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ANTI-HYPERLIPIDEMIC EFFECT OF A BRANDED NIGERIAN POLYHERBAL DRUG ON THE LIPID PROFILE OF HYDROGEN PEROXIDE-INTOXICATED RATS

Joseph Chinedum Ndefo[1], Innocent Uzochukwu Okagu[2], Precious Kelechi Izuchukwu[2]
[1]Department of Science Laboratory Technology, University of Nigeria, Nsukka, Enugu State, Nigeria.
[2]Medical Biochemistry, Parasitology, Toxicology and Drug Discovery Unit, Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria.

Email address: innocent.okagu@unn.edu.ng, okagu.innocent@yahoo.com

Abstract

The effects of a branded Nigerian polyherbal drug (NPD) on lipid profile of hydrogen peroxide (H_2O_2)-intoxicated rats were evaluated. Thirty male rats were divided into 6 groups of 5 rats each were used. Group 1 served as normal control while groups 2-6 were intoxicated with 0.2 ml of 5% H_2O_2 intraperitoneally on day 0. Rats in group 2 were H_2O_2-control while those in groups 3, 4 and 5 received 1, 2 and 3 ml/kg. b.w of NPD, and group 6 received 100 mg/kg b.w of silymarin orally for 14 days. All the rats were sacrificed on 15th day and their fasting blood samples were analyzed for total cholesterol (TC), high and low density lipoproteins (HDL and LDL) and triacylglycerol (TAG). A significantly ($p < 0.05$) higher TC, LDL and TAG, and lower HDL levels of rats in H_2O_2-control when compared with group 1 was observed in this study. The TC, LDL and TAG levels of rats intoxicated and NPD-treated rats was significantly ($p < 0.05$) reduced when compared with H_2O_2-control. However, there was a significant ($p < 0.05$) elevation in HDL levels in groups 3, 4 and 5 when compared with H_2O_2-control. This study demonstrated that the NPD exhibits anti-hyperlipidemic effect which may be attributed to the phytoconstituents.

Keywords: *Nigerian polyherbal drug, lipid profile, silymarin, hydrogen peroxide, phytochemicals*

Introduction

Lipids are required in all living cells where they play major role in the regulation of cellular processes such as cellular growth, differentiation and survival, as well as membrane homeostasis, response to chemotherapy and resistance to drugs [1]. Polyunsaturated fatty acids (the major component of membrane lipids) are susceptible to peroxidation. This results in several damages to biomolecules in the body. The impact of lipids oxidation in cell membrane and how these oxidative damages are involved in both physiological processes and major pathological conditions have been reported [2-4]. This has been linked with so many pathological situations such as cancers and cardiovascular diseases. The burden is worsened by toxicities associated with lipid-lowering drugs such as statins [5-7]. In view of this, there are needs to search for alternatives that can prevent or modulate such deleterious effects of oxidants with fewer or no side effects. The use of herbal medicine has been accepted because of perceived low toxicity, availability and affordability [8]. The manufacturer of a branded Nigerian polyherbal drug (NPD) that is taken as both drug and food supplements in Nigeria and other African countries claims that it has lipid-lowering potentials. The effect of NPD on the lipid status of hydrogen peroxide-induced hyperlipidemia in rat model was evaluated in this study. The phytoconstituents and acute toxicity were also determined.

Methods

Materials

Drugs used for this study were silymarin (Y.S.P. industries (M) Sdn. BHD), and the branded Nigerian polyherbal drug coded in this study as *NPD* (FESCO herbal mixture Nigerian Ltd., were purchased from drug store in Nsukka, Enugu State, Nigeria. NPD is registered by National Agency for Food and Drug Administration and Control with registration number A7-0912L and is composed of aqueous blend of *Cymbopogon citratus* (13%), *Carica papaya* leaves (12%), *Magnifera indica* back (11%), *Moringa oleifera* leaf (11%), *Citrus limonia* (9%), *Psidium* guajava (9%), *Zingiber officinale root* (9%) and *Allium sativum* (6%). Other chemicals used for this study were products of Química Clínica Aplicada, S.A. (QCA) (Spain), Sigma-Aldrich (USA) and BDH (India) and were of analytical grades.

Phytochemical analyses

The phytochemical constituents were determined using the methods of Harborne[9] and Trease and Evans[10] with little modifications.

Management of experimental animals

Animals used for the study were adult male albino mice of body weight 26-30 g and adult rats of body weight 185-200 g. They were obtained from the Breeding Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Before the experiment, the animals were acclimatized under standard laboratory condition in the animal farm of the Department of Nutrition and Dietetics, Faculty of Agriculture, University of Nigeria, Nsukka for 14 days with free access to water and fed with pelletized growers feed *ad libitum*. The experimental animals were handled carefully in accordance with institutional, national and international recommendations for the care and use of animals for research throughout the experimental period. The mice were used for acute toxicity and rats for anti-hyperlipidemic studies.

Acute toxicity test of the polyherbal preparation

Nine (9) mice were used and were randomized into 3 groups of 3 mice each and orally administered 1, 5 and 10 ml/kg body weight of NPD respectively. The mice were observed for 24 hours for behavioural and body weight changes as well as lethality.

Study design for anti-hyperlipidemic study

Thirty (30) adult male albino rats used in this study were grouped into 6 consisting of 5 rats per group. Rats in group 1 served as control and 5 ml/kg body weight of distilled water without intoxication. Rats in groups 2-6 were intoxicated with 0.2 ml of 5% H_2O_2 intraperitoneally on day 0. Rats in group 2 were not treated while those in groups 3, 4 and 5 were received 1, 2 and 3 ml/kg. b.w of NPD, and group 6 received 100 mg/kg b.w of silymarin orally for 14 days. On day 15, the experimental rats were sacrificed and fasting blood samples were collected into plain tubes, allowed to clot and spun at 4,000 rpm for 10 minutes. Sera generated from them were subjected

to lipid profile analysis. The doses used in this study were derived from the manufacturer's recommended dose.

Determination of biochemical lipid profile

Serum total cholesterol (TC) concentration was determined using Allain *et al.*[11] method as contained in QCA commercial kits. Serum high density lipoprotein (HDL) and triacylglycerols (TAG) concentrations were determined using Albers *et al.*[12] method as contained in QCA commercial kits. The levels of low density lipoprotein (LDL) was measured using the method of Friedwald *et al.*[13].

Statistical analysis

Raw data from the laboratory were entered into SPSS, Version 20 and were using analyzed one-way analysis of variance (ANOVA) to compare means across groups. Statistically significance was considered at 95% confidence interval.

Results

Phytochemical constituents of NPD

Result of the phytochemical constituents of NPD is shown in Table 1. The presence of alkaloids (3.50%), steroids (1.00%) and terpenoids (1.00%) were detected in high amount, glycosides (0.50%), anthocyanins (0.46%), anthraquinones (0.43%) and saponins (0.40%) were detected in moderate amount while flavonoids (0.18%), tannins (0.03%), phenols (0.22%) and carotenoids (0.11%) were detected in low amount. The observation showed that alkaloids, steroids and terpenoids are the major phytoconstituents of the herbal preparation (NPD).

24-Hour acute toxicity profile of NPD in mice

As shown in Table 2, there was no significant behavioural and body weight changes as well as mortality recorded in the mice used for acute toxicity test after 24 hours of observation.

Effects of NPD on the lipid profile of experimental rats

The results of the lipid profile of the experimental rats are shown in Table 3. The mean serum levels of total cholesterol (TC), low density lipoprotein (LDL) and triacylglycerol (TAG) in group 2 rats (H_2O_2 control) were significantly ($p < 0.05$) higher than groups 1 (normal control). However, treatment of intoxicated rats with varied doses of NPD in groups 3, 4 and 5 and silymarin in group 6 reduced the mean serum TC, LDL and TAG concentrations when compared to H_2O_2 control. On the other hand, the mean serum level of high density lipoprotein (HDL) in group 2 rats was significantly ($p < 0.05$) lower than normal control. However, treatment with NPD as seen in groups 3, 4 and 5, and with silymarin in group 6 increased the mean serum HDL concentration when compared with group 2 rats.

Discussion

The present study evaluated the phytochemical constituents of commercial herbal mixture (NPD) and lipid profile of hydrogen peroxide-intoxicated rats treated with the herbal mixture. The presence of terpenoids, alkaloids, steroids and saponins were detected in high concentration while others such as tannins and flavonoids were detected in low concentration in the herbal drug. Research has shown that saponins, flavonoids, tannins, steroids and alkaloids possess antioxidant. They are very useful in the scavenging of reactive species, preventing their damaging effects. Similarly, steroids possess analgesic properties [14]. The presence of these phyto-compounds in suggests that NPD could have some medicinal values such as hypolipidemic, antioxidant and hypoglycemic activities since they contain saponins, flavonoids, tannins, alkaloids and steroids. Oxidative stress was induced with hydrogen peroxide (H_2O_2), which is one of the pro-oxidants that causes damage on cellular biomolecules such as lipids, leading to dyslipidaemia [15].

A significant ($p < 0.05$) elevation in serum total cholesterol concentration in intoxicated and untreated rats group 2) when compared to normal control was observed. This increased total cholesterol concentration could be from decreased breakdown or increased synthesis of lipids while the body is trying to adjust to damages on the membrane lipid, receptors/transporters and enzymes responsible for lipid homoeostasis. The above findings suggest that the liver cells were damaged. The liver is a major target of most xenobiotics and as well the organ that regulates plasma lipid levels. Therefore, damaged liver cannot effectively catabolize cholesterol, leading to its accumulation in circulation.

Treatment of H_2O_2-intoxicated rats with different doses of the herbal preparation showed a dose-dependent significant ($p < 0.05$) decrease in the total serum cholesterol in groups 3, 4 and 5. This finding that NPD can reduce serum total cholesterol level infers that the herbal drug may have antioxidant and hypolipidemic potential against H_2O_2-induced lipid peroxidation, dyslipidaemia and oxidative stress. The herbal drug used in this study has 12% *Carica papaya* leaves and previous report has shown that treatment of hypercholesterolemic rats with *C. papaya* leaf extract significantly reduced the level of cholesterol in the liver homogenate as well as in serum. This effect was attributed to the presence of alkaloids and flavonoids which scavenge free radicals [16].

A significant ($p < 0.05$) higher triacylglycerol (TAG) concentration was observed in group 2 compared to group 1. Triacylglycerol are the major high-energy compound for energy storage; liberation of TAG from lipid stores begins under metabolic stress when circulating systemic nutrient supply is not sufficient to meet metabolic energy demand [17]. The metabolic stress seen in this study could be as a result of hydrogen peroxide intoxication which oxidizes liver membrane lipids via lipid peroxidation causing damage to the liver which is responsible for packaging of TAG into very low density lipoprotein for transport and release into the blood [18]. This marked increase in the concentration of TAG could also be due the mobilization of free fatty acid from peripheral depots [19]. Treatment of H_2O_2-intoxicated rats with different doses of NPD showed a significant ($p < 0.05$) decrease in serum TAG concentration as seen in groups 3, 4 and 5, in a dose-dependent manner. This observation implies that NPD possess anti-hyperlipidemic effect which could be linked to the synergistic effects of the free radical scavenging phytochemicals such as alkaloid, tannin, flavonoids and phenol.

Findings of the present study are in line with that of Ikeyi [20] who showed that *C. papaya* contains terpenoids, flavonoids, phenolic compounds, saponins, tannins, cardiac glycosides and alkaloids. The administration of flavonoids-rich extract of *C. papaya* to hyperlipidemic rats improved dyslipidaemia and decreased the intestinal absorption of cholesterol; this could be the mechanism for serum cholesterol improvement [21]. Earlier reports revealed that *C. citratus* is a rich source of phenolic compounds including flavonoids and tannin which are potent antioxidant and is known to reduce oxidative stress and is able to return the liver to normalcy [22].

The main function of LDL is to transport cholesterol from the liver to other tissues that incorporate it into the cell membranes. Result of this study showed that there was a significant ($p < 0.05$) elevation in the levels of LDL in group 2 compared to group 1. This may be due to intracellular cholesterol accumulation which reduces number of hepatic low density lipoprotein-receptor binding, and hence, LDL entry into cells decline and plasma concentration rises. Therefore, a reduced conversion of LDL to intermediate density lipoprotein particles occurs and the body would be unable to remove LDL from the blood. This leads to high concentration of LDL in the blood; a great target for reactive oxygen species such as hydrogen peroxide. Oxidized LDL may be involved in atherogenesis by inducing smooth muscle cell proliferation [23]. Treatment of H_2O_2-intoxicated rats with graded doses of the herbal drug (NPD) also caused a significant ($p < 0.05$) reduction in the serum levels of LDL in groups 3, 4 and 5 when compared with intoxicated-untreated rats. This suggests that NPD reduces serum LDL concentration and hence, its oxidation. This hypolipidemic effect could be linked to the phytoconstituents of this herbal product. Some of the plant constituents of the herbal drug such as *C. papaya* have high content of alkaloids, flavonoids and phenols which are good natural antioxidant that inhibit lipid oxidation thus reducing LDL oxidation [21]. Imaga [24] showed that *C. papaya* is rich in antioxidants compounds and possess hypolipidemic effects.

High density lipoprotein (HDL) is involved in the transport of lipids from tissues and back to the liver or excretion from the body. Thus, HDL has an anti-atherogenic and protective effect in the body. With its anti-inflammatory, antioxidant and vasodilatory properties, HDL is considered as an atheroprotective particle [25]. There was a significant ($p < 0.05$) decrease in HDL levels of group 2 rats compared to control. Hydrogen peroxide intoxication may have decreased lipase activity, reducing the Apo-A and HDL production. There is usually an inverse relationship between serum TAG and HDL

concentrations [26]. Treatment of hydrogen peroxide-intoxicated rats with graded doses of the herbal drug caused a significant ($p < 0.05$) increase in serum level of HDL in test groups (groups 3, 4 and 5 rats) when compared with control.

This demonstrates that the herbal drug could have lipase activating properties and may play an anti-atherogenic role through the elevation of HDL concentration, inhibition of lipid oxidation and promoting reverse cholesterol transport. The observed increase in HDL concentration with a dose, as low as 1 ml/kg b.w., could be attributed to the antioxidant effect exhibited by synergistic action of several antioxidant plant components of the herbal mixture. Previous study has shown that *Z. officinale* and *A. sativum*, which are components of NPD, have high phenolic and tannin concentration which has free radical scavenging ability [27]. In general, the effect of the standard drug, silymarin in modulating dyslipidaemia induced by hydrogen peroxide intoxication as seen in the present study agrees with previous findings [28-30]. In conclusion, the commercial herbal preparation evaluated NPD possesses lipid moderating potentials making it a good candidate of clinical trials for the management lipid-related diseases.

In conclusion, this research has demonstrated that NPD has hypolipidemic/anti-hyperlipidemic effect. Findings of this study suggest that NPD needs to undergo clinical trials to assess its usefulness in the prevention and management of lipid-associated conditions like cardiovascular diseases. However, further scrutiny on the adverse effects of chronic use of the herbal drug is warranted.

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Table 1: Phytochemical constituents of NPD

Phytochemicals	Bioavailability	Amount (%)
Saponins	++	0.40
Tannins	+	0.03
Alkaloids	+++	3.50
Flavonoids	+	0.18
Glycosides	++	0.50
Terpenoids	+++	1.00
Phenols	+	0.22
Steroids	+++	1.00
Carotenoids	+	0.11
Anthraquinones	++	0.43
Anthocyanins	++	0.46

Key: +++ = high content; ++ = moderate content; + = low content

Table 2: Acute toxicity test result of NPD

Group	Doses (ml/kg body weight)	Mortality	Behavioural Changes	Body weight change
Group 1	1	0/3	Nil	Not significant
Group 2	3	0/3	Nil	Not significant
Group 3	5	0/3	Nil	Not significant

(n = 3)

Table 3: Lipid profile of normal and H_2O_2-intoxicaed rats treated with NPD

Groups	TC (mmol/l)	TAGs (mmol/l)	LDL (mmol/l)	HDL (mmol/l)
Group 1	3.65 ± 0.13^{b}	1.23 ± 0.17^{a}	1.40 ± 0.14^{a}	1.95 ± 0.24^{d}
Group 2	4.98 ± 0.72^{c}	1.65 ± 0.24^{c}	3.95 ± 0.10^{d}	0.88 ± 0.10^{a}
Group 3	3.55 ± 0.13^{b}	1.43 ± 0.13^{bc}	2.33 ± 0.10^{c}	1.48 ± 0.10^{b}
Group 4	3.25 ± 0.48^{b}	1.35 ± 0.13^{ab}	1.90 ± 0.19^{b}	1.68 ± 0.13^{c}
Group 5	2.95 ± 0.13^{a}	1.23 ± 0.05^{a}	1.85 ± 0.24^{b}	1.93 ± 0.10^{d}
Group 6	3.50 ± 0.14^{b}	1.38 ± 0.05^{b}	1.93 ± 0.21^{b}	1.32 ± 0.21^{b}

Data are mean ± standard deviation (SD) (n = 4). Values with different superscripts in a column are significant at $p < 0.05$. TC = total cholesterol, TAGS = triacylglycerols, LDL = low density lipoproteins, HDL = high density lipoproteins.