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Effect of Terpenoid Fraction of Root Extract of *Physalis Angulata* on Cardiovascular and Renal System

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ABSTRACT

This study was designed to investigate the effect of terpenoid fraction of *Physalis angulata* on cardiovascular and renal parameters with a total of 20 male albino rats. These were determined using spectrophotometric methods. The rats were randomly divided into four groups of five (5) rats each. Group 1: was not administered with anything (Normal Control); Group 2: was administered with 50 mg/Kg b. w of vitamin C (standard drug); Group 3: was administered with 25 mg/Kg b. w of the isolated terpenoids; Group 4: was administered with 50 mg/Kg b. w. of the isolated terpenoids; Group 5: was administered with 100 mg/Kg b. w. of the isolated terpenoids for the period of 30 days. All the rats were given free access to water and commercial poultry feed *ad libitum*. Results of the analyses were expressed as mean ± standard deviation. Relative to the control group, the serum creatine kinase and lactate dehydrogenase activities increased non-significantly ($p>0.05$) with dose among groups of rats treated with terpenoid fraction. There was also a non-significant ($p>0.05$) increase in the serum level of creatine kinase activity of normal control animals compared to the group that received vitamin C (standard drug). The result of lipid profile showed that there was a significant ($p<0.05$) increase in the serum levels of total cholesterol (TC), low density lipoprotein (LDL) and triacylglycerol (TAG) while there was a non-significant ($p>0.05$) increase when compared to the normal control. The total cholesterol concentration of the group that received ascorbic acid (positive control) was significantly ($p<0.05$) lower than the group that received 100 mg/kg b.w terpenoids. A non significant ($p>0.05$) increase in high density lipoprotein was observed as the dose of terpenoids. The result equally revealed a significant ($p<0.05$) decrease in low density lipoprotein concentration of the group that received 50 mg/kg b.w terpenoids compared to the group that received 100 mg/kg b.w terpenoids. The low density lipoprotein concentration of the group that received ascorbic acid (positive control) was significantly ($p<0.05$) lower than the group that received 100 mg/kg b.w terpenoids. The evaluation of kidney function biomarkers revealed that there was a significant ($p<0.05$) decrease in the serum level of creatinine and urea when compared to the normal control. In conclusion, the study has shown that *Physalis angulata* terpenoid fraction could be used in the management of cardiovascular and renal disorders.

Keywords: *Physalis angulata*, Terpenoid fraction, Cardiovascular and Renal

INTRODUCTION

Today, the use of plant materials as functional foods and nutraceutics for the treatment of different diseases is on the increase all over the world.[1] These could be linked to their phytochemical constituents, which include phenolic acids, flavonoids, and alkaloids. These phytochemicals have several health benefits with little or no side effect compared to synthetic products [2], [3].

The use of herb in medicine has played an important role in nearly every culture on

earth, including Asia, Africa, Europe, and America. Medicinal plants have been based on fact that plant contains natural substances that can promote health and alleviate illness [4]. Several herbs can help to reduce high blood cholesterol concentrations [5]. Various organic compounds are derived from plants which are important in alleviating different diseases that humans are constantly exposed to, phytochemicals constituents of the plants and their usage immensely help in treatment of diseases both in the medical and pharmaceutical fields [6]. [7], [8], have reported that some commonly consumed traditional plants or herbs promote reduction in serum lipid.

Physalis angulata is the most common specie in the genus *Physalis* L. (family; Solanacaea) and highly distributed across regions of the world [9],[10]. It is native of the American continent [11] but, at present, it grows as an introduced plant in several Asian and African countries [12]. Currently, *P. angulata* is a wild species, for which recent plantations have been established in Mexico [13] and Brazil [14]. The species is considered as an alternative crop for its edible fruits and for its diversity of secondary metabolites, which have an important potential in the bio-products industry [15].The leaf of this plant is usually used in preparations of local dishes, as spices, and also consumed as vegetables in many Nigerian homes. This plant is also used for the management of diabetes, malaria, hepatitis, and inflammation [16], [17]. However, despite several reports on their biological activities, there is a dearth of information on their effects on cardiovascular and renal disorders.

Cardiovascular disease and other related disorders are the major cause of mortality or death in the populace all over the world-both in developed and developing countries. In the ethnology of cardiovascular disease, high level of total cholesterol (TC), and low density lipoprotein-cholesterol (LDL-C) in the serum have been implicated and seen as primary risk factor [18]. A number of epidemiological investigations have shown a clear association between dietary saturated fat, atherosclerosis, and coronary heart disease (CHD) [19].
The composition of human diet plays an essential role in the management of lipid and lipoproteins concentration in the blood [20]. However, disturbances in serum lipoprotein and abnormal lipid metabolism characterized by hyperlipoproteinemia or hyperlipidaemia have also been seen as an implicative risk factor in coronary heart disease (CHD) development [21].

MATERIALS AND METHODS

Materials

Equipment, Chemicals and Reagents used in this study were of analytical standards and grades

Collection and Preparation of Plant Materials

Fresh roots of *Physalis angulata* were collected from Yola South Local Government Area of Adamawa State, Nigeria. The plant material was identified and authenticated by Mr. Usman Gala of Botany Department, Ahmadu Bello University, Zaria Nigeria. The plant was assigned the voucher number: ABU2051. The root sample was washed and cut into smaller pieces and dried under direct sunlight. The sample was later pulverized to coarse powder using a hammer mill (Gallenkamp, U.S.A.).

Extraction of Plant Material

A known weight (6.952 kg) of the air-dried root powder was extracted with analytical grade ethanol in a soxhlet at 65°C. The mixture was vacuum-filtered through Whatman No 1 filter paper and concentrated using a vacuum rotary evaporator (Eyla N-1000, Japan) to afford 52.503g (0.755% w/w) of the extract. The extractive yield was calculated using the relation:

Fractionation of *Physalis angulata* Root Extract

The extract (52.50 g) was subjected to solvent-guided fractionation in a silica gel (60-120 mesh size) column (2 × 70 cm) successively eluted with 20% ethyl acetate in n-hexane, followed by 30, 40, 50, 60, 70, 80 and 100% ethyl acetate. The solvent fractions were collected in 100 ml volumes and screened for the presence of terpenoid using qualitative phytochemical test. Fractions that gave positive reaction to terpenoids were pulled together and concentrated in rotary evaporator under vacuum to yield (E: nH-F; 14017.50 mg: 26.70% w/w) fraction. A small quantity of the fraction (E: nH-F) was developed using percolated silica trial thin layer chromotography plates in a mixture of n-hexane: ethylacetate: methanol in different ratios, but (3:2:1) ratio which gave the best resolution (showing three distinct terpenoids T_1, T_2, T_3 and one steroid S_1 chromatographic spots) served as the solvent mixture for the final elution in the second column. The third terpenoid chromatographic spot (T_3) which appeared insoluble in n-hexane: ethylacetate: methanol (3:2:1) mixture, was eluted with 20% acetic acid in ethyl acetate. Consequently, the E:nH-Fraction (14017.50 mg) was subjected to further separation in silica gel (60-120 mesh size) column eluted with mixture of n-hexane : ethylacetate : methanol (3:2:1), followed by 20% acetic acid in ethyl acetate. The sub-fractions were collected in 100 ml volumes and screened for the presence of terpenoid using qualitative phytochemical test. The first 300 ml sub-fraction contained a mixture of T_1 and S_1, while the rest of n-hexane: ethyl acetate: methanol (3:2:1) sub-fractions contain only T_2 the largest amount of terpenoid. However, 20% acetic acid in ethyl acetate was used to elute T_3 and trace T_2. The sub-fractions were concentrated in rotary evaporator under vacuum to yield (T_2; 3.15 g: 22.471% w/w), (T_3 and T_2; 0.938 g: 6.69% w/w), sub-fractions.

Experimental design for the Study

In this study, twenty (20) albino rats were used. They were acclimatized for a period of one week and fed with commercial poultry feed and water *ad libtum*. The animals were divided into four groups of five (5) rats each, based on the similarity of their body weights and the extract were orally administered as shown below. The study lasted for 30 days and they were grouped as shown below: Group 1: Normal Control; Group 2: 50 mg/kg b. w of vitamin C (standard drug); Group 3: 25 mg/kg b. w of the isolated terpenoids; Group 4: 50 mg/kg b. w. of the isolated terpenoids; Group5: 100 mg/kg b. w. of the isolated terpenoids.

On day 30, blood samples were collected from all the rats through ocular puncture and then used for the analyses.

Biochemical Analysis

Determination of lactate dehydrogenase (LDH) and creatine kinase activities

LDH and creatine kinase activities were assayed by the method described by [22].

Determination Serum Cholesterol Concentration

The method of [18], was used for serum cholesterol determination. Commercially prepared [Quimica Clinica Applicada (QCA) test kit, Quimica Applicada, Spain] was used for determination.

Serum High Density Lipoprotein (HDL) Assay

The Dextran sulphate-mg (II) method for the *in vitro* determination of HDL-cholesterol in serum [23], using Quimica Clinica Applicada (QCA) HDL, test kit (QCA, Spain).

Serum Low Density Lipoprotein (LDL) Assay

The polyvinyl sulphate method for the *in vitro* determination of LDL-cholesterol in serum [24], using the Quimica Clinica Applicada (QCA) LDL test kit (QCA, Spain).

Determination of Serum Triacyglycerol

The glycerol phosphate oxidase method (enzymatic test) for the *in vitro* determination of triglycerides in serum [20], using Quimica Clinica Applicada (QCA) triacylglycerol test kit (QCA, Spain).

Determination of Serum Urea Concentration

The modified method of Berthelot-Searcy for the *in vitro* determination of urea in serum [21], using the Quimica Clinica Applicada (QCA) creatinine test kit (QCA, Spain).

Serum Creatinine Assay

The modified Jaffe method for the *in vitro* determination of creatinine in serum [22], using the Quimica Clinica Applicada (QCA) creatinine test kit (QCA, Spain).

Statistical Analysis

The statistical analysis was carried out using Statistical Product and Service Solution (SPSS 15.0) version. Statistical differences were evaluated using a one way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test to detect significant differences among the mean values of the dfferent groups.

RESULTS

Effect of Terpenoids on Cardiac Function Enzymes

The results of effects of terpenoids on cardiac function enzymes are presented in Table 1. Creatine kinase and lactate dehydrogenase were evaluated to ascertain the effect of the terpenoids on the cardiac function. The results showed that there was a non-significant ($p>0.05$) increase in the serum level of creatine kinase activity when the groups that received graded doses of the terpenoids were compared to the normal control (no treatment). There was also a non-significant ($p>0.05$) increase in the serum level of creatine kinase activity of normal control animals compared to the group that received vitamin C (standard drug). The evaluation of lactate dehydrogenase revealed a non-significant ($p>0.05$) increase when the groups that received the graded doses of the extract were compared to the normal control.

Table 1: Effect of Terpenoids on Cardiac Function Enzymes

S/N	GROUPS	CK (U/L)	LDH (U/L)
1	Normal Control	0.14±0.27	3.21±0.46
2	Positive Control	0.03±0.00	2.75±1.12
3	25 mg/kg b.w Terpenoid	0.02±0.01	3.34±1.75
4	50 mg/kg b.w Terpenoid	0.00±0.00	1.84±0.79
5	100 mg/kg b.w Terpenoid	0.00±0.00	2.75±0.97

Values are expressed as mean±SD. Values in the same column having different superscript are significantly different. Positive control = Vitamin C.

Effect of Terpenoids on Kidney Function Indices

The evaluation of kidney function biomarkers as shown in Table 2 revealed that there was a significant ($p<0.05$) decrease in the serum level of creatinine in the groups that received the graded doses of the terpenoids when compared

to the normal control. Also, there was a significant decrease in the serum level of urea when the group that received 50 mg/kg b.w terpenoids and vitamin C (standard drug) were compared to the normal control.

Table 2: Effect of Terpenoids on Kidney Function Indices

S/N	GROUPS	Creatinine (mg/dL)	Urea (mg/dL)
1	Normal Control	0.18±0.06[b]	24.92±1.87[b]
2	Positive Control	0.08±0.32[a]	11.67±2.28[a]
3	25 mg/kg b.w Terpenoid	0.12±0.05[ab]	22.14±1.79[b]
4	50 mg/kg b.w Terpenoid	0.07±0.03[a]	8.82±3.45[a]
5	100 mg/kg b.w Terpenoid	0.11±0.06[ab]	15.24±1.68[ab]

Values are expressed as mean±SD. Values in the same column having different superscripts are significantly different. n= 5.

Effect of Terpenoids on Lipid Profile

The lipid profile evaluation of the test animals showed that there was a significant ($p<0.05$) increase in the serum levels of total cholesterol (TC), low density lipoprotein (LDL) and triacylglycerol (TAG) while there was a non-significant ($p>0.05$) increase when the groups that received the graded doses of terpenoids and vitamin C were compared to the normal control as shown in Table 3. In addition, there was a significant ($p<0.05$) decrease in total cholesterol concentration of the group that received 50 mg/kg b.w terpenoids compared to the group that received 100 mg/kg b.w terpenoids. The total cholesterol concentration of the group that received ascorbic acid (positive control) was significantly ($p<0.05$) lower than the group that received 100 mg/kg b.w terpenoids. There was a non significant decrease in total cholesterol concentration when the group that received 25 mg/kg b.w terpenoids was compared to the group that received 100 mg/kg b.w terpenoids.

A non significant ($p>0.05$) increase in high density lipoprotein was observed as the dose of terpenoids increased from 25 to 100 mg/kg b.w.

On the other hand, there was a significant ($p<0.05$) decrease in low density lipoprotein concentration of the group that received 50 mg/kg b.w terpenoids compared to the group that received 100 mg/kg b.w terpenoids. The low density lipoprotein concentration of the group that received ascorbic acid (positive control) was significantly ($p<0.05$) lower than the group that received 100 mg/kg b.w terpenoids. There was a non significant decrease in total low density lipoprotein when the group that received 25 mg/kg b.w terpenoids was compared to the group that received 100 mg/kg b.w terpenoids.

Table 3: Effect of Terpenoids on Lipid Profile

S/N	GROUPS	Total cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TAG (mg/dL)
1	Normal Control	53.06±12.13^{b}	22.58±2.03^{a}	30.27±5.43^{c}	30.34±9.49^{b}
2	Positive Control	36.35±4.54^{a}	24.06±0.06^{a}	6.69±0.64^{a}	25.44±0.73^{ab}
3	25 mg/kg b.w Terpenoids	49.50±0.43^{b}	23.75±5.80^{a}	9.99±0.14^{ab}	17.80±2.80^{a}
4	50 mg/kg b.w Terpenoids	38.64±0.17^{a}	27.11±8.75^{a}	7.04±2.83^{a}	17.21±1.91^{a}
5	100 mg/kg b.w Terpenoids	45.81±4.49^{b}	27.25±0.18^{a}	12.90±3.92^{b}	23.37±1.87^{ab}

Values are expressed as mean±SD. Values in the same column having different superscript are significantly different. n= 5.

DISCUSSION

The results of effect of terpenoids and vitamin C (positive control) on selected kidney function biomarkers indicated that the normal control showed a significant ($p < 0.05$) increase in creatinine, urea and bilirubin when compared to the groups that received the vitamin C and graded doses of the terpenoids respectively. The administration of the graded doses of the terpenoids reduced the serum level of kidney function biomarkers below that of the normal control.

The toxicological profile of different chemical agents can be evaluated by routine toxicological testing which involves the examination of different organs such as the heart, liver and kidney. It has been reported that dose dependent reactions could be revealed in animal experiments. However, the assessment of kidney and heart function is ideal for toxicity evaluation of drugs and especially plant extracts as possible toxicities normally result in the alterations of their physiological function biomarkers. The cardiac function results indicated that normal control has a significant ($p < 0.05$) higher creatine kinase when compared with the groups that received the vitamin C and graded doses of the terpenoids respectively. The normal control also has a higher concentration of LDH though not significant when compared with the groups that received the vitamin C and graded doses of the terpenoids respectively. However, there was a sudden non-significant increase in cardiac biomarkers in the group that received 100 mg/kg b.w terpenoids.

Changes in the levels of major lipids such as TAG, TC, LDL-C and HDL-C could give useful information on the predisposition of the heart of animals to artherosclerosis and its associated coronary heart disease [7]. The administration of the graded doses of terpenoids and vitamin C (positive control) caused a significant ($p < 0.05$) decreases in the serum levels of total cholesterol, TAG, LDL while there was a significant increase in HDL level of the groups that received the vitamin C and graded doses of the terpenoids respectively compared to the normal control. The group that received 50 mg/kg b.w terpenoids showed a better lipid profile when compared to the groups that received 25 mg/kg and 100 mg/kg b.w respectively. This suggests that treatment with appropriate dose of terpenoids isolated from *Physalis angulata* could be used to ameliorate imbalance lipid profile.

Nephrotoxicity has been described as a major complication characterized by morphological damage of intracellular organelles and cellular necrosis with concomitant functional alterations such as the antioxidant defense system depletion and mitochondrial damage [11]. According to [3], nephrotoxicity occurs when there is derangement of kidney-specific detoxification and excretion due to the damage or destruction of kidney function by exogenous or endogenous toxicants. [19] reported that renal failure causes the retention of creatinine and other non-protein nitrogenous constituents of the blood. There were significant ($p< 0.05$) decreases in creatinine and urea levels of treatment groups compared to the normal control. The sudden increase in creatinine and urea level observed in the group that received 100 mg/kg b.w terpenoids could suggest that the terpenoid fraction may have a possible toxicity at higher doses.

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