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The Effect of Ethanolic Extract of *Cassia Fistula* Leaves on Triton X100 Induced Hyperlipidemic Male Albino Rats

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Hyperlipidemia is a disease condition characterized by the elevation of all lipid levels in the blood. It is an important risk factor in artherosclerosis which can lead to cardiovascular diseases (CVD). *Cassia fistula* linn (caesalpinaceae) tree is one of the most widespread in the forest of India, usually occurring in deciduous forests. The phytochemical determination of *Cassia fistula* plant revealed the presence of saponins, flavonoids, alkaloids and absence of phenols. Hyperlipidemia in rats was induced with Triton –X100 (a non –ionic detergent) which has been shown to elevate total cholesterol and triglyceride in the blood by altering hepatic lipid metabolism. Lipid profile of the hyperlipidemic control rats in this study showed higher levels of serum triglyceride, total cholesterol, low density lipoprotein followed by decrease in high density lipoprotein as compared to the normal control rats. Treatment of hyperlipidemic rats with low doses of astrovastatin (0.14mg/kg) showed a statistical difference (p< 0.05) in total cholesterol, triglycerides, very low density lipoprotein, low density lipoprotein (80.49± 10.00, 103±18.35, 20.68 ±3.56, 10.63±5.86 respectively) and increase in high density lipoprotein (49.24±2.92) compared to hyperlipidemic control while treatment with ethanolic extract of C. *fistula* at doses of 200mg/kg showed a reduction (91.41±27.88, 101.44±46.77, 20.16 ±9.14 and 23.48±18.73) in the levels of total cholesterol, triglycerides, very low density lipoprotein and low density lipoprotein respectively and increase in high density lipoprotein (48.30± 2.89). At 400mg/kg of the extract, the levels of total cholesterol, triglycerides, very low density lipoprotein, low density lipoprotein reduced to 82.09±12.89, 95.65±25.00, 19.12±5.00 and 14.05±10.41 respectively and increased the level of high density lipoprotein (48.92±1.93). At 600mg/kg the extract statistically reduced (p< 0.05) total cholesterol, triglyceride, very low density lipoprotein and low density lipoprotein (77.87±14.18, 91.53±33.65, 18.27±6.70 and 9.04±5.69 respectively) and increased the level of high density lipoprotein to 50.54±3.17 compared to hyperlipidemic control. This study showed that ethanolic extracts of *Cassia fistula* exhibited hypolipidemic effect on Triton X100 induced hyperlipidemia.

Keywords: *Cassia fistula* leaves, Hyperlipidemia, Triton X100, Ethanolic extract.

INTRODUCTION

Hyperlipidemia is a secondary metabolic dysregulation characterized by elevation of all lipid profile and/or lipoprotein in the body (Hassan, 2013). It is also called hyperlipoproteinemia because these fatty substances travel in the blood, attached to proteins and this is the only way that these fatty substances can remain dissolved while in circulation. It is also synonymously known as dyslipidemia (Chem, 2005). Increased plasma lipid level mainly total cholesterol, triglycerides and low density lipoprotein (LDL) with decrease in high density lipoprotein (HDL) are known causes of hyperlipidemia which is the reason for initiation and progression of atherosclerosis. Research has proved that increased lipid profile results from increased absorption of lipids through the gut or enhanced endogenous synthesis of lipids. Thus decreasing gut absorption and endogenous synthesis of lipid are ways of reducing hyperlipidemia.

The potency of herbal remedies in the reduction of hyperlipidemia thus cardiovascular diseases has been documented by many researchers (Ozumba *et al*., 2009; Igwilo *et al*., 2010; Nsofor *et al*., 2012; Igwilo *et al*., 2014).

Cassia fistula leaves (English: Golden shower, Indian laburnum, French: *Bâton casse, casse doux, casse espagnol*) belong to the family of *Fabacae* which has been shown to be potent in treatment of various diseases. The leaves posses laxative properties and are used in jaundice, piles, rheumatism, ulcers, ring worms, eczema and external skin eruption, the leaves back mixed with oil are applied to insect bites (Kiritikar and Basu 2006). The seeds are rich in glycerides with linoleic, oleic, stearic and palmitic acids as major fatty acids together with traces of caprylic and myristic acids. It has been reported that the stem bark of *Cassia fistula* is also a potential source of lupeol, ß-sitosterol and hexacosanol. A detailed biochemical analysis of the flower's pollen, suspected to play a significant allergenic role, showed a protein composition of 12% with appreciable amounts of free amino acids such as phenylalanine, methionine, glutamic acid and proline. Carbohydrate, lipid and free amino acid contents were of the order of 11.75%, 12.00% and 1.42%, respectively (Mondal *et al*., 1998). The aim of this study was to investigate the effect of ethanolic extract of *Cassia fistula* leaves on Triton X100 induced hyperlipidemic male albino rats.

MATERIALS AND METHODS

Triton X100 was purchased from National Chemicals Vadodara, India while the total cholesterol, triglyceride and high density lipoprotein kits were bought from Randox Laboratory Limited, 55 Diamond, Crumlim, Co. Antrim, United Kingdom. Other reagents used were of standard analytical grade and were procured locally.

Experimental animals

Thirty (30) Wistar albino male rats weighing between 100 to 200g and 12 mice were purchased from Chris Animal House, located at Nnamdi Azikiwe University temporary site, Awka, Anambra State. They were maintained and housed in cages in the same place. They were allowed to acclimatize with the environment for one week before use. The animals were kept on guinea growers mash pellets that were obtained from Eke Awka market, Awka before the experiment.

Extraction procedure

The leaves of *Cassia fistula* were washed properly and air dried at room temperature for two weeks. The dried leaves were ground into powder using Corona manual grinding machine. Three hundred grammes (300g) of ground leaves powder of *Cassia fistula* were soaked in 3 litres of 80% ethanol for 24 hours. The solution was sieved and filtered using whatman no 1 (125mm) filter paper. The filtrate was dried to powder by rotary evaporator and used as the crude ethanol leaf extract.

Phytochemical analysis of the extract

Phytochemical tests were carried out on the ethanolic extract of *Cassia fistula* leaves using standard phytochemical tests as described by Harbone (1973), Sofowora (1993), Trease and Evans (1989). The following phytochemicals were assayed: saponins, tannins, flavonoids, alkaloids and phenolic groups.

Acute toxicity study

The LD_{50} of the extract was determined in mice using the Lorke method (1983). The animals were administered with the extract and monitored for 24 hours for signs and symptoms and LD_{50} was calculated.

Experimental procedure

The animals were divided into six groups, each group containing 5 rats. The first dose of the drug treatment was given immediately after triton administration to the animals from group 3 to group 6. Second and third doses

were administered after 24 hours and 44 hours respectively. After 4 hours of the third dose, the animals were used for the study of various biochemical parameters. Under anaesthesia, blood samples were collected through heart puncture and centrifuged at 2000rpm to obtain serum for the analysis of lipoproteins like high density lipoprotein (HDL-C), low density lipoprotein (LDL-C), very low density lipoprotein (VLDL), Total Cholesterol and Triglyceride.

Biochemical analysis

Serum lipid levels of total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-C) were determined using respective diagnostic commercial kits from Randox diagonistic, United Kingdom and low density lipoprotein cholesterol (LDL-C) in the plasma was calculated as per friedewald estimation.

LDL-C= (TC-(TG/5+HDL)-C) mg/dl, VLDL = (TG/5).

Statistical analysis

The results were expressed as mean ± SD. The Triton control was compared with normal and the experimental results were compared with Triton control. Statistical analysis was carried out using paired t-test and one-way ANOVA followed by Dunnett test. Differences below $P<0.05$ implied statistically significance

RESULTS

The qualitative phytochemical investigation of the leaves extract of *Cassia fistula* showed the presence of saponins, tannins, flavonoids, alkaloids and absence of phenols.

The effect of the ethanolic extract of *Cassia fistula* (EECF) on serum Total Cholesterol and Triglyceride on the male albino rats are shown in Figure 1. There was a significant ($p>0.05$) decrease in total cholesterol treated groups when compared to the hyperlipidemic control group except the group treated with 200mg/kg of ethanolic extract of *Cassia fistula* (EECF) which showed reduction but was not statistically significant. This result proves the ability of Triton X100 to induce hyperlipidemia as demonstrated by Chakraborty *et al*., (2012). When compared to the hyperlipidemic control, there was a reduction in astrovastatin, 200mg/kg EECF, 400mg/kg EECF and 600mg/kg EECF treated groups but was not statistically significant.

The effect of the ethanolic extract of *Cassia fistula* on serum HDL, LDL and VLDL are shown in Figure 2. There was a statistical ($p>0.05$) decrease in serum HDL in hyperlipidemic control group when compared to the 600mg/kg EECF treated group while there was increase in astrovastatin, 200mg/kg EECF and 400mg/kg EECF treated groups but was not statistically significant. Among the entire treated groups *Cassia fistula* at 600mg/kg showed the best effect which could be as a result of dose dependency.

From Figure 2, there was a significant ($p>0.05$) decrease in low density lipoprotein values of 200mg/kg EECF, 400mg/kg EECF, 600mg/kg EECF and 0.14mg/kg astrovastatin low dose groups when compared to the hyperlipidemic control group.

There was no statistical ($p<0.05$) decrease in very low density lipoprotein of astrovastatin 0.14mg/kg, 200mg/kg EECF, 400mg/kg EECF and 600mg/kg EECF treated groups when compared to the hyperlipidemic treated group. This could be as result of the animals not being fed during the study.

DISCUSSION

Hyperlipidemia is the greatest risk factor of coronary heart disease and studies have shown that an increase in HDL cholesterol and decrease in total cholesterol, LDL cholesterol and triglycerides is associated with decrease in the risk of ischemic heart disease. Antihyperlipidemic drugs target the reduction of total cholesterol and increase the level of high density lipoproteins (HDL) known as the good cholesterol by either blocking intestinal absorption of lipid or inhibiting 3-hydroxyl-3-methyl-glutary CoA reductase enzyme thereby preventing hepatic cholesterol synthesis (Saravanakumar *et al*., 2010).

Triton X100 (a non- ionic detergent) was used to induce hyperlipidemia and has been shown by studies to elevate TC and TG in the blood by altering the hepatic lipid metabolism (Charkraborty *et al*., 2012). The result of this study showed that the extract of *Cassia fistula* leaves produced a statistical significant decrease in total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and a statistical increase of high density lipoprotein (HDL-C) at a dose of 600mg/kg which could be as a result of dose dependency.

The lowering of cholesterol by the extract could be as a result of the extract interacting with hepatic receptors leading to rapid catabolism of LDL-C and final elimination in the form of bile acid as demonstrated by Khana *et al*.,(2002). It could also be as a result of the extract inhibiting 3-hydroxyl-3-methyl glutaryl CoA reductase enzyme which catalyzes the conversion of 3-hydroxyl-3-methyl glutarly CoA to mevalonate which is a committed step in the hepatic synthesis of cholesterol or by blocking the intestinal absorption of lipid.

The quantitative phytochemical analysis of the *Cassia fistula* leaves extract showed the presence of saponins, tannins, flavonoids, alkaloids and absence of phenol

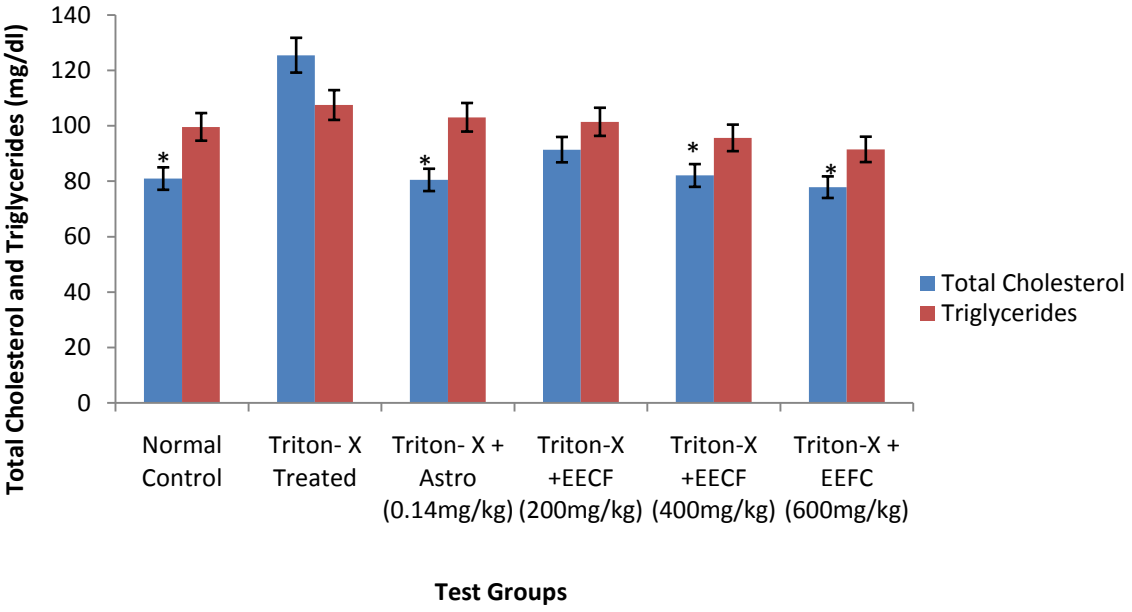

Figure 1: Effect of *Cassia Fistula* Leaves Extract on Serum Total Cholesterol and Triglycerides

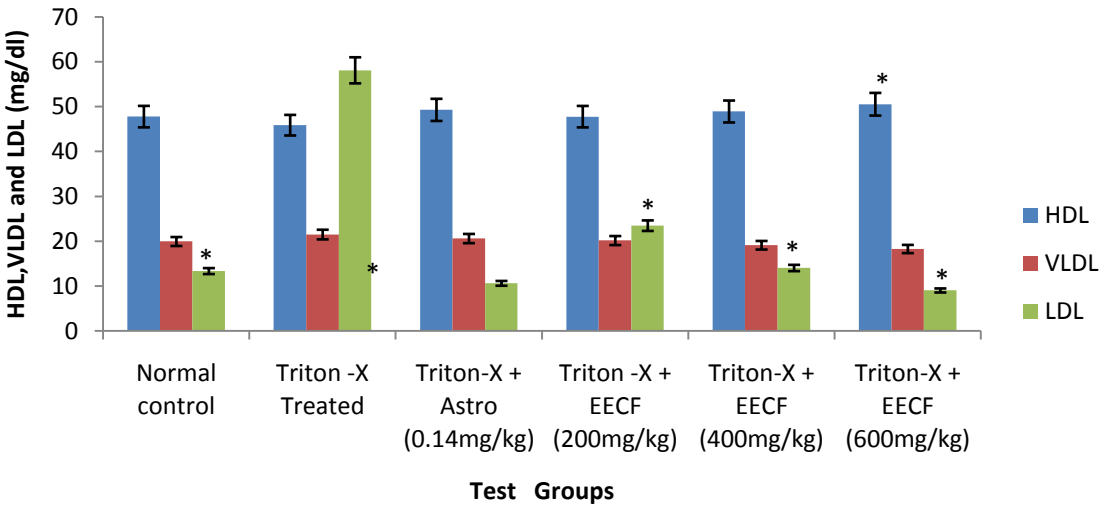

Figure 2: Effect of *Cassia Fistula* Leaves Extract on Serum High Density Lipoprotein, Very Low Density Lipoprotein and low density Lipoprotein

which could be the reason behind the extracts' hypolipidemic properties.

CONCLUSION AND RECOMMENDATION

From the result of this study, it showed that the ethanolic extract of *Cassia fistula* at doses of 200mg/kg, 400mg/kg and 600mg/kg have hypolipidemic properties. But among all treated groups, the 600mg/kg dose had the best effect and could be used in the treatment of hyperlipidemia. Further research work is necessary to investigate the bioactive factors present in *Cassia fistula* leaves extract

that confer the hypolipidemic properties and possibly its mechanism of action.

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