Evaluation of *in vivo* Synergistic Hypoglycemic & Hypolipidemic Activity of Ethanolic Extract of *Calotropis gigantean* Leaves in Combination to Metformin in Alloxan Induced Rats .

ABSTRACT:

Aim: The present study was designed to investigate the antidiabetic & hypolipidemic activity of *Calotropis gigantean* (Family: Apocynaceae) in alloxan-induced diabetic rat model.

Study Design: *in vivo* study was carried out by ethanolic leaf extract was administered in 250mg/kg body weight concentration and then subjected to different rats models to authenticate the antidiabetic and hyperlipidimic properties of the plant.

Place and Duration of Study: Department of Pharmacy, Southeast University, Banani, Dhaka-1213,Bangladesh within a period of July 2018 to December, 2018.

Methodology: Diabetes was induced in rats by an intraperitoneal injection (i.p) of alloxan (100 mg/kg B.W). Ethanolic leaf extract of *C. gigantean* (250 mg/kg B.W) was administrated orally as a single dose per day to the diabetic rats for 7 days. The negative control group received 0.5 ml of sterile normal saline water & positive control group received metformin with 100 mg/kg B.W & 50 mg/kg B.W doses in combination to extract to evaluate synergistic effect. After 7 days study period, fasting blood glucose, total cholesterol, triglyceride, high-density lipoprotein cholesterol, liver weight & body weight were measured

Results: Individual plant extract & standard reduced FBG significantly by 52% (P<0.001) & 55.3% (P<0.001) correspondingly. Metformin (100mg/kg B.W) potentiated reduction (68%) (P<0.001) when combined to plant extract (250 mg/Kg B.W). Significant dose dependent manner was followed when metformin (50 mg/kg B.W) was combined to plant extract (250mg/Kg B.W). Our results clearly suggest that *C. gigantean* exhibit hypoglycemic & hypolipidemic activity with an alteration in body-liver weight. The present study also suggested to develop a combination therapy of extract along with metfromin in different doses to minimize the intake of synthetic drug. Significant reduction of TG, TC were noted by extract (250 mg/kg B.W) with 32.42% (P<0.001) & 41.32% (P<0.001) respectively where standard shown the diminution 43.43% (P<0.05) & 47.21% (P<0.001) respectively as compare to Untreated diabetic rats. 50.21% (P<0.01) & 42.38% (P<0.001) reduction of TG & TC were estimated by *C.gigantea* extracts (250 mg/kg B.W) when combined with Metformin (100 mg/kg B.W). 34.53% (P<0.05) & 41.54% (P<0.001) reduction of TG & TC by *C.gigantea* extracts (250 mg/kg B.W) were confirmed when combined to Metformin (50 mg/kg B.W). Combination therapy also shown synergistic effect in elevation of plasma HDL-cholesterol.

Conclusion: The results of the study concluded that *Tricosanthes tricuspidata and Clematis montana* leaf and root extracts have potential antidiabetic and antioxidant properties.

Keywords: *Calotropis gigantean, diabetes mellitus, hypolipidemic activity & antidiabetic activity.*

1. BACKGROUND

As Diabetes mellitus is a public health challenge the complications are raising day to day life. According to World Health Organization the diabetic population is likely to increase up to 300

million or more by the year 2025 [1]. Diabetes mellitus (DM) is a severe physiological problem being one of the major causes of death all over the world, and if not treated, it can lead to many complications [2] such as long term damage, dysfunction, and failure of various organs [3]. This disease is caused by the destruction or dysfunction of pancreatic of *β*-cell and insulin resistance which results in elevating blood glucose level, known as hyperglycemia [4, 5]. Aldose reductases, a key enzyme in the polyol pathway catalyze the glucose to be reduced to sorbitol. Accumulation of sorbitol in the body causes various complications [6]. Over time, diabetic patients with poor glycemic control undergo various life threatening difficulties which include nephropathy, retinopathy, neuropathy, and cardiovascular diseases [7]. Alongside with exercise, modern drugs such as pioglitazone, biguanides, meglitinides, thiazolidinedione, alpha glucosidase inhibitors and sulphonylureas shows considerable benefits with side effects like hypoglycemia, GIT disturbance, , water intoxication, and hyponatremia, obesity with high cost for long term use [8]. Numerous agents that are currently used for the treatment of type 2 diabetes are facing limited efficacy and tolerability [9]. For instance, sulfonylureas induce *β*-cell death in isolated rodent and human islets while glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors have potential risks for pancreatitis, pancreatic, and thyroid cancers [10]**.** So natural compounds could be great substitute when taken with synthetic drugs.

Now a days medicinal plants show the proof to be used as hypoglycemic agent as most of plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids **etc.**[11] that significantly posses antidiabetic effect. Antihyperglycemic activity of the plants is mainly due to their ability to renovate the function of pancreatic tissues by causing an elevation in insulin output or hindering the intestinal absorption of glucose, facilitating of metabolites in insulin dependent or amylase and glucosidase inhibitor as these enzymes are responsible for breaking *α*- 1, 4 bonds in complex carbohydrate to elevate FBGL [12].

Calotropis gigantean (Family: Apocynaceae) is a common weed in open waste ground, roadsides, village surroundings and railway lines. *It* is native to continental Asia and South-East Asia and has been introduced in the Pacific Islands, Australia, Central and northern South America and Africa [13]. Mainly known as crown flower, crown plant, giant milkweed or rubber bush [14]. Different parts of the plant contains stigmasterol, ß-sitosterol [15], mudarine, glycosides (calotropin uscharin, calotoxin), lupeol, calotropin, uscharin, calotoxin, calactin and uscharidin; gigantin, protease such as calotropin DI and DII and calotropin FI and FII [16]. Calotropnaphthalene, calotropises juiterpenol, calotropisesterterpenol and calotropbenzofuranone along with sucrose, have been isolated from *Calotropis gigantean* [17]. Traditionally different parts of the plant are used such as in leprosy, eczema, syphilis, elephantiasis, ulceration, and cough [18] purgative and gastrointestinal irritant and abortion inducer [19], paralysis, swellings, intermittent fevers, asthma, anorexia, helmintic infections, inflammations, cutaneous infections, intestinal worms, ascites, bronchitis and dyspepsia and promotes gastric secretions [20], in poisonous snake or rat bites, periodic fever, vatha diseases, ulcers, cures dental problems, gonococcal arthritis and other rheumatic complaints[21]. The plant proves to hold some pharmacological effects like antipyretic [22], proteolytic activity [23] antiamoebic [24] wound healing [25], hepatoprotective [26] and anti-oxidant [27] properties. Other reported potentials are analgesic activity [28], antimicrobial [29] and cytotoxic activity [30], anti-diarrhoeal activity anti-Candida activity [31], anti-pyretic activity [32], insecticidal activity [33], CNS activity [34] and pregnancy interceptive properties [35], procoagulant activity [36]. *C. gigantea* is reported to possess major phytochemical groups as alkaloids, cyanogenic, glycosides, phenolics, tannins [37], cardenolides, ester [38,39], flavonoids [40], terpenes [41] (antimosquito larvicidal activity) sterols (Campesterol ,Stigmasterol, gamma-Sitosterol, Desmosterol anticervical cancer property), Proteinases [42] and nonprotein amino acid [43]. Acetates and the benzoates, α-and β-calotropeols and β-amyrin, tetracyclic triterpene compounds and traces of sterols, Giganteol acetate, Giganteol are also reported by P. Bhaskara *et al*. [44].

Therefore, the aim of this study was to find out the scientific basis of the use *C. gigantean* in the management of diabetes & hyperlipidemia used by traditional practitioners using ethanol extracts on alloxan-induced diabetic mice.

2. MATERIALS AND METHODS

2.1 Experimental Animals

30 Long Evan rats with (gender: male, wg: 80±10g) were obtained from ICDDR, B Mohakhali, Dhaka, Bangladesh. Rats were housed under standard laboratory conditions (22-25^{0}C, humidity 40-60%,12 hr light:12 hr dark cycle) and housed in standard size metallic cages (5 rats/ cages) in properly ventilated room. Through the experiments all rats were fed with standard laboratory diet. Prior to the beginning of the study, animals were allowed for two weeks to acclimatize to laboratory conditions.

2.2 Collection of Plant Material and Preparation of Extracts

C. gigantea plant was collected from the natural population growing in the Gazipur, Dhaka, Bagladesh & authenticated by the expert taxonomist from Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh (Accession number: 45130). Leaves were washed and shade dried for several days followed by grinding using mechanical grinder. About 200 gm dried powder were soaked in 800 ml ethanol and kept for a period of about 7 days with occasional shaking and stirring. The whole mixture is then filtered through Whitman No.1 filters paper and concentrated by a rotary evaporate under reduced pressure at 50^{0}C temperature to afford crude extract with gummy or semisolid appearance. The concentrate was stored in an airtight container and kept in a cool, dark and dry place until the next course of action.

2.3 Chemicals

Alloxan and metformin were purchased from Sigma-Aldrich and Merck company (Germany) respectively. All other used chemicals were of analytical grade and were obtained from standard commercial suppliers.

2.4 Induction of Diabetes

Diabetes was induced in overnight fasted Evan rats by single-dose intraperitoneal injection of freshly prepared alloxan at 140 mg/kg body weight dissolved in 0.5 ml of sterile normal saline water and drink 10% glucose solution to overcome drug induced hypoglycemia. After 72 hours blood glucose level was measured by using tail blood sample. Rats with fasting blood glucose level above 7.0 mmol/L were selected for further study.

2.5 Experimental Design

Long Evan rats were randomly assigned into group I, II, III, IV, V, VI with 5 rats in each group for the respective one week treatment due to determination of blood glucose, lipid profile test studies.

Group I: Non Diabetic Normal Control (Only water & normal diet)

Group II: Diabetic Control (Only water & normal diet)

Group III: Diabetic Control+ Metformin (100 mg/kg B.W in 0.5 ml 99% DMSO)

Group IV: Metformin(50 mg/kg B.W) + Ethanolic Extract of *C.gigantea* (250mg/kg B.W in 0.5 ml 99% DMSO)

Group V: Metformin (100 mg/kg B.W) + Ethanolic Extract of *C.gigantea* (250 mg/kg B.W in 0.5 ml 99% DMSO)

Grroup VI: Diabetic Control+ Ethanolic Extract of *C.gigantea* (250 mg/kg B.W in 0.5 ml 99% DMSO)

2.6 Collection of blood and determination of Biochemical Parameters

At 0^{th}, 3^{th}, 5^{th} & 7^{th} day, blood samples were collected from tail vein after the administration of metformin & ethanolic extract of *C.gigantea* and blood glucose levels were determined by using by glucose meter. After completing the one week treatment the rats were at first anesthetized with chloroform and 3 ml of blood was directly collected from heart by syringe. Immediately after blood samples collection, serum was isolated by centrifugation at 4000 rpm for 20 min and then analyzed for various biochemical parameters. The serum samples were stored at −80 °C in a freezer until they were analyzed. The concentration of TC, TG, HDL-Cholesterol were measured colorimetrically by blood analyzer using commercially available wet reagent diagnostic kits (HUMAN GmbH, Germany).

2.7 Statistical Analysis

The results were expressed as mean ± SD. Data analysis was performed by the SPSS version 24 (SPSS/IBM, Chicago, IL) using one-way analysis of variance (ANOVA) and Dunnett's test. To assess the individual variations between the control and treatment groups, $P < 0.05$ was considered significance level.

3. RESULTS

3.1 Antidiabetic Activity:

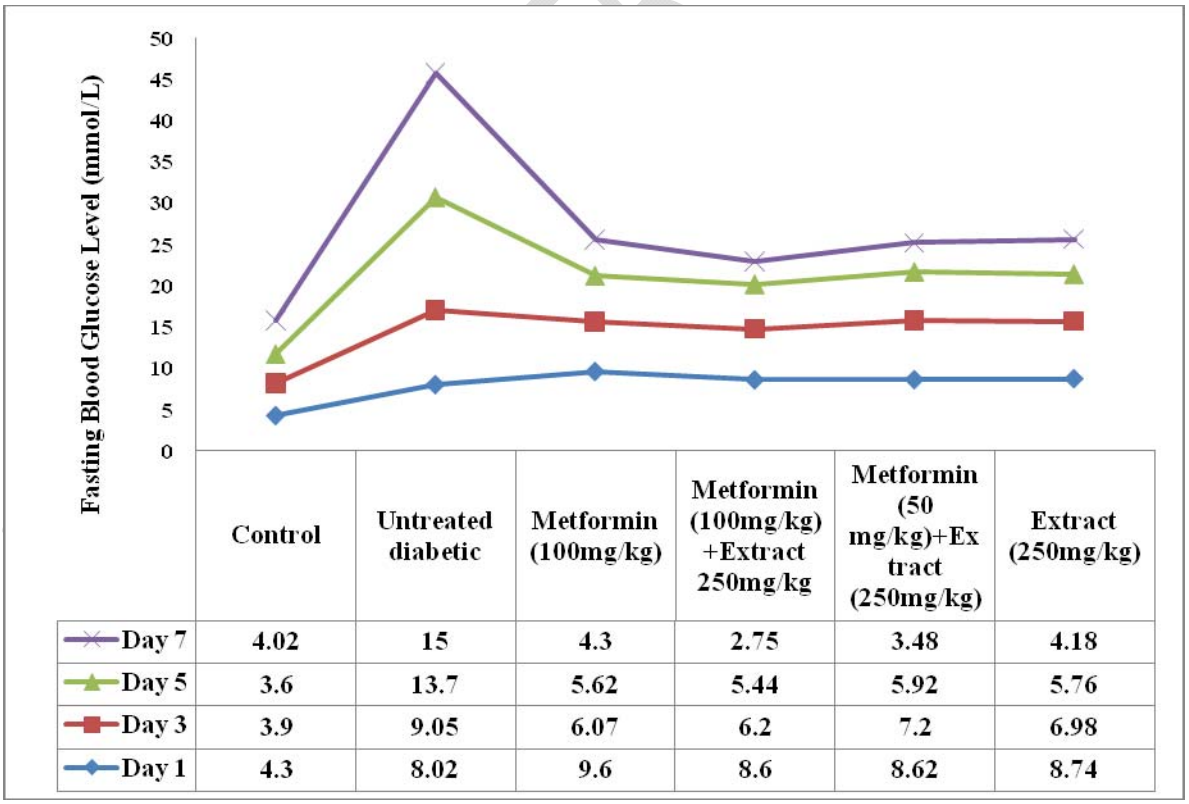
Table 1: Effect of *C. gigentea* on fasting blood glucose levels in alloxan induced diabetic rats.

Groups	Day of treatment			
	Day 1	Day 3	Day 5	Day 7
Control	4.3±0.29	3.9±0.37	3.7±0.20	4.02±0.26
Untreated diabetic	8.02±0.53	9.05±1.02 (12.84)	13.6±1.02 (69.58)	15.00 ±3.15 (87.03)
Diabetic+Metformin (100 mg/kg B.W)	9.6±0.98***	6.07±0.37*** (36.77)	5.62±0.07*** (41.46)	4.30±0.07*** (55.21)
Metformin (100 mg/kg B.W)+Extract (250 mg/kg B.W)	8.6±0.37***	6.2±0.12*** (27.91)	5.44±0.17*** (36.74)	2.75±0.35*** (68.02)
Metformin (50 mg/kg)+Extract (250	8.62±0.28***	7.2±0.12*** (16.47)	5.92±0.09*** (31.32)	3.48±0.37*** (59.63)

mg/kg B.W)				
Extract (250 mg/kg B.W)	8.74±0.46***	6.98±0.24*** (20.14)	5.76±0.29*** (34.1)	4.18±0.24*** (52.17)

Values are expressed as mean ± SD (*n* = 5 rats). Significance level among different groups at $P < 0.05$. ($^{*}P<0.05$; $^{**}P<0.01$, $^{***}P<0.001$); Diabetic rats were compared with normal rats. **Metformin** and ***C.gigantea*** treated diabetic rats were compared with diabetic rats.

At all-time points, blood glucose concentration remain unchanged ($p<0.001$) in normal rats treated with distilled water. BGL were gradually decreased for each group at 1st, 3rd, 5th & 7th day. The FBGL of all groups were compared to untreated diabetic group **(Table: 01, Figure: 01).** At 7th day, oral administration of *C.gigantea* extracts (250 mg/kg B.W) significantly decreased the blood glucose level 52.17% ($P<0.001$). Combination therapy was performed to establish synergistic effect with two doses. Metformin (100 mg/kg B.W) has shown significant FBGL reduction by 55.21% ($P<0.001$) individually but potentiated reduction (68.02%) ($P<0.001$) when combined to plant extract (250 mg/Kg B.W). Dose dependent manner (59.63%) ($P<0.001$) was followed when metformin (50 mg/kg B.W) was combined to plant extract (250 mg/Kg B.W) with reduced dose. The possible mechanism by which *C.gigantea* brings about its hypoglycemic action may be stimulating the insulin effect of serum by increasing either the pancreatic secretion of insulin from the beta - cells of islets of langerhans or its release from bound insulin. Thus, the significant antidiabetic effect of the extracts of *A.remota* could be due to the presence of the flavonoids, tannin and alkaloid in the extracts, which could act synergistically and/or independently to enhance the activity of glycolytic enzymes.



	Control	Untreated diabetic	Metformin (100mg/kg)	Metformin (100mg/kg) +Extract 250mg/kg	Metformin (50 mg/kg)+Extract (250mg/kg)	Extract (250mg/kg)
Day 7	4.02	15	4.3	2.75	3.48	4.18
Day 5	3.6	13.7	5.62	5.44	5.92	5.76
Day 3	3.9	9.05	6.07	6.2	7.2	6.98
Day 1	4.3	8.02	9.6	8.6	8.62	8.74

Figure 01: Effect of *C.gigentea* extracts & metformin on fasting blood glucose level in alloxan induced diabetic rats.

3.2 Hypolipidemic Activity:

Table 02: Effect of *C.gigentea* on lipid profile in alloxan induced diabetic rats.

Groups	Organ weight (mg/g)	Lipid profile (mmol/l)		
	Liver	TG	TC	HDL-C
Control	4.73	3.75±0.95	8.86±0.95*	8.12±0.41
Untreated diabetic	4.15*** (12.26)	4.72±0.64*** 25.87	9.51±0.76*** 7.34	5.43±0.20*** 33.13
Diabetic+ Metformin (100 mg/kg B.W)	4.49* (8.19)	2.67±0.29*** (43.43)	5.02±0.12*** (47.21)	6.78±0.415** (24.86)
Metformin (100 mg/kg)+Extract (250 mg/kg B.W)	4.61** (11.08)	2.35±0.37** (50.21)	5.48±0.46*** (42.38)	6.81±0.26** (25.23)
Metformin (50 mg/kg B.W)+Extract (250 mg/kg B.W)	4.31** (3.86)	3.09±0.49 (34.53)*	5.56±0.62*** (41.54)	6.64±0.98** (22.28)
Extract (250 mg/kg B.W)	4.25*** (2.41)	3.19±0.40 (32.42)*	5.58±0.35*** (41.32)	6.60±0.415** (21.55)

Values are expressed as mean ± SD (n = 5 rats). Significance level among different groups at $P < 0.05$. ($^{*}P<0.05$; $^{**}P<0.01$,$^{***}P<0.001$); Diabetic rats were compared with normal rats. **Metformin** and ***C.gigantea*** treated diabetic rats were compared with diabetic rats.

Table 03: Effect of *C.gigentea* on mean weight of liver in alloxan induced diabetic rats.

Groups	Mean weight of animals at sacrificial time (Grams)	Mean weight of Liver at sacrificial time (Grams)	Weight of liver in grams/kg body weight
Control	110	4.73	43 gm/kg
Untreated diabetic	95	4.15	43.68 gm/kg

Table 02 & Figure 02 showed that the effect of the *C.gigantea* extract on TG,TC, HDL in alloxanized diabetic rats. After alloxan induced, the result showed that TG, TC increased while HDL decreased compare to Untreated diabetic rats (Table: 02). Highest reduction of TG, TC were shown by Metformin (100 mg/kg B.W) like 43.43% ($P<0.05$) & 47.21% ($P<0.001$) respectively where extracts shown significant diminution by 32.42% ($P<0.001$) & 41.32% ($P<0.001$) respectively. Combination study of Extract to Meformin was performed to develop the synergistic effect with different doses in dose dependent activity.

Metformin (100 mg/kg B.W) with *C.gigantea* extracts (250mg/kg) reduced TG & TC by 50.21% (*P*<0.01) & 42.38% (*P*<0.001) respectively & metformin (50 mg/kg B.W) with *C.gigantea* extracts (250mg/kg) lessened TC & TC level by 34.53% (*P*<0.05) & 41.54% (*P*<0.001) The administration of the extract of *C.gigantea* produced a significant increase in the level of High-density lipoprotein-cholesterol (HDL-C) in individual & combination groups.

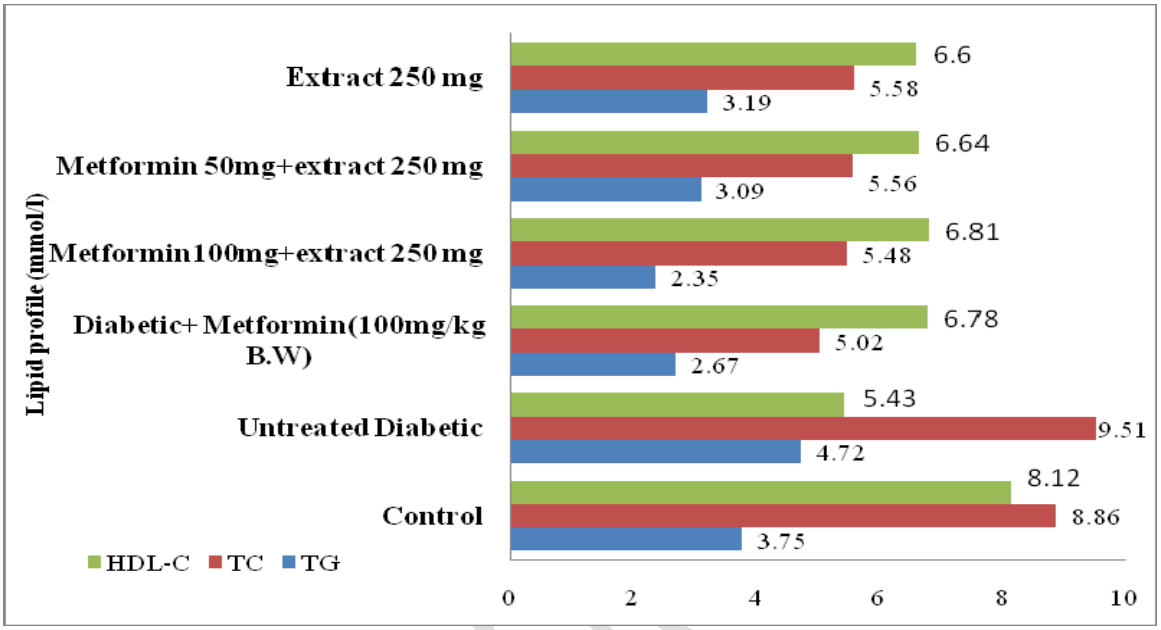


Figure 02: Effect of *C.gigentea* on lipid profile in alloxan induced diabetic rats.

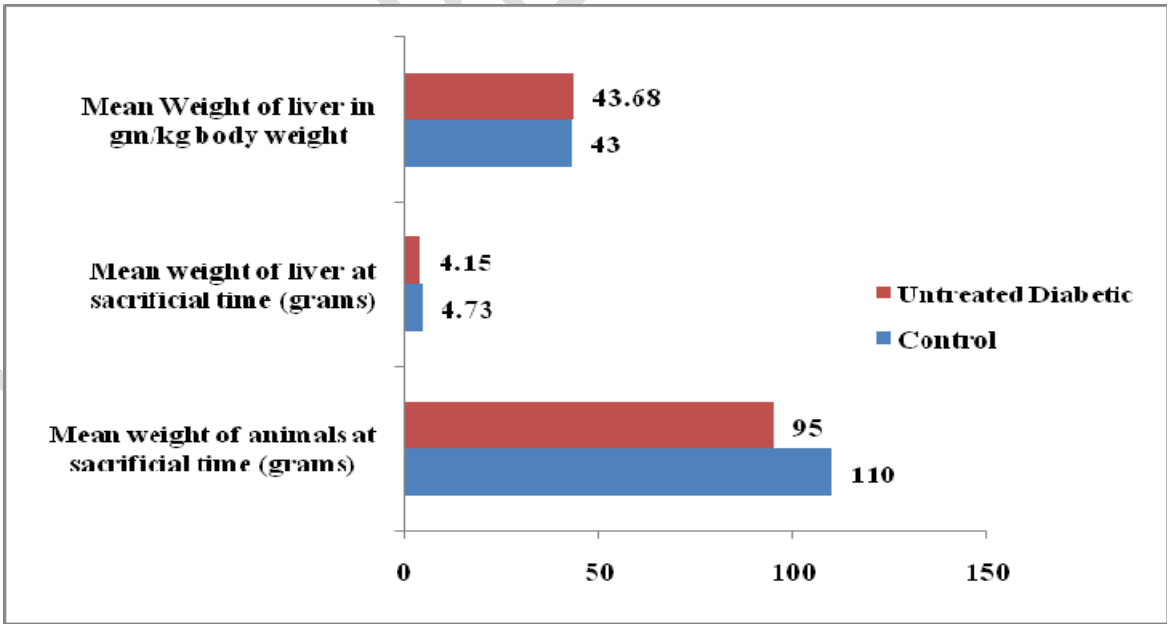


Figure 03: Effect of *C.gigentea* on mean weight of liver in alloxan induced diabetic rats.

Significant decrease of liver weight was revealed in diabetic rat (12.26%) (*P*<0.001) as compared to control. The tested compound confirmed slightly significant increase (2.41%) (*P*<0.001) of liver weight in

comparison to untreated diabetic. Metformin (100 mg/kg B.W) with *C.gigantea* extracts (250 mg/kg B.W) & Metformin (50 mg/kg B.W) with *C.gigantea* extracts (250 mg/kg) increase liver weight 11.08% & 3.86% (*P*<0.01). During sacrificial time mean liver weight & body weight of alloxanized group also compared to that of control group **(Table: 03, Figure: 03).** Liver weight was slightly increased in diabetic rats (43.00 gm/kg B.W & 43.68 gm/kg B.W) when compared with non-diabetic rats.

The observed significant reduction in serum total lipids, total cholesterol and LDL cholesterol by the extract which can be attributed to the phytochemical constituents that propose the use of the plant to prevent cardiovascular complications arising from hyperlipidemia [45]

Discussion

New antidiabetic drugs from natural plants are already in search that contain phytochemical compounds with high efficacy with minimum toxicity. As most of plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, *etc.*, that are significantly posses antidiabetic effect [12] Plant extracts are evaluated to balance the liberation and absorption of glucose is becoming a striking therapeutic choice in the treatment of diabetes mellitus.

Alloxan, a beta cytotoxic agent, rapidly and selectively accumulates in pancreatic beta cells] and causes beta cell death and apoptosis by generation of reactive oxygen species (ROS), super oxide radicals and hydrogen peroxide [46]. Sequential injection of alloxan caused a significant increase ($p<0.05$) in blood glucose concentration for 7 days in all group of rats compared with their respective baseline blood glucose and to control values. Single & combination therapy was performed to establish synergistic effect with two doses of metformin for 7 days. The estimated results were taken after 7^{th} days. Individual plant extract & standard reduced FBG significantly by 52% (*P*<0.001) & 55.3% (*P*<0.001) correspondingly. Metformin (100mg/kg B.W) potentiated reduction (68%) (*P*<0.001) when combined to plant extract (250 mg/Kg B.W). Significant dose dependent manner was followed when metformin (50 mg/kg B.W) was combined to plant extract (250mg/Kg B.W) with reduced dose. This results can led to a development of new drug design with reduced dose of standard when taken with leaf extract of *C.gigantea*. The studied plant may reduce absorption of glucose from the small intestine as glucose liberation from disaccharides is reduced. In our study, it is found that extract have also hypoglycemic effect in glucose induced hyperglycemic rats. Diabetes induction mainly alters morphological changes & level of enzymatic metabolism. In the study liver size were measured for extract along with standard. Significant & dose dependant increase of liver size were found in rats in comparison to untreated diabetic.

Hyperlipidemia is a recognized outcome of Diabetes mellitus [47]. Abnormal high concentration of serum lipids result from increase in the mobilization of free fatty acids from the peripheral storehouse. The marked hyperlipidaemia that characterizes the diabetic state is the consequence of the dysfunction of lipolytic hormones on the fat depots [48]. Hyperlipidemia associated with diabetes mellitus is reduced by limited absorption of free fatty acids and free cholesterol following inhibition of pancreatic lipase and pancreatic cholesterol esterase [49]. Significant reduction of TG, TC were noted by extract (250 mg/kg B.W) with 32.42% (*P*<0.001) & 41.32% (*P*<0.001) respectively where Standard shown the diminution 43.43% (*P*<0.05) & 47.21% (*P*<0.001) respectively as compare to Untreated diabetic rats. 50.21% (*P*<0.01) & 42.38% (*P*<0.001) reduction of TG & TC were studied by *C.gigantea* extracts (250 mg/kg B.W) with Metformin (100 mg/kg B.W). TG (34.53%) (*P*<0.05) & TC (41.54%) (*P*<0.001) reduction by *C.gigantea* extracts (250 mg/kg B.W) were expressed when combined to Metformin (50 mg/kg B.W). Combination therapy alsoshown synergistic effect in elevation of plasma HDL-cholesterol that prevent risk of developing cardiovascular disease. The administration of the extract of *C.gigantea* produced a significant increase in the level of High-density lipoprotein-cholesterol (HDL-C). The plant demonstrated a

cardioprotective effect via an increase in HDL-cholesterol levels. The extracts of *C.gigantea* prove to have a hypolipidemic potential. Alteration of liver weight is also related to diabetic. The present study has shown related reduction of liver weight according to the dose of studied sample & standard in individual & combination design. The liver is an insulin-sensitive organ that undergoes functional abnormalities in individuals with untreated diabetes [50]. In this study, the liver of diabetic animals & control animals were compared. An increase (hypertrophy) in the weight of liver in proportion to the body weight was observed despite the reduction of the mean weight of all the animals in Alloxan induced group. It could be ascribed to increased triglyceride accumulation that can lead to liver enlargement by reason of increased entry of fatty acids into the liver induced by hypoinsulinemia [51] and the less elimination of lipoprotein from liver. Previous research articles also present the same agreement with the present findings [52].

4. Conclusion

It had been concluded that in our study, decrease in the concentration of glucose, total triglyceride, total cholesterol, and increase in HDL cholesterol were observed for ethanolic extract of *C.gigantea* leaves along with metformin. The antidiabetic and hypolipidemic activity of the plant source is due to the phyto chemical constituents present in the plant. this justifies its use in ethnomedicine and can be exploited in the management of diabetes induced hyperlipidemia. Further studies are in progress for isolation and identification of lead compound to design a combination therapy in conjunction with synthetic drug.

Ethical Considerations

This was carried out in strict compliance with the National Research council guidelines on the care and use of laboratory animals to minimize research animal pain and suffering [53].

Conflict of Interests

The authors declare that they have no conflicts of interest.

Data Availability

The data used to support the findings of this study are included within the article.

Reference

1. Sy GY, Cissé A, Nongonierma RB, Sarr M, Mbodj NA, Faye B. Hypoglycaemic and antidiabetic activity of acetonic extract of *Vernonia colorata* leaves in normoglycaemic and alloxan-induced diabetic rats. J Ethnopharmacol. 2005;98(1–2):171–175.

2. El-Hilaly J, Tahraoui A, Israili ZH, Lyoussi B. Acute hypoglycemic, hypocholesterolemic, and hypotriglyceridemic effects of continuous intravenous infusion of a lyophilised aqueous extract of Ajuga iva L. Schreber whole plant in streptozotocin-induced diabetic rats. Pakistan Journal of Pharmaceutical Sciences, 2007; 20(4): 261–268.

3. Lyra R, Oliveira M, Lins D, Cavalcanti N. Prevention of type 2 diabetes mellitus. Arquivos Brasileiros de Endocrinologia & Metabologia. 2006;50(2): 239–249.

4. American Diabetes Association. “Diagnosis and classification of diabetes mellitus,” *Diabetes Care*. 2010; 33(1): 62–69.

5. M. E. Cerf, Beta cell dysfunction and insulin resistance. *Frontiers in Endocrinology*. 2013; 4:37.

6. D. Deshpande, M. Harris-Hayes, and M. Schootman. Epidemiology of diabetes and diabetes-related complications. *Physical therapy*. 2008;88(11):1254–1264.

7. Lee HS. Rat lens aldose reductase inhibitory activities of *Coptis japonica* root-derived isoquinoline alkaloids. J Agric Food Chem. 2002;50(24):7013–7026.

8. Deepa VS, Rajaram K and Kumar PS. *In vitro* and *In vivo* antidiabetic effect of *Andrographis lineate* Wall. Ex.Nees and Andrographis serphyllifolia Wt.Ic leaf extracts. *African Journal of Pharmacy and Pharmacology.* (2013);7: 2112–2121.

9. X. Xu, G. Wang, T. Zhou, L. Chen, J. Chen, and X. Shen. Novel approaches to drug discovery for the treatment of type 2 diabetes. *Expert Opinion on Drug Discovery*. 2014; 9(9): 1047–1058.

10. Jonathan Emeka Emordi , Esther Oluwatoyin Agbaje, Ibrahim Adekunle Oreagba and Osede Ignis Iribhogbe. Antidiabetic Effects of the Ethanolic Root Extract of *Uvaria chamae* P. Beauv (Annonaceae) in Alloxan-Induced Diabetic Rats: A Potential Alternative Treatment for Diabetes Mellitus. Hindawi Advances in Pharmacological Sciences. 2018:1-13.

11. Malviya N, Jain S, Malviya S. Antidiabetic potential of medicinal plants. Acta Pol Pharm. 2010;67(2):113–118.

12. An overview on antidiabetic medicinal plants having insulin mimetic property, DK Patel, SK Prasad, R Kumar, and S Hemalatha* Asian Pac J Trop Biomed. 2012; 2(4): 320–330.

13. Ahmed, K.K.M., Rana, A.C. & Dixit, V.K. *Calotropis* species (Ascelpediaceae) - a comprehensive review. Pharmacognosy Magazine. 2005; 1(2): 48–52.

14. Ajay, K., Patil, P.A. Purnima, A. & Basavaraj, H. Anti-inflammatory and anti-ulcer effects of *Calotropis gigantea* R.Br flowers in rodent. Journal of Natural Remedies. 2008; 8(2): 183–190.

15. http://www.hear.org/pier/species/calotropis_gigantea.htm.

16. http://www.stuartxchange.org/Kapal-kapal.html.

17. Malaya k. misra, manoj k. mohanty1 and pradeep k das. Studies on the method ethnobotany of *calotropis gigantea* and *c.procera*. Ancient science of life. 1993; 1 & 2: 40 – 56.

18. Chitme HR, Chandra M, Kaushik S. Studies on anti-diarrhoeal activity of *Calotropis gigantea* R.Br. in experimental animals. J Pharm Pharm Sci 2004. Feb;7(1):70-75.

19. Srivastava SR, Keshri G, Bhargavan B, Singh C, Singh MM. Pregnancy interceptive activity of the roots of *Calotropis gigantea* Linn. in rats. Contraception 2007.;75(4):318-322.

20. Mahajan RT, Badgujar SB, Phytochemical Investigations of some laticiferous plants belonging to Khandesh Region of Maharashtra. Ethnobotanical Leaflets 2008;12:1145-1152.

21. A Review on Pharmacological and Phytochemical Profile of *Calotropis Gigantea* Linn Gaurav Kumar, Loganathan Karthik and Kokati Venkat Bhaskara Rao, *Pharmacologyonline.*2011;1: 1-8.

22. Sengupta A, Bhattacharya D, Pal G, Sinha NK. Comparative studies calotropin DI and DII from the latex of *Calotropis gigantea.* Arch Biochem Biophys. 1984;1:17-25.

23. Rajesh R, Raghavendra Gowda CD, Nataraju A, Dhananjaya BL, Kemparaju K, Vishwanath BS. Procoagulant activity of *Calotropis gigantea* latex associated with fibrin(ogen)olytic activity. Toxicon. 2005;46(1):84-92.

24. Singh S, Bharti N, Chugh M, Naqvi F, Azam A. Activity of extracts and procesterol from *Calotropis gigantea* against Entamoeba histolytica. Nat Prod Commun. 2010.;5(6):867-868.

25. Deshmukh PT, Fernandes J, Atul A, Toppo E. Wound healing activity of Calotropis gigantea root bark in rats. J Ethnopharmacol. 2009;125(1):178-181.

26. Lodhi G, Singh HK, Pant KK, Hussain Z. Hepatoprotective effects of *Calotropis gigantea* extract against carbon tetrachloride induced liver injury in rats. Acta Pharm 2009. ; 59(1):89-96.

27. Bedekar A, Shah K, Koffas M. Natural products for type II diabetes treatment. Adv Appl Microbiol. 2010;71:21-73.

28. Pathak AK, Argal A, Analgesic activity of *Calotropis gigantea* flower. Fitoterapia 2007;78(1):40-42.

29. MR Habib; MR Karim, Antimicrobial and Cytotoxic Activity of Di-(2-ethylhexyl) Phthalate and Anhydrosophoradiol-3-acetate Isolated from *Calotropis gigantea* (Linn.) Flower. Mycobiology. 2009; 37(1):31-36.

30. Kumar G, Karthik L, Bhaskara Rao KV, *In vitro* anti-Candida activity of *Calotropis gigantea* against clinical isolates of *Candida*. Journal of Pharmacy Research. 2010;3(3):539-542.

31. Kumar G, Karthik L, Bhaskara Rao KV, Antibacterial activity of aqueous extract of *Calotropis gigantea* leaves – an *in vitro* study. International Journal of Pharmaceutical Sciences Review and Research. 2010;4(2):141-144.

32. Chitme HR, Chandra R, Kaushik S, Evaluation of antipyretic activity of *Calotropis gigantean* (Asclepiadaceae) in experimental animals. Phototherapy Research. 2005;19(5):454-456.

33. Alam MA, Habib MR, Nikkon F, Khalequzzaman M, Karim MR, Insecticidal activity of root bark of *Calotropis gigantea* L. against *Tribolium castaneum* (Herbst). World Journal of Zoology. 2009;4(2):90-95.

34. Argal A, Pathak AK, CNS activity of *Calotropis gigantea* roots. J. Ethnopharmacol. 2006;106(1):142-145.

35. Srivastava SR, Keshri G, Bhargavan B, Singh C, Singh MM, Pregnancy interceptive activity of the roots of *Calotropis gigantea* Linn. in rats. Contraception. 2007;75(4):318-322.

36. Rajesh R, Raghavendra Gowda CD, Nataraju A, Dhananjaya BL, Kemparaju K, Vishwanath BS, Procoagulant activity of *Calotropis gigantea* latex associated with fibrin(ogen)olytic activity. Toxicon. 2005;46(1):84-92.

37. Mahajan RT, Badgujar SB, Phytochemical Investigations of some laticiferous plants belonging to Khandesh Region of Maharashtra. Ethnobotanical Leaflets. 2008;12:1145-1152.

38. Lhinhatrakool T, Sutthivaiyakit S, 19-Norand 18, 20-Epoxy-cardenolides from the leaves of *Calotropis gigantea*. J. Nat. Prod. 2006;69(8):1249-1251.

39. Seeka C, Sutthivaiyakit S, Cytotoxic cardenolides from the leaves of *Calotropis gigantea*. Chem. Pharm. Bull. 2010;58(5):725-728.

40. . Sen S, Sahu NP, Mahato SB, Flavonol glycosides from *Calotropis gigantea*. Phytochemistry. 1992;31(8):2919-2921.

41. Gupta J, Ali M, Rare chemical constituents from *Calotropis gigantea* roots. Indian J. Pharm.Sci. 2000;62(1):29-32.

42. Abraham KI, Joshi PN, Studies on proteinases from *Calotropis gigantea* latex. Purification and some properties of two proteinases containing carbohydrate. Biochim Biophys Acta. 1979;568(1):111-119.

43. Pari K, Rao PJ, Devakumar C, Rastogi JN, A Novel Insect antifeedant nonprotein amino acid from *Calotropis gigantea.* J. Nat. Prod. 1998;61(1):102-104.

44. P. Bhaskara Rama Murti, T. R. Seshadri. Chemical composition of Calotropis gigantean. Proceedings of the Indian Academy of Sciences - Section A. 1945; 21(1): 8–18 .

45. Kwiterovich PO Jr, The metabolic pathways of high-density lipoprotein, low-density lipoprotein, and triglycerides: a current review, Am J Cardiol. 2000, 86(12A):5-10

46. Szkudelski, T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res. 2001;50: 536-546.

47. Sharma, R.D., A. Sarkar, D.K. Hazra, B. Misra and I.B. Singh. Hypolidemic effect of fenugreek seeds. Phytotherapy Res. 1996; 10: 332-334.

48. Al-Shamaony, L.S.M. Al-Khazrajoi and H.A.D. Twajii. Hypoglycemic effect of *Artemisia herba Alba* - II. J. Ethnopharmacol. 1994;43: 167-171.

49. R. B. Birari and K. K. Bhutani. Pancreatic lipase inhibitors from natural sources: unexplored potential. *Drug Discovery Today*. 2007; 12(19-20): 879–889.

50. H. Berredjem, Y. Reggami, M. Benlaifa, M. Berredjem and N. Bouzerna. Antidiabetic and Hypolipidemic Potential of 3, 4-dihydroisoquinolin-2(1H)- Sulfonamide in Alloxan Induced Diabetic Rats. International Journal of pharmacology. 2015;11 (3): 226-235.

51. Merzouk, H.; Madani, S.; Chabane, Sari, D.; Prost, J.; Bouchenak, M. & Belleville, J. Time course of changes in serum glucose, insulin, lipids and tissue lipase activities in macrosomic offspring of rats with Streptozotocin induced diabetes. Clin. Sci. (Lond). 2000;98 (1):21-30.

52. Paola Loria, Amedeo Lonardo and Frank Anania. Liver and diabetes. A vicious circle. Hepatol Res. 2013; 43(1): 51–64.

53. Council N. R. *Guide for the Care and Use of Laboratory Animals*. Washington, DC, USA: National Academies Press; 2010.