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EFFECTS OF COMMERCIAL HERBAL PREPARATION (HP) USED IN NIGERIA ON LIPID PROFILE OF ACETAMINOPHEN-INTOXICATED RATS

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Abstract

Objective: The present study evaluated the phytoconstituents and protective effects of commercial herbal preparation (HP) on acetaminophen (APAP)-induced lipid profile aberrations in Wistar albino rats. *Materials and Methods:* Twenty four (24) male wistar albino rats were divided into 6 groups of 4 rats each as follows: Group 1 (Normal control- no induction and treated with distilled water only), group 2 (APAP-intoxicated and untreated), group 3 (Pre-treated with 100 mg/kg b.w. of silymarin and APAP-intoxicated), group 4 (Pre-treated with 1 ml/kg b.w. HP and APAP-intoxicated), group 5 (Pre-treated with 2 ml/kg b.w. of HP and APAP- intoxicated) and group 6 (Pre-treated with 3 ml/kg b.w. of HP and APAP-intoxicated). Pre-treatment lasted for 10 days. On day 11, after 30 minutes of HP and standard drugs administration, the animals in groups 2 to 6 were intoxicated with 2 g/kg b.w. of APAP orally after which treatment continued. The rats were sacrificed on day 15 after an overnight fasting and blood samples were collected for biochemical analysis through the jugular vein. The lipid profile: Total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and triacylglycerol (TAG) concentrations of the experimental rats and the phytoconstituents of HP were evaluated using standard methods. *Results:* The presence of alkaloids, saponins, flavonoids, tannins, glycosides, steroids, carotenoids, anthocyanins, anthraquinones, terpenoids and phenols were detected in the extract. There was a significantly ($p < 0.05$) higher mean serum TC, LDL and TAG concentrations of rats in group 2 compared to that of group 1. Also, rats in group 2 (APAP-induced and untreated) had significantly ($p < 0.05$) lower mean serum HDL concentration compared to that of rats in group 1. Pre-treatment with 1 and 2 ml/kg body weight of HP and 100 mg/kg body weight of silymarin significantly ($p < 0.05$) prevented the elevation in the mean serum TC and LDL concentrations of rats in the treated rats compared to that of group 2. Rats pre-treated with 100 mg/kg b.w. of silymarin, and 2 and 3 ml/kg b.w. of HP (groups 3, 5 and 6 respectively) had significantly ($p < 0.05$) higher mean serum HDL concentration compared with APAP-induced and untreated rats (group 2). *Conclusion:* These findings showed that the studied commercial herbal preparation (HP) is rich in phytochemicals and possess modulatory effect on lipid profile of APAP-intoxicated rats which is comparable to the standard drug used.

Keywords: *Herbal preparation; lipid profile; silymarin; lipoprotein; phytochemical*

Introduction

Traditional medicine is a key element among the rural communities in developing countries for the provision of primary health care especially where there are inadequate primary health care systems. The existence of traditional medicine depends on plant species diversity and related knowledge of their use as herbal medicines [1]. Herbal products are used only after some kind of processing, which may include, for example, stir-frying or soaking in vinegar or wine. They include finished labelled products containing herbal materials as the active ingredient, their traditional use and effectiveness have been verified by pharmacological and clinical evaluation, and generally used as either complementary or alternative medicine in health care [2]. Due to limited availability and/or affordability of pharmaceutical medicines in many tropical countries, the majority of the populations depend on traditional remedies mainly from plants. One of the manifestations of acetaminophen (APAP) toxicity is hyperlipidaemia [3]. Lipids are transported by lipoprotein particles in the human body due to their hydrophobicity. Defects or alterations in the enzymatic metabolism of lipids have been related to pathogenesis of important common diseases such as Alzheimer disease, atherosclerosis, insulin-resistant diabetes, cancer or schizophrenia [4]. Obesity and hyperlipidaemia are well recognized risk factors to coronary heart diseases and infertility. However, with the increasing prevalence of sedentary lifestyles and dietary changes, obesity and hyperlipidaemia are well recognized risk factors to coronary heart diseases and infertility [1]. The available hypolipidemic drugs such as statins are toxic [5. 6, 7,] and hence, there are needs to search for herbal principles with lipid-lowering potentials and low toxic effects.

Aim and Specific Objectives of the Study

The aim of the study was to evaluate the effect of commercial herbal preparation (HP) on lipid profile of APAP-intoxicated Wistar rats. The specific objectives of this study were to determine: the phytochemical constituents of the HP, serum total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL) and triacylglycerol (TAG) concentrations of APAP-intoxicated rats pre-treated with commercial herbal preparation (HP).

Materials and Methods

Commercial herbal preparation: the commercial herbal preparation (HP) used in this study according to the manufacturer, is composed of Cymbopogon citrates (13%), Carica papaya leaves (12%), Mangifera indica bark (11%), Moringa oleifera leaves (11%), Citrus limonia (9%), Psidium guajava (9%), Zingiber officinale root (9%), Allium sativum (6%) and water. Standard drugs used for this study were silymarin and acetaminophen. They were purchased from a drug store in Nsukka.

Phytochemical Analysis

The phytochemical analyses were determined using the method of Harborne (1973) and Trease and Evans (1989).

Management of Experimental Animals and Parasite Inoculation

Animals used for the study were male albino rats of weight 120-200 g. The rats were obtained from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Before the experiment, the rats were acclimatized under standard laboratory condition in the animal farm of the Department of Home Science and Nutrition, Faculty of Agriculture, University of Nigeria, Nsukka for 7 days with free access to water and fed with pelletized growers feed ad libitum. They received human care throughout the experimental period in accordance with the ethical rules and recommendations of the University of Nigeria committee on the care and use of laboratory animals and the revised National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No.85-23, revised 1985) at the Animal house, Department of Biochemistry.

Study Design for Animal Study

Twenty four (24) male Wistar albino rats, divided into 6 groups of 4 rats each were acclimatized for 7 days and treated as follows: Group 1: Normal Control (No induction, no treatment)

- **Group 2**: Positive (APAP-induced without treatment)
- **Group** 3: (APAP-induced and pre-treated with 100 mg/kg b.w of silymarin).
- **Group 4**: Low Dose of Drug (APAP-induced and pre-treated with 1 ml/kg b.w. of HP Drug)

- **Group 5**: Mid-dose of Drug (APAP-induced and pre-treated with 2 ml/kg b.w of HP Drug)
- **Group 6**: High Dose of Drug (APAP-induced and pre-treated with 3 ml/kg b.w of HP Drug)

Animals in groups 4, 5 and 6 were treated with HP while those in group 3 were treated with silymarin for 10 days. On day 11, after 30 minutes of HP and standard drug administration, the animals in groups 2-6 were intoxicated with 2 g/kg b.w. of APAP orally. Treatment continued respectively and the animals were sacrificed on day 15 after an overnight fasting and blood samples were collected for biochemical analysis through the jugular vein.

Determination of Biochemical Lipid Profile

Serum total cholesterol (TC) concentration was determined using the method of Allain et al. [8] as contained in QCA commercial kits. Serum high density lipoprotein (HDL) and triacylglycerol (TAG) concentrations were determined using the method of Albers et al. [9] as contained in QCA commercial kits. Low density lipoprotein (LDL) was determined by modified method of Friedwald et al. (1972), as the difference between total cholesterol and the cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in the presence of polyethylene glycol monomethyl ether:

LDL concentration = Total cholesterol – Cholesterol in the supernatant

Statistical Analysis

Data were reported as mean ± SD. One way analysis of variance (ANOVA) were used to analyze the experimental data and Duncan multiple test range was used to compare the group means obtained after each treatment with control measurements. Differences were considered significant when $p < 0.05$.

Results

Phytochemical constituents of HP

Result of the phytochemical constituents of HP 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 mixture.

Serum lipid profile of APAP-intoxicated rats pre-treated with commercial herbal preparation (HP)

The result of lipid profile of normal and APAP-intoxicated rats pre-treated with HP is shown in Table 2. There was a significantly ($p < 0.05$) higher mean serum total cholesterol concentration in the APAP-intoxicated and untreated rats (group 2 = 5.25 ± 0.73 mmol/l) when compared to that of normal control rats (group 1 = 3.65 ± 0.13 mmol/l). Treatment with 1 and 2 ml/kg body weights of HP, and 100 mg/kg b.w. of silymarin significantly reduced the mean total cholesterol concentrations (group 4 = 3.20 ± 0.08 mmol/l, group 5 = 3.15 ± 0.13 mmol/l and group 3 = 3.63 ± 0.26 mmol/l respectively) compared to that of group 2. However, the mean serum total cholesterol concentration of APAP-intoxicated rats pre-treated with 3 ml/kg b.w. of HP (group 6 = 2.97 ± 0.18 mmol/l) was significantly ($p < 0.05$) lower than that of normal control rats (group 1). Induction of rats with APAP significantly ($p < 0.05$) increased the mean serum low density lipoprotein (LDL) concentration in the APAP-intoxicated and untreated rats (group 2) compared to that of normal control rats (group 1). Treatment with 100 mg/kg b.w. of silymarin, and 1, 2 and 3 ml/kg b.w. of HP significantly ($p < 0.05$) reduced the mean serum LDL concentration of APAP-intoxicated rats in groups 3, 4, 5 and 6 respectively towards that of normal control rats when compared with APAP-intoxicated and untreated rats.

Similarly, induction of rats with APAP significantly increased the mean serum triacylglycerols (TAG) concentration in the APAP-induced and untreated rats (group 2) compared to that of normal control rats (group 1). Treatment with 100 mg/kg b.w. of silymarin, and 1 and 2 ml/kg b.w. of HP non-significantly ($p > 0.05$) reduced the mean serum TAG concentration of APAP-intoxicated rats in groups 3, 4, and 5 respectively compared to that of normal control rats. Meanwhile, treatment with 3 ml/kg b.w. of HP significantly ($p < 0.05$) reduced the mean serum TAG concentration of APAP-intoxicated rats in group 6 towards that of normal control rats compared with APAP-intoxicated and untreated rats (group 2). Rats

in groups 2 and 4 had significantly ($p < 0.05$) lower mean serum high density lipoprotein (HDL) concentration compared to that of rats in other experimental groups. Rats pre-treated with 100 mg/kg body weight of silymarin, and 2 and 3 ml/kg b.w. of HP (groups 3, 5 and 6 respectively) had significantly ($p < 0.05$) higher mean serum HDL concentration compared with APAP-intoxicated and untreated rats (group 2).

Discussion

The present study evaluated the phytoconstituents and protective effects of deep root herbal mixture on APAP-induced lipid profile aberrations in rats. 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 the herbal mixture. Some of these phytochemicals act as antioxidants and prevent damages to cellular components which, if not prevented or repaired, may give rise to diseases. They also possess other bioactivities such as antimalarial, antimicrobial and anti-inflammatory effect. Natural polyphenols from plants have been found to exert their beneficial effect by removing free radicals, chelating metal catalysts and activating antioxidant enzymes [10]. In recent times, antioxidants from plant sources have received a lot of attention and are even preferred to synthetic ones, especially due to their multiple potential health benefits, availability, and affordability and in many cases, reduced toxicity [11]. In adipocytes, quercetin, a flavonoid, inhibited glucose transport, oxidation, and incorporation into lipids, reducing lipid accumulation in the system, and other flavonoids active lipid metabolism [12].

There was a significantly ($p < 0.05$) higher mean serum total cholesterol (TC), LDL and TAG concentrations in the APAP-intoxicated and untreated rats (group 2) when compared to other rats. Also, rats in group 2 had significantly ($p < 0.05$) lower mean serum high density lipoprotein (HDL) concentration compared to rats in group 1 (normal control). Dyslipidaemia is a disorder characterized by alterations in the levels and composition of plasma lipids. According to Adult Treatment Panel III (2001), plasma levels ≥ 200 mg/dl for TC, ≥130 mg/dl for LDL, <40 mg/dl for HDL, and ≥150 mg/dl for TAG are dyslipidaemic. Dyslipidaemia may result from in born defects of lipoprotein production or metabolism; but in most cases, it is secondary to an unhealthy lifestyle (e.g., excessive cigarette smoking or alcohol consumption), other health disorders (e.g., obesity, diabetes, Infection, obstructive liver disease), or medication (e.g., β blockers, steroids) [13]. Besides hypertension, chronic dyslipidaemia is a major cause of atherosclerosis, a vascular disease affecting blood circulation in the coronary, central, and peripheral arteries. The pathology is initiated by irritation of the arterial endothelium by high level of circulating LDL, which leads to over expression of adhesion and chemo-attraction molecules (e.g., vascular cell adhesion molecule-1, intercellular adhesion molecule, P and E selectins, monocyte chemo-attractant protein-1) to injured sites, and the recruitment and capture of circulating monocytes to these sites. These immune cells penetrate into the sub-endothelium and differentiate into tissue macrophages, which take up oxidized LDL (oxLDL) via scavenger receptors (e.g., CD36, scavenger receptor-A), becoming the lipid-laden foam cells characteristic of atheromatous plaques [13]. In response to growth factors, resident vascular smooth muscle cells (VSMC) proliferate and form a fibrous cap overlying the plaques. The oxidative process that leads to oxLDL production also contributes to atherogenesis, as this modified lipoprotein and its by-products (oxysterols and oxPL) act as monocyte chemo-attractants and VSMC mitogens. Clinical complications of this process include a narrowing of the arterial lumen, plaque rupture, and formation of circulating thrombi. These complications could lead to coronary artery disease (CAD), myocardial infarction, thrombo-embolic stroke, and peripheral artery disease [14].

Pre-treatment with 1 and 2 ml/kg b.w. of HP and 100 mg/kg b.w. of silymarin significantly ($p < 0.05$) prevented the elevation in the mean TC and LDL concentrations of rats in the HP and silymarin-treated rats compared to that of group 2. Rats pre-treated with 100 mg/kg b.w. of silymarin, and 2 and 3 ml/kg b.w. of HP (groups 3, 5 and 6 respectively) had significantly ($p < 0.05$) higher mean serum HDL

concentration compared with APAP-intoxicated and untreated rats (group 2). Silymarin is a flavonolignan (polyphenolic fraction) extracted from the seeds and fruits of Silybum marianum plant. It is composed of mainly silybin, isosilybin, silydianin and silychristin all of which possess structural similarity to steroids and could be linked to their protein synthesis stimulating effects [15]. It is reported to have antioxidant, anti-inflammatory, antifibrotic, anti-lipid peroxidative, membrane stabilization and liver regenerating activities. The mechanism of action includes inhibition of hepatotoxin binding to receptor sites on the hepatocyte membrane, and stimulating the ribosomal RNA polymerase and subsequent protein synthesis, leading to enhanced hepatocyte regeneration [16]. Studies have reported that M. oleifera [17, 18], which is 11%, M. indica [19], which is 11%, P. guajava [20], which is 9%, Z. officinale (Goyal and Kadnur, 2006) which is 9%, and C. citratus, which is 13% composition of HP possess hypolipidaemic and cholesterol-lowering effect. These plant components and their phytochemicals may be responsible for the observed lipid-lowering effects seen in this study.

Conclusion

Findings from the present study showed that the commercial herbal preparation (HP) is rich in phytochemicals and possess modulatory effect on dyslipidaemia induced by APAP in rats which is comparable to the standard drug (silymarin).

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Table 1: Phytochemical constituents of HP

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

Table 2: Lipid profile of normal and APAP-induced rats pre-treated with HP

Groups	TC (mmol/l)	TAG (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^{c}
Group 2	5.25 ± 0.73^{c}	2.58 ± 0.33^{b}	2.35 ± 0.06^{b}	1.32 ± 0.09^{a}
Group 3	3.63 ± 0.26^{b}	1.93 ± 0.38^{b}	1.05 ± 0.13^{a}	1.84 ± 0.10^{c}
Group 4	3.20 ± 0.08^{b}	1.80 ± 0.42^{b}	1.25 ± 0.13^{a}	1.40 ± 0.04^{a}
Group 5	3.15 ± 0.13^{b}	1.60 ± 0.32^{ab}	1.20 ± 0.41^{a}	1.64 ± 0.04^{b}
Group 6	2.97 ± 0.18^{a}	1.33 ± 0.51^{a}	1.08 ± 0.15^{a}	1.95 ± 0.03^{c}

Data are mean ± standard deviation (SD) (n = 4). Values with different superscripts in a column are significant at $p < 0.05$

HP = commercial herbal preparation; APAP = Acetaminophen (or paracetamol)

TC = Total cholesterol; TAG = Triacylglycerol; HDL/LDL = High/Low density lipoprotein

Group 1 = Normal control (3 ml/kg b.w. of distilled water)

Group 2 = Positive control (APAP-induced and untreated)

Group 3 = Standard control (100 mg/kg b.w. of Silymarin + APAP-induced)

Group 4 = Low dose treatment (1 ml/kg b.w. of HP + APAP-induced)

Group 5 = Mid-dose treatment (2 ml/kg b.w. of HP + APAP-induced)

Group 6 = High dose treatment (Pre-treated with 3 ml/kg b.w. of HP + APAP-induced)