International Journal of Sciences

Research Article Volume 7 – August 2018 (08)

Impact of Ethanolic Extract of *Tecoma stans* and *Costus afer* Leaves on Lipid Profile Status of Streptozotocin Induced Diabetic Wistar Rats

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Abstract: The lipid profile status of streptozotocin induced diabetic rats treated with ethanolic extract of *Tecoma stans* and *Costus afer* leaves using seventy (70) male wistar rats divided into five (5) parallel group consisting of diabetic (DC) and non diabetic (ND) groups of seven (7) rats per group. Group I received placebo (Control) Group II received insulin (humulin 5mg/kg body weight) Group II received Ts 250mg/kg Body weight) Group IV, received Ca (200mg/kg Body weight) Group V received combined extracts TS (150mg/kg body weight) A and a (100mg/kg body weight). The extracts were administered orally while insulin was subcutaneously. The treatment lasted for 21 days after which the animals were sacrificed and whole blood was collected for biochemical analysis. There was no significant increase in total cholesterol among insulin, *Tecoma stans* and combined therapy when compared to NC. HDL-C was significantly ($P<0.05$) higher in non diabetic group treated with *Costus afer* and combined therapy compared to NC, insulin and *Tecoma stans* in the non diabetic group while the total cholesterol levels in the serum was significantly ($p<0.05$) higher in diabetic control group and those administered insulin, Tc and Ca compared in NC. HDL-C concentration in *Tecoma stans* and *Costus afer* treated group were significantly increased ($P<0.005$) compared to NC in diabetic study group. From the result obtained from the study, it may be concluded that the ethanolic extracts of *Tecoma stans* and *Costus afer* leaves has hypolipidemic, hepatoprotective properties and may be able to alleviate oxidative stress induced by streptozotocin in wistar rats.

Keywords: Ts-*Tecoma stans*, Ca-*Costus afer*, Cardiovascular Disease, Antioxidant

Introduction

The concept of poly herbal formulation was originally peculiar to Ayuverda- the oldest healing system of medicine, but it has been practiced and accepted today as an effective therapeutic approach in sourcing for medications for most degenerative ailments (Singh, 2005).

The herbs are selected based on the disease and are advantageous over monotheraphy such as reduced toxicity and side effects e.g powdered of *Rauwolfia serpentine* (the biological source of the antihypertensive alkaloids, resepine) is devoid of unpleasant side effect while reserpine an isolate from this plant once used in the treatment of hypertension was later withdrawn from circulation due to unpleasant side effect particularly of bone resorption. Salicylic acid isolated from salicin (aglycoside used as an anti - inflammatory drug) is known to cause gastric irritation and hence cannot be used by ulcer patient this calling for the so called gastric coated aspirin. This plant when used alone is without this side effect probably due to the presence of tannis (Singh, 20005). World Health Organization WHO (1980) recommended evaluation of effectiveness of plants to replace modern drugs that are not safe. WHO (2002) also stated that developing countries cannot achieve effective health care with the use of western medicine alone without complementing with traditional herbal therapies.

Costus afer ker-gano (Costaceae) is among 150 species of stout, perennial and rhizomatous genus *Costus* (Edeoga and Okoli, 2000). It can be found in the forest belt of Senegal, South Africa, Guinea, Niger, Sierra Leone and Nigeria (Burkill, 1985, Edeoga and Okoli 2000). The plant is community called bush cane (*Ireke omoda* Yoruba Western part of Nigeria) *Opete* (Igbo Eastern part of Nigeria and *Mbritem* (Ibibio/Efik Southern part of Nigeria) *C. afer* which belongs to the family *Zingiberaceae* is a monocot and a relatively tall, herbaceous, unbranched tropical plant with creeping rhizome. It is commonly found in most or shady forest of west and tropical Africa (Iwu, 1983). *C. afer* is a perennial, rhizomatous herb that can attain a height up to 4m. It

Published at: http://www.ijsciences.com/pub/issue/2018-08/
DOI: 10.18483/ijSci.1740; Online ISSN: 2305-3925; Print ISSN: 2410-4477

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is often planted in home garden for medicinal purposes (Aweke, 2007). Many scientists have reported on ethno pharmacological characteristics of medicinal plants. Their finding revealed different properties of these medicinal plants. *C. afer* is a useful medicinal plant that is highly valued for its anti-diabetic, anti inflammatory and anti-anthritic properties in south – east and south – west Nigeria (Soladoye and Oyesika, 2008). It is also used for other socio cultural purposes. *Tecoma stans* from *Bignoniaceae* family is a semi ever green ornamental tropical shrub or small tree originally from Latin America which has been cultivated in Nigeria recently. It is found in Nigeria, India and Iran. Its primary application have been in treating diabetes and digestive problem. Flower infusion can be taken orally as a diuretic to treat syphilis or for intestinal worms. The roots is considered and effects remedy for snake and scorpion sting. The root are as diuretic, vermifuge and tonic.

Flower and leaves have some medicinal value for the treatment of various cancers (Kiritikar and Basu, 1999). It leaves are used traditionally in Mexico to control diabeties (Roman – Ramos *et al*., 1991, Costantino *et al*., 2003). The plant contains tecomononine, tannis, flavonoid, alkaloids, quinones and traces of saponins (Lozoya and Mellado-Campus, 1985). Extracts from *Tecoma Stans* leaves have been found to inhibit the growth of yeast infection. (Marzouk *et al.,* 2006), have studies the anticancer activity of *Tecioma stans* and antioxidant constituent. (Alanso-Castro *et al.,* 2010) have reported that the *Tecoma stan*s extract exhibited antidiabetic activity while (Sonthil Kumar *et al.*, 2010) have reported that the extracts having antibacterial activity on human pathogenic bacteria.

Materials and methods

Several chemicals and analytical grade reagents used for this research include: ethanol(98%) Sodium hydroxide, sodium chloride, dimethylsulphoxide (DMSO), streptozotocin (STZ) was obtained from Sigma St. Louis, MO USA, etc.

Collection and preparation of plant materials

Fresh leaves of *Tecoma stans* and *Costus afer* were collected at Malabo Campus, University of Calabar, Calabar, and from Eman-Uruan local government area, Akwa Ibom, Nigeria respectively. The leaves were rinsed severally with tap water to remove dust particle and debris and allowed to completely drain. The plant materials were dried on laboratory surfaces. The dried leaves were crushed into fine powder and stored in clean capped bottles.

Animals

Seventy albino male rats weighing between 125 – 200g were obtained from the animal house of the department of Zoology and environmental Biology, University of Calabar, Calabar, Nigeria. The animals were allowed to acclimatize for two weeks in the animal house of the department of Biochemistry. The animals were housed in well ventilated cages and kept under controlled environmental conditions of temperature (28 ± 2^0C) relative humidity (50±59) and 12 hour light/dark cycles.

Experimental design

70 rats were divided into 5 parallel groups consisting of a diabetic and non-diabetic pair of 7 animals each. The doses used were based on the predetermined LD50 values obtained from preliminary studies.

Groups	Mean body weight (g)	Treatment administered
1	198.8±9.7	Placebo (0.5ml DMSO) diabetic control (DC)
2	140.34±0.7	5 unit of insulin per kg body weight
3	165.00±3.18	250mg *Tecoma stans* leaves
4	170.4±3.04	200mg *Costus afer* leaves
5	127.8±3.74	200mg *Tecoma stans* leaves + 10mg *Costus afer*
6	128.8±5.22	Placebo (0.5ml DMSO) non-diabetic control (NC)
7	136±4.01	5 units of insulin per kg body weight
8	138.6±2.52	250mg *Tecoma stans* leaves
9	142.1±3.55	2000mg *Costus afer* leaves
10	125.00±2.71	200mg *Tecoma stans* leaves + 100mg *Costus afer*

Induction of experimental diabetes

Prior to diabetes induction, the rats were subjected to 12hr fast, and then diabetes was induced by intraperitoneal injection of 65mg STZ per kg body weight. Streptozotocin (STZ) (Sigma St. Louis, MO, USA) reconstituted in sodium citrate buffer (P^H 4.5). Control animals received 0.5ml DMSO only. Four days after STZ treatments, diabetes was confirmed in STZ treated rats with a fasting blood sugar concentration > 200mg/dl. This was estimated using one touch Glucometer (life span Inc. 1995 Milpas. California, USA) with blood obtained from the tail vein of the rats.

Extraction of plant material

The dried powdered leave of the plant 500g were separately soaked in 80% ethanol for 72 hours with occasional agitation after which they were filtered

into clean beakers through chess material and the whatman No 1 filter paper to obtain a homogenous filtrate. The filtrate was concentrated in vacuo at low temperature 37^0C – 40^0C to about one tenth of the original volume. The concentrates were allowed in an open water bath (40^0C) to evaporate to complete dryness. Evaporation was maintained at 40^0C to prevent denaturation of bioactive constituents inherent in the crude extract. Semi solid dark green coloured concentrate were obtained from the leaves. They were stored in clean capped bottles in refrigerator for further use.

Experimental protocol

Diabetic and non-diabetic animals were grouped as shown in the table above and also accordingly treated with extracts and insulin. The insulin dose adopted was as previously reported by Sonia and Srinivasan (1999) and also to stimulate human regimen. The plant extracts were administered via oral gastric intubation twice per day (7:00am, 7:00pm) and insulin once per day post prandial (7:00am) subcutaneously.

Treatment lasted for 21 days and throughout this period changes in blood glucose were monitored. The animals were maintained on water and pellets prepared with growers feed from Vital Feed, Jos Plateau state, Nigeria.

Collection of sample for analysis

At the end of the 21 days food was withdrawn from the rats and they were fasted overnight but had free access to water. They were euthanized under chloroform vapour and sacrificed. Whole blood was collected via cardiac puncture using sterile syringes and needles for biochemical analysis.

Statistical analysis

The result were analysed for statistical significance by one way ANOVA using SPSS. All data were expressed as Mean ±SEM, $P < 0.05$ were considered significant.

Results

Serum lipid concentration of non diabetic group

Treatment Group	TC (mg/dl)	HDL-c (mg/dl)	HDL/TC	TG (mg/dl)	VLDL (mg/dl)
NC	52.8 ± 0.20	32.3 ± 3.84	0.61 ± 0.07	168.9 ± 7.72	33.8 ± 54
Insulin	52.5±0.34	35.4±4.20	0.658±0.08	141.00±2.02	28.2±0.46
Ts	54.2±0.26	32.2±0.60	0.59±0.01	73.4±1.08	14.7±0.22
Ca	134.8±9.13	62.2±2.69	0.46±0.02	160.5±2.13	32.1±0.43
Ts + Ca	49.6 ±1.64	40.7±0.83	0.81±0.03	134.4±0.99	26.9±0.20

Values are expressed as mean ± SEM, n=7 significantly different from NC at P<0.05, a= significantly different from insulin at P<0.05, b = significantly different from Ts at P<0.05, C = significantly different from Ca at P<0.05, Tc = total cholesterol, HDL-c = High density lipoprotein cholesterol, TC = Triglyceride, VLDL=Very low density lipoprotein.

Serum lipid profile of non diabetic test animals

Administration of Ca caused a significant (P<0.05) increase in total cholesterol in serum compared to NC, insulin and Ts in normal glycemic study group as shown in the table above. No significant difference was observed in total cholesterol among insulin, Ts and combined therapy compared to NC. Serum lipids concentration following a 21 days treatment with the extracts HDL cholesterol (HDLC) was significantly (P<0.05) higher in non diabetic groups treated with Ca and combined therapy compared to NC, insulin and Ts. VLDL was significantly (P<0.05) reduced in non diabetic rats treated with insulin Ts and combined therapy when compared to NC. VLDL was significantly (P<0.05) lower in Ts and Ca group of normal glycemic study group compared to insulin group. Significantly (P<0.05) lower concentration of VLDL when compared to single extracts of TC and Ca in a non diabetic study group was also recorded. There was a significant (P<0.05) increase in atherogenic index (HDLC/TC) in combined therapy group of non diabetic treated group relative to NC, Ts, and Ca.

Serum lipid concentrations of diabetic group

Treatment Group	TC (mg/dl)	HDL-c (mg/dl)	HDL/TC	TG (mg/dl)	VLDL (mg/dl)
NC	52.8 ± 0.20	32.3±3.84	0.61±0.07	168.9±7.72	33.8±1.54
DC	90.82±9.72	41.8±4.08	0.48±0.04	116.12±7.08	23.2±1.42
Insulin	77.71±1.16	48.7±2.18	0.63±0.03	116.9±2.13	23.4±0.43
Ts	107.75±24.27	45.2±4.52	0.54±0.09	139.51±7.00	27.9±1.40
Ca	103.36±9.21	51.7±4.89	0.53±0.08	142.00±8,43	28.5±1.69
Ts + Ca	80.72±10.44	46.4±3.41	0.63±0.08	121.44±3.40	24.0±0.89

Values are expressed as shown mean +_SEM, n = 7 significantly different from Nc at (P<0.05), a = significantly from DC at (P<0.05), b = significantly different from insulin at (P<0.05) c = significantly different from Ts at (P<0.05), d = significantly different from Ca at (P<0.05), Tc = total cholesterol, HDL-c = high density lipoprotein cholesterol, Tg = Triglycerids, VLDL = very low density lipoprotein.

Serum lipid profile of diabetic test animals

Total cholesterol levels in the serum was significantly (P<0.05) higher in diabetic control group and those administered insulin, Ts and Ca compared to Nc. However administration of Ca caused a more significant (P<0.05) increase in total cholesterol in serum compared to NC in diabetic study group.

Changes in serum lipids concentration following a 21 days treatment with the extracts as shown in the table above. Serum high density lipoprotein cholesterol concentration in diabetic control rats increased though not significantly (P<0.05) higher compared to non diabetic control. HDL-c concentration in insulin Ts, and Ca treated group were significantly increased (P<0.05) compared to Nc in diabetic study group.in the diabetic study group VLDL was significantly (P<0.05) lower in all treatment group as compared to Nc. Combined therapy recorded lowest value (24.0±0.87mg/dI) when compared to *Tecoma stans* (27.9±1.40 mg/dI) and *Costus afer* (28.5±1.69 mg/dI). These was an increase in atherogenic index in combined therapy group compared to all other groups in this diabetic study group although this increase was not statistically significant.

Discussion

Some herbal plants have been reported to have effects in blood lipid (Campillo *et al.*, 1994, Dominquez *et al.*, 1996, Jones *et al.*, 1997, Cigneralla *et al.*, 1998, Perez *et al.*, 1999, Duze *et al.*, 2012, Ranyan *et al.*, 2012 and Kameshwaran *et al.*, 2013) have reported that *Tecoma stans* flower extract has therapeutic effects in the treatment of hypolipidemia and obesity.

The management of de lipidemia remains a key factor in the multifactional approach to prevent cardiovascular disease (CVD) (Maria & Ronald, 2006). In this study Ca was observed to increase total cholesterol and HDL-c levels significantly compared to NC, insulin and Ts in the normoglycemic study group. Combined therapy also elevated HDL-c compared to NC, insulin and *Tecoma stans*, triglycerides and VLDL levels were reduced by the extract and insulin although combined therapy was shown to exert a more significant decrease of more than 100% compared to NC and other treatment groups while insulin, Ca and combined therapy was between 20% and 30% reduction.

Triglycerides in this study has been shown to be reduced by the extract and TG is the main form of which fat is stored in the body. High TG levels is a contributing factor to atherosclerosis, hence the result of triglycerides in this study indicates that the plant extracts especially *Tecoma stans* has a protective effect against cardiovascular disease since low TG will reduce the risk of coronary heart disease (CHO), this was the observation of (Duze *et al.*, 2012) in their work with G. Kola on the other and an increase in HDL-c observed in this study also indicates a protective effect against CHD since HDL helps to remove excess cholesterol from circulation and carries it back to the liver for degradation or conversion into bile acids. HDLC is considered one of the strongest predictor of CHD (Maria & Roland, 2006).

Although the mechanism of hypolipidemic effect of these extracts is not yet known, it may however be attributed to phytochemical constituents inherent in them. These phyto-constituents may have reduced blood lipids by competing with cholesterol biosynthesis in the liver by inhibiting the key enzyme hydroxymethylglutaryl co-enzyme (HMG Co-A) at the regulatory site during cholesterol biosynthesis. HDL-c plays a direct role in the atherogenic process which agrees with findings of (Amaechi *et al.*, 2015) therapeutic intervention by raising HDL-c is widely encouraged.

Conclusion

The results of this study show that the ethanolic extracts of *Tecoma stans* and *Costus afer* leaves possess hypolipidemic, hepatoprotective properties by their abilities in the decrease in serum lipid profile when compared with the diabetic control group.

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