



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

**Nisrat Jahan<sup>1\*</sup>, Nasreen Akter<sup>1</sup> and Mosiqur Rahman<sup>1</sup>**

<sup>1</sup>Department of Pharmacy, Southeast University, Banani, Dhaka, Bangladesh.

### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author NJ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author NA managed the analyses of the study. Author MR managed the literature searches. All authors read and approved the final manuscript.*

### **Article Information**

DOI: 10.9734/JOCAMR/2019/v7i230096

#### Editor(s):

- (1) Dr. Francisco Cruz-Sosa, Metropolitan Autonomous University, Iztapalapa Campus Av. San Rafael Atlixco, Mexico.  
(2) Dr. Sachin Kumar Jain, Associate Professor, IPS Academy College of Pharmacy, India.

#### Reviewers:

- (1) Walter Antônio Roman Junior, Community University of the Region of Chapecó, Brazil.  
(2) Dennis, Amaechi, Veritas University, Nigeria.  
(3) Muruganandan Shanmugam, Harvard University, USA.  
(4) Uchendu, Mbah Okuwa, Michael Okpara University of Agriculture, Nigeria.  
(5) Javier Rodríguez Villanueva, Alcalá University, Spain.

Complete Peer review History: <http://www.sdiarticle3.com/review-history/47917>

**Original Research Article**

**Received 13 January 2019**  
**Accepted 02 April 2019**  
**Published 12 April 2019**

### **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 250 mg/kg body weight concentration and then subjected to different rats models to authenticate the antidiabetic and hyperlipidemic 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.

\*Corresponding author: E-mail: [nisrat\\_honey@yahoo.com](mailto:nisrat_honey@yahoo.com);

**Methodology:** Diabetes was induced in rats by an intraperitoneal injection (i.p) of alloxan (100 mg/kg B.W). Ethanollic 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 orally & positive control group received metformin orally. Synergistic effect of plant was evaluated by combination with 100 mg/kg B.W & 50 mg/kg B.W oral administration of metformin. After 7 days study period, fasting blood glucose, total cholesterol, triglyceride, high-density lipoprotein cholesterol, liver weight & body weight were measured only for diabetic group to observe the effects of diabetes induction.

**Results:** Individual plant extract (250 mg/Kg B.W) & Metformin (100 mg/kg B.W) reduced FBG significantly by 52% ( $P<0.001$ ) & 55.3% ( $P<0.001$ ) correspondingly. Metformin (100 mg/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 (250 mg/Kg B.W). Our results clearly suggests 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 metformin 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 has shown synergistic effect in elevation of plasma HDL-cholesterol.

**Conclusion:** The results of the study concluded that *C. gigantean* have potential antidiabetic and antioxidant properties.

**Keywords:** *Calotropis gigantean*; diabetes mellitus; hypolipidemic activity and 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  $\beta$ -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 when used for long term [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  $\beta$ -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]. Alone some synthetic drugs have various side effects due to its high dose, low solubility, low bioavailability [11,12]. So, it is important to deliver the synthetic drugs along with the natural supplement to overcome their problems. In this scenario, combination therapy is expected to reduce the dosage regimen such that the cost of the treatment and associated adverse events are reduced considerably [13].

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 [14]. that significantly posses antidiabetic effect. Antihyperglycemic activity of the plants is mainly due to their ability to restore 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  $\alpha$ - 1, 4 bonds in complex carbohydrate to elevate FBGL [15].

*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 [16]. Also known as crown flower, crown plant, giant milkweed or rubber bush [17]. Different parts of the plant contains stigmaterol,  $\beta$ -sitosterol [18], mudarine, glycosides (calotropin uscharin, calotoxin), lupeol, calotropin, uscharin, calotoxin, calactin and uscharidin; gigantol, protease such as calotropin DI and DII and calotropin FI and FII [19]. Calotropnaphthalene, calotropis juiterpeneol, calotropis esterterpeneol and calotrop benzofuranone along with sucrose, have been isolated from *C. gigantean* [20]. Traditionally different parts of the plant are used such as in leprosy, eczema, syphilis, elephantiasis, ulceration, cough [21], purgative, gastrointestinal irritant, abortion inducer [22], paralysis, swellings, intermittent fevers, asthma, anorexia, helminthic infections, inflammations, cutaneous infections, intestinal worms, ascites, bronchitis, dyspepsia (promotes gastric secretions) [23], poisonous snake or rat bites, periodic fever, ulcers, cures dental problems, gonococcal arthritis and other rheumatic complaints [24]. The plant proves to hold some pharmacological effects like proteolytic activity [25,26], anti amoebic [27] wound healing [28], hepatoprotective [29] and anti-oxidant [30] properties. Other reported potentials are analgesic activity [31], antimicrobial [32] and cytotoxic activity [33], anti-diarrhoeal activity, anti-candida activity [34], anti-pyretic activity [35], insecticidal activity [36], CNS activity [37], pregnancy interceptive properties [38] and procoagulant activity [39]. *C. gigantea* is reported to possess major phytochemical groups as alkaloids, cyanogenic, glycosides, phenolics, tannins [40], cardenolides, ester [41,42], flavonoids [43], terpenes [44] (antimosquito larvicidal activity), sterols (campesterol, stigmaterol, gamma-sitosterol, desmosterol) with anticervical cancer property), proteinases [45] and nonprotein amino acid [46]. Acetates, benzoates,  $\alpha$ - and  $\beta$ -calotropeols,  $\beta$ -amyrin, tetracyclic triterpene compounds, traces of

sterols, giganteol acetate and giganteol are also reported from this plant [47].

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 (International Centre for Diarrhoeal Disease Research, Bangladesh) Mohakhali, Dhaka, Bangladesh. Rats were housed under standard laboratory conditions (22-25°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, Bangladesh & 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 Whatman No.1 filters paper and concentrated by a rotary evaporate under reduced pressure at 50°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.



Fig. 1. *Calotropis gigantea*

## 2.5 Experimental Design

Long Evan rats were randomly assigned into group I, II, III, IV, V, VI (n=5) for 7 days treatment due to determination of blood glucose, lipid profile tests.

**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 (Dimethyl sulfoxide)).

**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).

**Group 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

A long term use of alloxan can be toxic and may cause the loss of many animals due to tubular

cell necrotic toxicity in kidney. For this reason a 7days study has been carried out for clinical trial on animal [48].

At 0<sup>th</sup>, 3<sup>th</sup>, 5<sup>th</sup> & 7<sup>th</sup> 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 [49] by blood analyzer using commercially available wet reagent diagnostic kits (HUMAN GmbH, Germany).

## 2.7 Statistical Analysis

The results were expressed as mean  $\pm$  SD. Data analysis was performed by the SPSS (Statistical Package for the Social Sciences) version 20 using one-way analysis of variance (ANOVA) and Dunnett's test. To assess the individual variations between the control and treatment groups,  $P \leq 0.05$  was considered significance level.

## 3. RESULTS

### 3.1 Antidiabetic Activity

At all-time points, blood glucose concentration remain unchanged in normal rats treated with distilled water. Table 1 indicates gradual decrease of FBGL for each group at 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup> & 7<sup>th</sup> day. The FBGL of all groups were compared to untreated diabetic group. At 7<sup>th</sup> 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

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

Animal Grouping	Fasting Blood Glucose Level (FBGL) (mmol/l)			
	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 mg/kg B.W)	8.62±0.28***	7.2±0.12*** (16.47)	5.92±0.09*** (31.32)	3.48±0.37*** (59.63)
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

**Table 2. Effect of *C.gigantea* on lipid profile in alloxan induced diabetic rats**

Animal Grouping	Liver Weight (mg/gm)	Lipid profile (mmol/l)		
		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 3. Effect of *C.gigantea* 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

*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 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.

### 3.2 Hypolipidemic Activity

After alloxan induced, the result showed that TG, TC increased while HDL decreased compare to Untreated diabetic rats (Table: 2). 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 (250 mg/kg B.W) 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 (250 mg/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. Individual extract showed elevation of HDL-C by 21.55%. Metformin (100 mg/kg B.W) with *C.gigantea* extracts (250 mg/kg B.W) increased 25.23% & metformin (50 mg/kg B.W) with *C.gigantea* extracts (250 mg/kg B.W) increased 22.28% of HDL-C.

Significant decrease of liver weight was revealed in diabetic rat (12.26%) ( $P<0.001$ ) as compared to control (Table: 3). Liver weight was slightly increased by 1.58% ( $P<0.001$ ) with 43.68 gm/kg B.W in diabetic rats when compared with non-diabetic rats (43.00 gm/kg B.W). The observed significant reduction in serum total lipids, total cholesterol and LDL cholesterol by the extract which can attribute the presence of phytochemical constituents like flavonoid [50] which is a active biological principle of most medicinal plants with hypoglycemic and antidiabetic activities.that propose the cardioprotective features with prevention of cardiovascular complications arising from hyperlipidemia [51].

### 4. 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 [52]. Sequential injection of alloxan caused a significant increase 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<sup>th</sup> 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 (250 mg/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*. It can be due to probable reduced absorption of glucose from the small intestine as glucose liberation from disaccharides is reduced. In our study, it is found that extract have hypoglycemic effect in glucose induced hyperglycemic rats.

Hyperlipidemia is a recognized outcome of Diabetes mellitus [53]. 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 [54]. 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 [55]. When compare to untreated diabetic rats 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 by 43.43% ( $P<0.05$ ) & 47.21% ( $P<0.001$ ). 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) when combined to Metformin (100 mg/kg B.W). Dose dependent manner was followed by the extract (250 mg/kg B.W) when combined with metformin at lower dose (50 mg/kg B.W) where TG & TYC were lessened by (34.53%) ( $P<0.05$ ) & (41.54%)

( $P < 0.001$ ) respectively. 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 Metformin was performed to develop the synergistic effect with different doses in dose dependent activity. Metformin (100 mg/kg B.W) with *C.gigantea* extracts (250 mg/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 (250 mg/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). The plant demonstrated a cardioprotective effect via an increase in HDL-cholesterol levels. Combination therapy also shown synergistic effect in elevation of plasma HDL-cholesterol that prevent risk of developing cardiovascular disease.

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 [56]. 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 [57] and the less elimination of lipoprotein from liver. Previous research articles also present the same agreement with the present findings [58].

## 5. CONCLUSION

In the present study, reduction in the concentration of glucose, TG (total triglyceride), TC (total cholesterol) and increase in HDL cholesterol were observed for ethanolic extract of *C.gigantea* leaves. Synergistic effect was estimated in combination with metformin. The results propose the probability of dose reduction of synthetic drug with required pharmacological activity when taken with *C.gigantea* leaves. The antidiabetic and hypolipidemic activity of the plant source is due to the phyto chemical constituents present in the plant. This study

justifies ethnomedicinal use of the plant 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.

## CONSENT

It is not applicable.

## 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 [59].

## ACKNOWLEDGEMENT

The authors are thankful to Department of Pharmacy, Southeast University, Banani, Dhaka-1213, Bangladesh, for providing lab facilities to carry out this research work.

## DATA AVAILABILITY

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

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

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. Cerf ME. Beta cell dysfunction and insulin resistance. *Frontiers in Endocrinology*. 2013;4:37.
  6. Deshpande D, Harris-Hayes M, Schootman M. 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, Kumar PS. *In vitro* and *In vivo* antidiabetic effect of *Andrographis lineate* Wall. Ex.Nees and *Andrographis serphyllifolia* Wt.lc leaf extracts. *African Journal of Pharmacy and Pharmacology*. 2013;7:2112–2121.
  9. Xu X, Wang G, Zhou T, Chen L, Chen J, Shen X. 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, 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. Conforti F, Ioele G, Statti GA, Marrelli M, Ragno G. Antiproliferative activity against human tumor cell lines and toxicity test on Mediterranean dietary plants. *Food Chem Toxicol*. 2008;46:3325-32.
  12. Cicerale S, Lucas L, Keast R. Biological Activities of Phenolic Compounds Present in Virgin Olive Oil. *I J Mol Sci*. 2010;11: 458-479.
  13. Bharti Mangla, Kanchan Kohli. Combination of Natural Agent with Synthetic Drug for the Breast Cancer Therapy. *Combination of Natural Agent with Synthetic Drug for the Breast Cancer Therapy*. *Int J Drug Dev & Res*. 2018;10: 22-26
  14. Malviya N, Jain S, Malviya S. Antidiabetic potential of medicinal plants. *Acta Pol Pharm*. 2010;67(2):113–118.
  15. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian Pac J Trop Biomed*. 2012; 2(4):320–330.
  16. Ahmed KKM, Rana AC, Dixit VK. *Calotropis* species (Asclepiaceae) - A comprehensive review. *Pharmacognosy Magazine*. 2005;1(2):48–52.
  17. Ajay K, Patil PA, 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.
  18. Available:[http://www.hear.org/pier/species/calotropis\\_gigantea.htm](http://www.hear.org/pier/species/calotropis_gigantea.htm).
  19. Available:<http://www.stuartxchange.org/Kapal-kapal.html>.
  20. Malaya k. misra, Manoj k. mohanty, Pradeep k das. Studies on the method ethnobotany of *calotropis gigantea* and *c.procera*. *Ancient science of life*. 1993; 1&2:40 – 56.
  21. Chitme HR, Chandra M, Kaushik S. Studies on anti-diarrhoeal activity of *Calotropis gigantea* R.Br. in experimental animals. *J Pharm Pharm Sci*. 2004; 7(1):70-75.
  22. 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.
  23. Mahajan RT, Badgujar SB. Phytochemical Investigations of some laticiferous plants belonging to Khandesh Region of Maharashtra. *Ethnobotanical Leaflets*. 2008;12:1145-1152.
  24. Gaurav Kumar, Loganathan Karthik, Kokati Venkat Bhaskara Rao. A Review on Pharmacological and Phytochemical Profile of *Calotropis Gigantea* Linn. *Pharmacology online*. 2011;1:1-8.
  25. 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.
  26. 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.
  27. 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.



28. 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.
29. 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.
30. Bedekar A, Shah K, Koffas M. Natural products for type II diabetes treatment. *Adv Appl Microbiol.* 2010;71:21-73.
31. Pathak AK, Argal A, Analgesic activity of *Calotropis gigantea* flower. *Fitoterapia.* 2007;78(1):40-42.
32. 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.
33. 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.
34. 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.
  - a. *Calotropis gigantea* leaves – an *in vitro* study. *International Journal of Pharmaceutical Sciences Review and Research.* 2010; 4(2):141-144.
  - b. *Calotropis gigantea* leaves – an *in vitro* study. *International Journal of Pharmaceutical Sciences Review and Research.* 2010; 4(2):141-144.
35. Chitme HR, Chandra R, Kaushik S, Evaluation of antipyretic activity of *Calotropis gigantea* (Asclepiadaceae) in experimental animals. *Phototherapy Research.* 2005;19(5):454-456.
36. 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.
37. Argal A, Pathak AK, CNS activity of *Calotropis gigantea* roots. *J. Ethnopharmacol.* 2006;106(1):142-145.
38. 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.
39. Das S, Das S, Das MK, Basu SP, Evaluation of anti-inflammatory effect of *Calotropis gigantea* and *Tridax procumbens* on Wistar albino rats. *J. Pharm. Sci. & Res.* 2009;1(4):123-126.
40. Mahajan RT, Badgujar SB, Phytochemical Investigations of some laticiferous plants belonging to Khandesh Region of Maharashtra. *Ethnobotanical Leaflets.* 2008;12:1145-1152.
41. Lhinhatrakool T, Sutthivaiyakit S, 19-Norand 18, 20-Epoxy-cardenolides from the leaves of *Calotropis gigantea*. *J. Nat. Prod.* 2006;69(8):1249-1251.
42. Seeka C, Sutthivaiyakit S, Cytotoxic cardenolides from the leaves of *Calotropis gigantea*. *Chem. Pharm. Bull.* 2010;58(5): 725-728.
43. Sen S, Sahu NP, Mahato SB, Flavonol glycosides from *Calotropis gigantea*. *Phytochemistry.* 1992;31(8):2919-2921.
44. Gupta J, Ali M, Rare chemical constituents from *Calotropis gigantea* roots. *Indian J. Pharm.Sci.* 2000;62(1):29-32.
45. 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.
46. 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.
47. Bhaskara Rama Murti P, Seshadri TR. Chemical composition of *Calotropis gigantea*. *Proceedings of the Indian Academy of Sciences - Section A.* 1945; 21(1): 8–18.
48. Kumar NP, Annamalai AR, Thakur RS. Antinociceptive property of *Emblica officinalis* (Amla) in high fat dietfed/low dose streptozotocin induced diabetic neuropathy in rats. *Indian Journal of Experimental Biology.* 2009;47(9):737–742
49. Antia BS, Okokon JE, Umoh EE, Udobang JA. Antidiabetic activity of ethanolic leaf extract of *Panicum maximum*. *Int J Drug Dev & Res.* 2010;2(3):488–492.
50. Trivedi NA, Mazumdar B, Bhatt JD, Hemavathi KG. Effect of shilajit on blood glucose and lipid profile in alloxan-induced diabetic rats. *Indian J. Pharmacol.* 2004;36:373-376.
51. 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
52. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the

- rat pancreas. *Physiol Res.* 2001;50:536-546.
53. Sharma RD, Sarkar A, Hazra DK, Misra B, Singh IB. Hypolipidemic effect of fenugreek seeds. *Phytotherapy Res.* 1996;10:332-334.
54. Al-Shamaony LSM, Al-Khazrajoi, Twajii HAD. Hypoglycemic effect of *Artemisia herba Alba* - II. *J. Ethnopharmacol.* 1994; 43:167-171.
55. Birari RB, Bhutani KK. Pancreatic lipase inhibitors from natural sources: unexplored potential. *Drug Discovery Today.* 2007; 12(19-20):879–889.
56. Berredjem H, Reggami Y, Benlaifa M, Berredjem M, Bouzerna N. 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.
57. 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.
58. Paola Loria, Amedeo Lonardo and Frank Anania. Liver and diabetes. A vicious circle. *Hepatol Res.* 2013;43(1):51–64.
59. Council NR. Guide for the Care and Use of Laboratory Animals. Washington, DC, USA: National Academies Press; 2010.

© 2019 Jahan et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Peer-review history:*

*The peer review history for this paper can be accessed here:*  
<http://www.sdiarticle3.com/review-history/47917>