

EVALUATION OF ANTI-DIABETIC ACTIVITY OF LEAVES OF CASSIA OCCIDENTALIS

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ABSTRACT

Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins. Many are secondary metabolites, of which at least 12,000 have been isolated — a number estimated to be less than 10% of the total. In many cases, substances such as alkaloids serve as plant defense mechanisms against predation. Diabetes mellitus often simply referred to as diabetes—is a condition in which a person has a high blood sugar (glucose) level as a result of the body either not producing enough insulin, or because body cells do not properly respond to the insulin that is produced. Insulin is a hormone produced in the pancreas which enables body cells to absorb glucose, to turn into energy. *Cassia occidentalis* is an annual shrub. The leaves, roots & entire plant as such has been used in various countries like China, Brazil, Sri Lanka & India in the treatment of the variety of ailments such as malaria, liver diseases, fungal infections etc. Thorough literature survey for the chemical nature of the plant reveals that the plant contains quinines, flavonoids, saponins and alkaloids. The ethnomedical information reveals the plant has been used to treat diabetes.

Keywords: Insulin, Diabetes, Hyperglycemia, Pancreas, Hormone.

INTRODUCTION

Many of the herbs and spices used by humans to season food yield useful medicinal compounds (Lai & Roy, 2004). The use of herbal medicines in the right way provides effective and safe treatment for many ailments. The effectiveness of the herbal medicines is mostly subjective to the patient. The potency of the herbal medicines varies based on the genetic variation of the herbs, growing conditions of the herbs, timing and method of harvesting of the herbs, exposure of the herbs to air, light and moisture, and type of preservation of the herbs. Herbal medicines can be used for healing purposes and to promote wellness. Herbal medicines are not

addictive or habit forming, but are powerful nutritional agents that support the body naturally. Herbal medicines promote health and serve as excellent healing agents without side effects. Chinese herbs are taken as tonics to enhance physical and mental well-being. Herbal medicines are safe and effective for health, healing, weight loss/ gain/ maintenance, etc. Herbal medicine can nourish the body's deepest and most basic elements. Herbal medicines are great body balancers that help regulate body functions. Natural therapies such as herbal medicines can be used to support balance process of our body.

Herbal medicines offer the nutrients that the

body fails to receive due to poor diet or environmental deficiencies in the soil and air. If the body cells do not absorb the glucose, the glucose accumulates in the blood (hyperglycemia), leading to various potential medical complications (Rother, 2007).

There are many types of diabetes, the most common of which are

Type 1 diabetes

Results from the body's failure to produce insulin, and presently requires the person to inject insulin.

Type 2 diabetes

Results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency.

Gestational diabetes is when pregnant women, who have never had diabetes before, have a high blood glucose level during pregnancy. It may precede development of type 2 DM. As of 2009 at least 271 million people worldwide suffer from diabetes, or 2.8% of the population. Type 2 diabetes is by far the most common, affecting 80 to 85% of the total diabetes population.

Classification

Most cases of diabetes mellitus fall into the three broad categories of type 1 or type 2 and gestational diabetes. A few other types are described:

The term *diabetes*, without qualification, usually refers to diabetes mellitus, which roughly translates to excessive sweet urine (known as "glycosuria"). Several rare conditions are also named diabetes. The most common of these is diabetes insipidus in which large amounts of urine are produced (polyuria), which is not sweet (insipidus meaning "without taste" in Latin). The term "type 1 diabetes" has replaced several former terms, including childhood-onset diabetes, juvenile diabetes, and insulin-dependent diabetes mellitus (IDDM). Likewise, the term "type 2 diabetes" has replaced several former terms, including adult-onset diabetes, obesity-related diabetes, and non-insulin-dependent diabetes mellitus (NIDDM). Beyond these two types, there is no agreed-upon standard nomenclature. Various sources have defined "type 3 diabetes" as: gestational diabetes, insulin-resistant type 1 diabetes (or

"double diabetes"), type 2 diabetes which has progressed to require injected insulin, and latent autoimmune diabetes of adults (or LADA or "type 1.5" diabetes).

Overview of the most significant symptoms of diabetes

The classical symptoms of DM are polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger) (Cooke & Plotnick, 2008). Symptoms may develop quite rapidly (weeks or months) in type 1 diabetes, particularly in children. However, in type 2 diabetes symptoms usually develop much more slowly and may be subtle or completely absent. Type 1 diabetes may also cause a rapid yet significant weight loss (despite normal or even increased eating) and irreducible mental fatigue. All of these symptoms except weight loss can also manifest in type 2 diabetes in patients whose diabetes is poorly controlled, although unexplained weight loss may be experienced at the onset of the disease. Final diagnosis is made by measuring the blood glucose concentration. When the glucose concentration in the blood is raised beyond its renal threshold (about 10 mmol/L, although this may be altered in certain conditions, such as pregnancy), reabsorption of glucose in the proximal renal tubuli is incomplete, and part of the glucose remains in the urine (glycosuria). This increases the osmotic pressure of the urine and inhibits reabsorption of water by the kidney, resulting in increased urine production (polyuria) and increased fluid loss. Lost blood volume will be replaced osmotically from water held in body cells and other body compartments, causing dehydration and increased thirst. A rarer but equally severe possibility is hyperosmolar nonketotic state, which is more common in type 2 diabetes and is mainly the result of dehydration due to loss of body water. Often, the patient has been drinking extreme amounts of sugar-containing drinks, leading to a vicious circle in regard to the water loss. A number of skin rashes can occur in diabetes that is collectively known as diabetic dermadromes.

Causes

Type 2 diabetes is determined primarily by lifestyle factors and genes (Riseruset *al*, 2009).

Lifestyle

A number of lifestyle factors are known to be important to the development of type 2 diabetes. In one study, those who had high levels of physical activity, a healthy diet, did not smoke, and consumed alcohol in moderation had an 82% lower rate of diabetes. When a normal weight was included the rate was 89% lower. In this study a healthy diet was defined as one high in fiber, with a high polyunsaturated to saturated fat ratio, and a lower mean glycemic index (Mozaffarian *et al*, 2009) Obesity has been found to contribute to approximately 55% type 2 diabetes, and decreasing consumption of saturated fats and trans fatty acids while replacing them with unsaturated fats may decrease the risk. The increased rate of childhood obesity in between the 1960s and 2000s is believed to have lead to the increase in type 2 diabetes in children and adolescents (Roselbloom & Silverstein, 2003). Environmental toxins may contribute to recent increases in the rate of type 2 diabetes. A positive correlation has been found between the concentration in the urine of bisphenol A, a constituent of some plastics, and the incidence of type 2 diabetes (Lang *et al*, 2008).

Medical conditions

Subclinical Cushing's syndrome (cortisol excess) may be associated with DM type 2 (Iwasaki *et al*, 2008). The percentage of subclinical Cushing's syndrome in the diabetic population is about 9%. Diabetic patients with a pituitary microadenoma can improve insulin sensitivity by removal of these microadenomas (Taniguchi *et al*, 2008). Hypogonadism is often associated with cortisol excess and testosterone deficiency is also associated with diabetes mellitus type 2 (Saad & Gooren, 2009) even if the exact mechanism by which testosterone improve insulin sensitivity is still not known.

Pathophysiology

The pathophysiology of type 2 diabetes mellitus is characterized by peripheral insulin resistance, impaired regulation of hepatic glucose production, and declining β -cell function, eventually leading to β -cell failure. The primary events are believed to be an initial deficit in insulin secretion and, in many patients, relative insulin deficiency in

association with peripheral insulin resistance (Reaven, 1998).

Herbal anti-diabetic drugs

It has become increasingly evident in recent years that a full spectrum of therapeutic agents for prevention & treatment of diabetes is far from being complete. In an attempt to fill this gap, drug development focus has now shifted to traditional herbal drugs for new & more effective drug therapies. The search for indigenous anti-diabetic plants is still going on. Several plants have been identified as potential source of drugs in Indian system of ayurvedic medicine for treatment of diabetes. Extracts of various plants have been shown to possess hypoglycaemic properties in experimental diabetic as well as normal animals

MATERIAL AND METHOD

Introduction of Diabetes in Mice

Diabetes was induced in animals by a single intraperitoneal injection of alloxan (40 mg/kg) dissolved in 0.1 M citrate buffer (pH 4.5) after fasting for 24 hour. Blood samples for glucose, cholesterol and other estimations were obtained retro-orbitally from the inner canthus of eyes using microcapillaries. Rats with fasting blood glucose of more than 220 mg/dl were considered to be diabetic and were included in the present study.

ALLOXAN

The cellular site of action of alloxan and exact mechanisms involved in its toxicity are not completely understood. Number of studies have shown that alloxan disrupts the integrity of the β -cell plasma membrane. The site at which alloxan interacts with the cell membrane is uncertain. Some evidence indicates that alloxan acts at the site for sugar transport into the cell. On the other hand, there is also evidence to suggest that alloxan acts at a glucoreceptor site responsible for insulin release, which is separate from the transport site. It has also been proposed that alloxan, leads to mitochondrial dysfunction and interferes with intracellular glucose oxidation. There is a large variability in dosage of alloxan required to produce long-standing diabetes in different species, which is also compatible with life. In many experiments, a single dose (32 to 200 mg/kg) was adequate to produce desired degree of hyperglycemia, while in certain other studies

it had to be repeated after 3 days. In the chronic state, hyperglycemia remains constant and blood glucose levels of 400 mg or more can be expected after standard diabetogenic doses of alloxan and streptozotocin (STZ), high dosages of the β -cell toxin streptozotocin and alloxan induce severe insulin deficiency and IDDM with ketosis. Lower dosages calculated to cause a partial reduction of β -cell mass could be used to produce a mildly insulin-deficient state of NIDDM, without a tendency to ketosis. The dosage is difficult to judge to create stable NIDDM without either gradual recovery or deterioration into IDDM. Streptozotocin is preferred because it has more specific cytotoxicity, to β -cell of the pancreas.

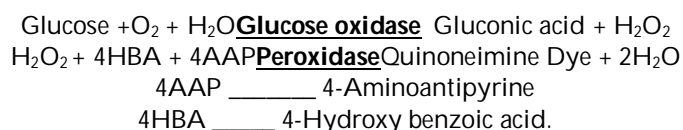
Quantitative Estimation of Blood Glucose

Blood glucose level was estimated colorimetrically at 505 nm by Glucose

Oxidase-Peroxidase (GOD-POD) method (Trinder's method) using a commercially available kit (Transasia Bio Medicals Ltd. Daman).

Principle

Quantitative estimation of blood glucose level by GOD-POD method is based on the principle that enzyme glucose oxidase reacts with glucose in the presence of oxygen and water to form gluconic acid and hydrogen peroxide. This hydrogen peroxide reacts with 4-amino antipyrine and 4-hydroxybenzoic acid in the presence of the enzyme peroxidase to form quinoneimine dye and water. The pink coloured end product has absorption maxima at 505 nm. The intensity of the pink colour formed is proportional to the glucose concentration.



Serum separation

Blood was withdrawn retro-orbitally with the help of microcapillaries. The collected blood was kept for 30 mins at room temperature. Serum from blood after clotting was separated out by centrifugation at 3000 rpm for 15 minutes. The serum thus obtained was used for glucose and cholesterol estimations.

Blank

To 10 μl of distilled water, 1000 μl of glucose working reagent was added and the contents were thoroughly mixed.

Standard

To 10 μl of glucose standard (100 mg/dl), 1000 μl of glucose working reagent was added and the contents were thoroughly mixed.

Test

To 10 μl of serum, 1000 μl of glucose working reagent was added and were mixed thoroughly. The above solution was mixed well and incubated for 15 minutes at 37 $^{\circ}\text{C}$. The absorbance of standard and each test tube was read against blank at 505 nm.

$$\text{Concentration of glucose (mg/dl)} = \frac{\text{O.D. Test}}{\text{O.D. Standard}} \times 100$$

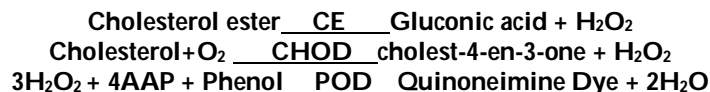
Quantitative Estimation of Serum Cholesterol

Serum cholesterol was estimated at 505 nm by CHOD-PAP method (Modified Roeschlau's method) using a commercially available kit (Transasia Bio Medicals Ltd. Daman).

Principle

Quantitative estimation of serum cholesterol level by CHOD-PAP method is based upon the principle that enzyme cholesterol esterase reacts with cholesterol ester to form

cholesterol and fatty acid. The cholesterol formed reacts with enzyme cholesterol oxidase in the presence of oxygen give rise to cholest-4-en-3-one and hydrogen peroxide. This hydrogen peroxide reacts with 4-aminoantipyrine and phenol in the presence of quinoneimine dye and water. The pink colored end product has absorbance maxima at 505 nm. The intensity of pink color formed is proportional to the cholesterol concentration.



CE _____ Cholesterol esterase
 CHOD _____ Cholesterol oxidase
 4AAP _____ 4-Aminoantipyrine
 POD _____ Peroxidase

Blank

To 20 μl of distilled water, 1000 μl of cholesterol working reagent was added and the contents were thoroughly mixed.

Standard

To 20 μl of cholesterol standard (5.14mmol/l), 1000 μl of cholesterol working reagent was

added and the contents were thoroughly mixed.

Test

To 20 μl of serum, 1000 μl of cholesterol working reagent was added and were mixed thoroughly. The above solution was mixed well and incubated for 15 minutes at 37 $^{\circ}\text{C}$. The absorbance of standard and each test tube was read against blank at 505 nm.

Concentration of cholesterol (mmol/l) =

O.D. Test

————— x 5.14mmol/l

O.D. Standard

Serum Lipids

Total cholesterol, LDL- and HDL- cholesterol and triacylglycerol were estimated using kits from Dr. Reddy's Pathology Lab-Hyderabad following manufacturer's instructions.

available weighing balance, prior to alloxan treatment and after the administration of alloxan.

HDL-CHOLESTEROL

Principle: Low-density lipoproteins (LDL and VLDL) and chylomicron fraction was precipitated quantitatively by addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation. The cholesterol concentration in HDL fraction, which remains in the supernatant, is estimated.

Experimental Protocol

Four groups were included in the present study and each group comprised of six animals.

Group 1: Normal control

Group 2: Diabetic control (alloxan treated)

Group 3: Diabetic mice treated with butanolic extract

(20 mg/kg/once a day, orally)

Group4 : Diabetic mice treated with aqueous extract

(30 mg/kg/once a day, orally)

LDL- Cholesterol

After estimating total cholesterol, HDL cholesterol and triacylglycerol, Triacylglycerols The LDL cholesterol (mg/100ml)=Total Cholesterol+ HDL (Cholesterol) /5(Friedelwald 1972).

Estimation of the concentration of low - density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge.

Statistical Analysis

The data for blood glucose, cholesterol, body weight and were expressed as mean \pm S.E.M. Data was analysed using one-way ANOVA. Multiple range Tukey's test was employed as post-hoc test for comparison between various groups and with control group. $p \leq 0.05$ was considered to be statistically significant.

Determination of Body Weight

The body weight of the experimental animals was determined using a commercially

RESULTS AND DISCUSSION

Effect of *Cassia occidentalis* extracts on the plasma glucose values of various groups

Plasma glucose levels of all the experimental animals were examined at the end of the study. The diabetic control group showed significant increase (300.7 ± 10.88) as compared to normal control group (84 ± 9.30). DTB group showed significant reduction in plasma glucose levels (95.2 ± 7.46). DTA group (119.6 ± 29.03) showed significant reduction but was less as compared with the DTB group (Fig. 1) Table 1.

Effect of *Cassia occidentalis* extracts on the Total cholesterol values of various groups

Total cholesterol levels of all the experimental animals were examined at the end of the study. The diabetic control group showed significant increase (246 ± 14.4) as compared to normal control group (168.6 ± 16.9). DTB group showed significant reduction in plasma glucose levels (186 ± 14.8). DTA group (190 ± 14.81) significant reduction which was slightly less as compared with the DTB group (Fig. 2).

Effect of *Cassia occidentalis* extracts on the HDL values of various groups

The HDL levels remained largely unchanged in all the groups throughout the study (Fig. 3).

Effect of *Cassia occidentalis* extracts on the LDL values of various groups

LDL values of all the groups were examined at the end of the study. The diabetic control group showed significant increase (152.2 ± 6.0) as compared to normal control group (78 ± 6.2). DTB group showed significant reduction in LDL levels (99.7 ± 7.3). Reduction in LDL levels in DTA group (111 ± 5.1) was also significant as compared to DTB group (Fig. 4)

Effect of *Cassia occidentalis* extracts on the TAG values of various groups

TAG levels of diabetic control group (181.8 ± 18.8) showed significant increase as compared to the normal control group. TAG values of DTB and DTA remained largely unchanged. (Fig. 5) Table 2.

Effect of *Cassia occidentalis* extracts on the body weights of various groups

Body weight of the diabetic control group was reduced significantly as compared to the normal group while body weights of the DTA and DTB groups remained largely unchanged. (Fig. 6) Table 3.

CONCLUSION

The transverse section of stem showed greatly thickened and heavily cutinized outer walls of the epidermis, rectangular cells of the epidermis, pericycle and vascular bundles.

The leaf constants such as vein islet number, vein termination number, stomatal index, stomatal number were determined and recorded.

Extractive values such as butanolic extractives and water soluble extractives were determined and recorded.

The butanolic extracts and the aqueous extracts brought down the plasma glucose values to normal values with the butanolic extracts having more profound effect. So we can expect that the *Cassia occidentalis* extracts either repaired the damaged pancreas or the extract stimulated directly the utilization of glucose by various tissues.

Hence, it was concluded that the leaf extracts have anti-diabetic activity. The various photochemical present in the extracts may be isolated to study which of them is responsible for anti-diabetic activity.

Table 1: Effect of Butanolic and Aqueous Extracts on the Plasma Glucose Levels

Group	Plasma glucose levels (after 4 weeks)
Normal(control)	84.0 ± 9.30
Diabetic control	300.7 ± 10.88 a
Diabetic treated (butanolic)	95.2 ± 7.46 b
Diabetic treated (aqueous)	119.6 ± 13.03 b

Data are expressed in Mean \pm SEM (n=6)

a = p < 0.05 vs normal control group

b = p < 0.05 vs diabetic control group

Table 2: Effect of Butanolic and Aqueous Extracts on the Serum Lipid Profile

Group	Total cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	TAG (mg/dl)
Normal(control)	168.6±16.9	78.0±12.2	44.9±13.3	115.6±18.6
Diabetic control	246.0±14.4 a	152.2±12.6 a	45.0±12.0	181.8±18.8 a
Diabetic treated (butanolic)	186.0±14.8 b	99.7±16.8 b	51.3±8.4	175.0±15.8
Diabetic treated (aqueous)	190±14.81 b	111±12.8 b	47.2±6.87	187.6±15.17

Data are expressed in Mean± SEM(n=6)
 a=p<0.05 vs normal control group
 b=p<0.05 vs diabetic control group

Table 3: Effect of Butanolic and Aqueous Extracts on the Body Weights After 4 Weeks

Group	Body weight (g)
Normal	210 ± 6
DC	180 ± 2
DTB	200 ± 7
DTA	196 ± 2.9

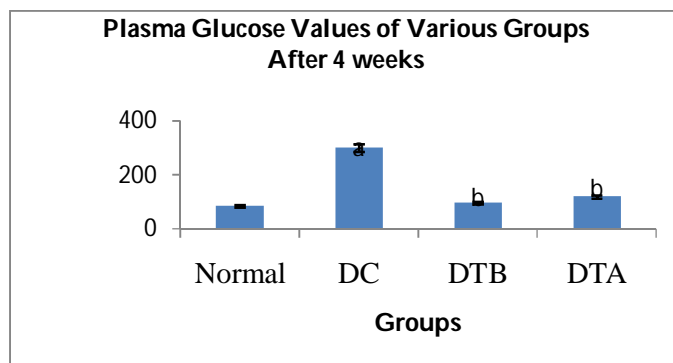


Fig. 1: Effect of *Cassia Occidentalis* Extracts on the Plasma Glucose Values of Various Groups

DC- Diabetic control
 DTB- Diabetic treated with butanolic extracts
 DTA- Diabetic treated with aqueous extracts.

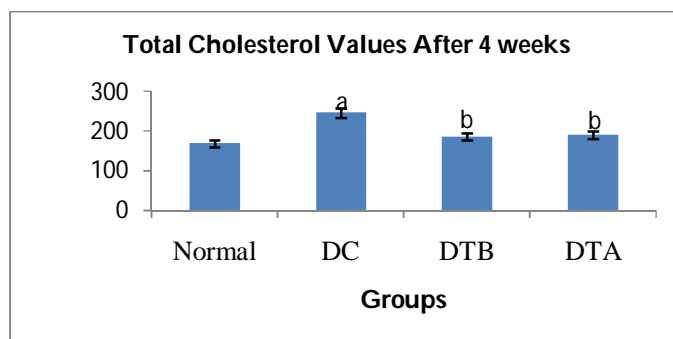


Fig. 2: Effect of *Cassia Occidentalis* Extracts on the Total Cholesterol Values of Various Groups

DC- Diabetic control
 DTB- Diabetic treated with butanolic extracts

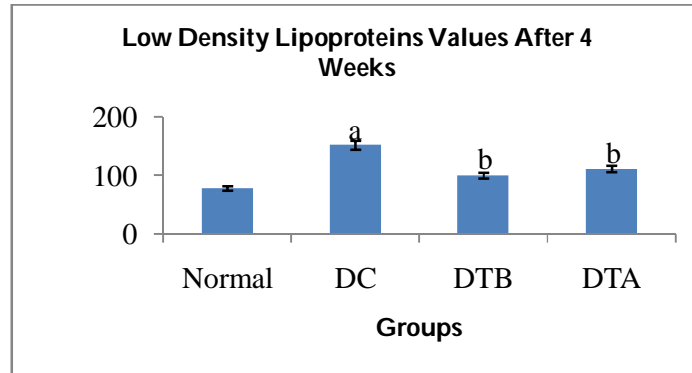


Fig. 3: Effect of *Cassia Occidentalis* extracts on the Low density Lipoprotein Values of Various Groups

DC- Diabetic control
 DTB- Diabetic treated with butanolic extracts
 DTA- Diabetic treated with aqueous extract.

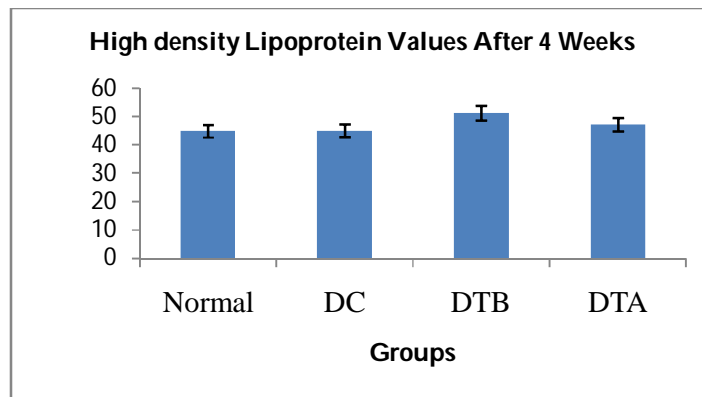


Fig. 4: Effect of *Cassia Occidentalis* extracts on the High density Lipoprotein Values of Various Groups

DC- Diabetic control
 DTB- Diabetic treated with butanolic extracts

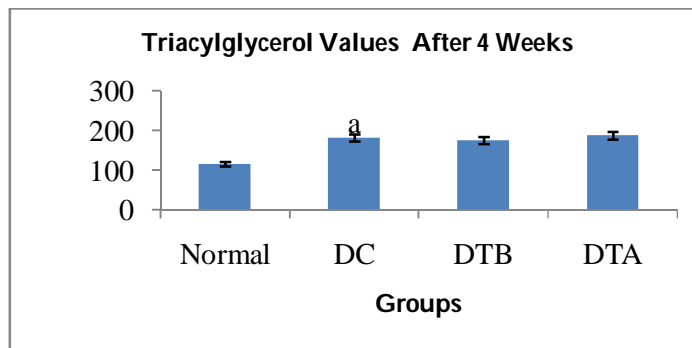


Fig. 5: Effect of *Cassia Occidentalis* Extracts on the Triacylglycerol Values of Various Groups

DC- Diabetic control

DTB- Diabetic treated with butanolic extracts
 DTA- Diabetic treated with aqueous extract

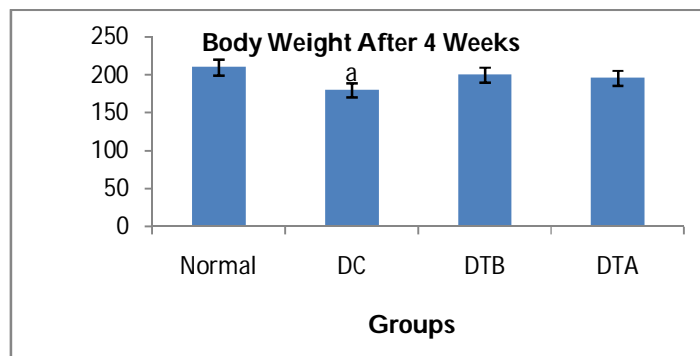


Fig. 6: Effect of *Cassia occidentalis* Extracts on the Body Weights of Various Groups

DC- Diabetic control
 DTB- Diabetic treated with butanolic extracts
 DTA- Diabetic treated with aqueous extracts

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