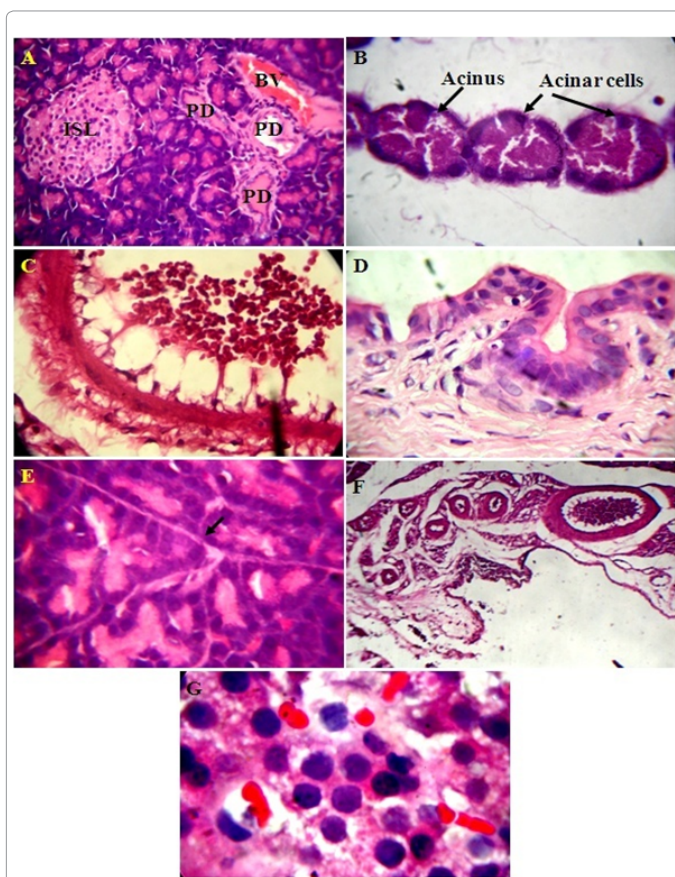


Figure 5: A photomicrograph of normal control of rat pancreatic tissue (NC). A) Normal flow of Islet's cells, blood vessel and pancreatic ducts B) Normal acini and acinar cells, C) Blood vessel having normal connective tissue and RBC cells, D) Normal exocrine pancreatic wall, E) Interlobular septa, F) More number of pancreatic ducts and blood vessels, G) Islet's cells along with blood capillaries and RBC cells (at 100x oil immersion).

inhibitors [17-19]. Fraction 20 yielded 11 different compounds in which three compounds (ar-tumerone, 3,7,11,15-tetramethyl-2-hexadecanol, trans-13-octadecenoic acid) have already exhibited antidiabetic, α -glucosidase inhibitors [20,21].

Animals are been extensively used as study model to carry out diabetes research *in vivo*. Similarly medicinal plant extracts also used to treat diabetes since from long ages. Column fractions CF-17 and CF-20 were selected for animal studies based upon the results exhibited by GC-MS studies and literature survey indicating the presence of different antidiabetic agents. After the treatment of 14 day schedule it was noticed that, the column fractions CF-17 and CF-20 of *L. micranthus* extract are efficient antidiabetic agents. Since diabetes mellitus is a state of multiple disorders, CF-17 and CF-20 were having the normal body weight, blood glucose level, serum lipid profile and also normal pancreatic histological characters in alloxan induced rats.

Slight increase in the body weight of normal control rats may be due to their normal growth. But diabetic control rats showed 5.16% reduction in their body weight. Body weight is also an important determinant in diabetes mellitus. The body weight usually decreases as diabetes progresses [22]. Significant increase in the body weight by 16.52% and 12.43% for CF-17 and CF-20 of *L. micranthus* correlates with the work carried out by Ahmed et al. [23].

Several workers have shown the hyperglycemia and hyperlipidemia,

the common characteristics of Alloxan-induced diabetes mellitus in experimental rats [24,25]. And also plant extracts have been valuable anti-diabetic agents and may involve one or more active components responsible for blood glucose reduction [26,27]. After the 14 day treatment schedule, the result clearly indicates that, CF-17 and CF-20 of *L. micranthus* have significant hypoglycaemic effect. The antihyperglycemic results are highly correlated with the work of Harikiran et al. [28] with *Bombax malabaricum* and Tyagi et al. [29] with *Anacyclus pyrethrum* in alloxan induced rats.

Hyperlipidemia, the common characteristic of alloxan induced diabetes mellitus in experimental rats and in diabetes patients [24,25] and serum triglycerides play a role in the development and progression of renal disease in type I diabetes mellitus [30,31]. Derangements in lipid metabolism in diabetes mellitus are an important determinants and status of the disease [32]. Significant decrease in the hyperlipidemic parameters like elevated serum cholesterol and serum triglycerides by CF-17 and CF-20 of *L. micranthus* attributes antihyperlipidemic activity in relation to antidiabetic activity. Flavonoids isolated from different sources [33,34] and polyphenolic extracts of *Ichnocarpus frutescens* [35] have been documented to show antidiabetic and antihyperlipidemic activities in diabetic rats in a similar way. Thus, *L. micranthus* signifies its antidiabetic and antihyperlipidemic property and strengthen the presence of various triterpenoids, flavonoids and phenolic compounds.

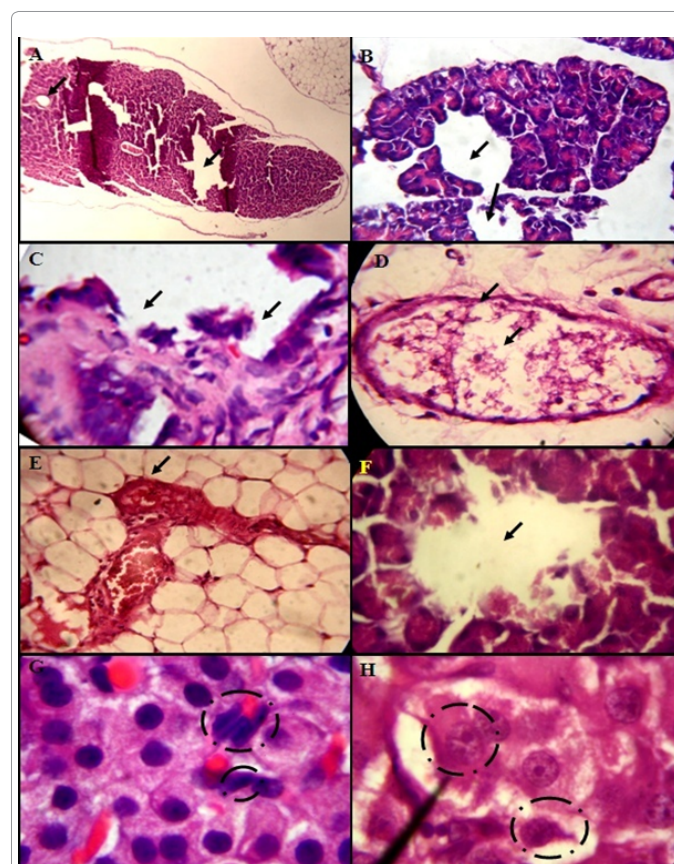


Figure 6: A photomicrograph of the effect of alloxan induction on rat pancreatic tissue in diabetic control (DC). A) Acinar cell damage and enlarged blood vessel, B) Depletion of Islet's cells, C) Depletion of exocrine layer, D) Necrosis of connective tissue of blood vessels, E) Inflammation of blood vessel, F) Islet's cells damage, G) Depletion of Islet's cells and H) Amyloid formation on Islet's cells (at 100x oil immersion).

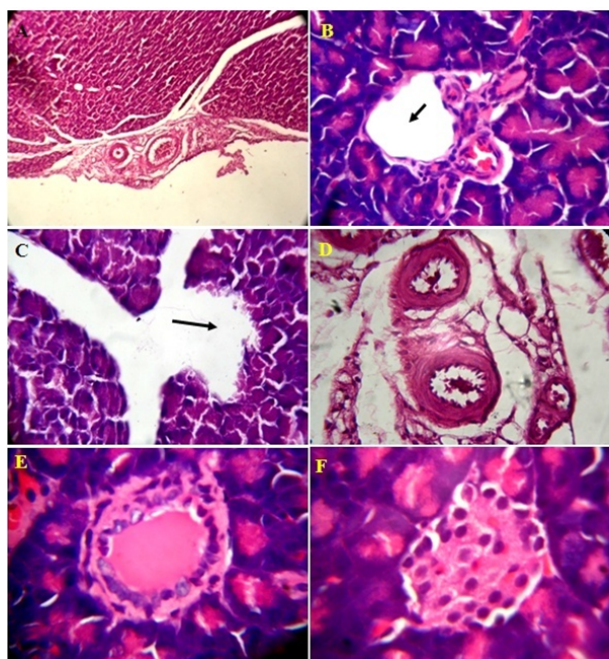


Figure 7: A photomicrograph of the effect of glibenclamide on rat pancreatic tissue (STD=0.5 mg/kg bw), A) Blood vessel having normal connective tissue, pancreatic ducts, blood vessels, B) Recovery of Islet's cells with proper pancreatic duct and blood capillaries, C) Recovery of Islet's cells D) Increase in the number of pancreatic ducts and proper connective tissue E) Normal pancreatic duct and F) Normal acini with Islet's cells and RBC cells (at 100x oil immersion).

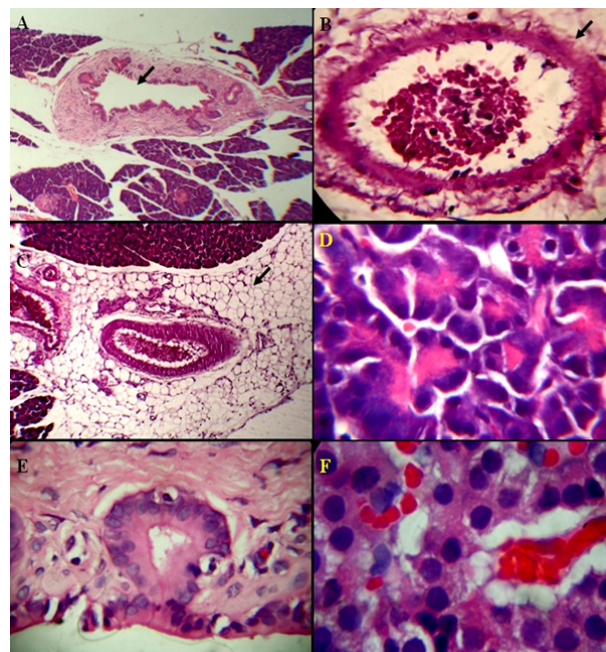


Figure 9: A photomicrograph of the effect of CF-20 of *Loranthus micranthus* extract on rat pancreatic tissue, A) Normal flow of connective tissue between exocrine part of pancreatic lobes, B) Blood vessel having normal connective tissue and RBC cells, C) Normal pancreatic ducts, blood vessels of exocrine pancreas D) Normal acini with acinar cells, E) Normal exocrine pancreatic wall, F) Islet's cells along with blood capillaries and RBC cells (at 100x oil immersion).

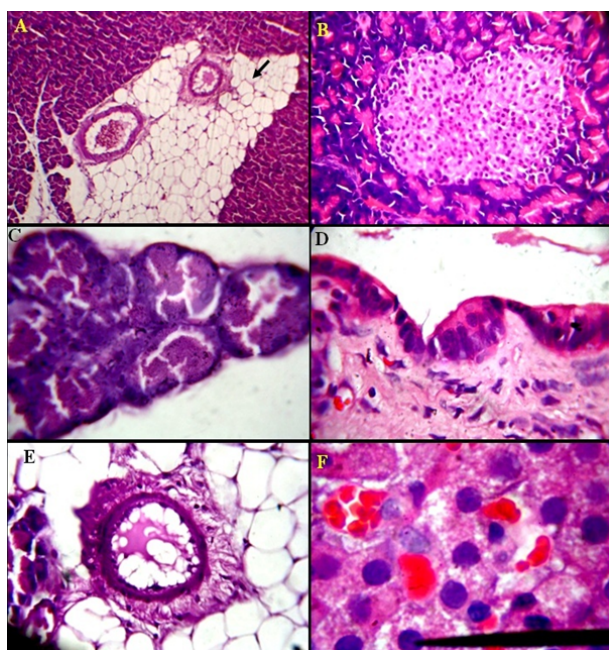


Figure 8: A photomicrograph of the effect of CF-17 of *Loranthus micranthus* extract on rat pancreatic tissue, A) Normal flow of connective tissue and proper blood vessels with exocrine part of pancreas, B) Prominent Islet's cells in Islets of Langerhans, C) Normal acini with proper adherence, D) Prominent exocrine pancreatic wall, E) Normal pancreatic duct having proper connective tissue and F) Distinguishable Islet's cells along with blood capillaries and RBC cells (at 100x oil immersion).

Alloxan is a hydrophilic and unstable analogue of glucose and selectively destroys beta cells of islets of pancreas, results in the elevation of blood glucose level, decreases protein content, and increases levels of cholesterol and triglycerides [36]. Alloxan and its product dialuric acid establish a redox potential and forms superoxide radicals. These reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of B-cells [37]. Decreasing the islets cell numbers, cell damage, and cell death [38], thickened and hyalinized blood vessels causing hypoxia and resulting in degenerative changes and necrosis [39] and structural and functional alterations such as disorganization of pancreatic architecture and depletion of insulin producing cells [40-42] are the significant pancreatic histological changes observed in alloxan induction. Histopathology reports of CF-17 and CF-20 of *L. micranthus* have shown clear confirmative results of regeneration of pancreatic connective tissue, acini, blood vessels, blood capillaries, pancreatic ducts and their walls, islets capsule, islets cells in accordance with their organization, number, texture and dispersion in the pancreatic lobes. The results were correlated with the work carried out by Prince and Kamalakkannan (2006) [43] reported the beneficial effects of rutin on islet morphology, oxidative status and glycemia in diabetic rats. The general mechanisms of regeneration include replication of existing mature β -cells and differentiation or neogenesis by ductal or intra-islet pancreatic precursor cells [44]. In alloxan-induced diabetes, (-)-epicatechin [45] and *Vinca rosea* extract [46] have also shown to act by β -cell regeneration and decrease in blood glucose.

Conclusion

The present histological study strongly provides evidence that the two fractions exert protective effect in Alloxan induced diabetic rats.

Hence, it is concluded that the two fractions possess preventive and useful effect. And it can be used as herbal medicine to protect islet cells or manage diabetes.

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