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Research Article

Effect of Methanolic Extract of Ginger (Zingiber officinale) on Lipids Profile, Liver Enzymes Activities and Kidney Functions of P407 induced Hyperlipidemic Albino Rats

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Abstract

The aim of the research was to investigate the effect of methanolic extract of ginger (*Zingiber officinale*) on the liver enzymes activities, lipid profile, kidney function in P407 induce hyperlipidemic albino rats. Liver enzymes, lipid profile and kidney function activity of rat blood were measured. Liver enzymes activity increases from 15.23±2.22 to 110.30±3.22 and from 20.45±4.5 to 40.11±6.7 in AST and ALT enzymes respectively, a decrease was observed from 120.12±1.22 to 92.12±5.6 in the ALP enzymes activity. An increase in the lipid profile from 133.30 ± 33, 154.23±4.55, 104.10±1.23 ± 120.23 ± 4.5C and from 92.34±3.67 to 113.34±5.67 in TG, LDL and Cholesterol level. However, a significant decrease was observed in the HDL from 123.45 ± 3.4 to 80.01 ± 2.0. An induction with poloxamer 407 led to an increase from 35.34±3.35 to 55.56±1.34 in Urea and from 1.33±0.45 to 4.54±0.50 in creatinine level respective. The findings of the study demonstrated that poloxamer 407 induced hyperlipidemia is associated with elevated lipids profile, liver enzymes and kidney disturbance, which were ameliorated by treatment with ginger.

Keywords: *Zingiber officinale*; Poloxamer; Methanolic extract; Lipid profile; Kidney function activity; Hyperlipidemia.

Introduction

Ginger has a very long history of use in various forms of traditional/alternative medicine. It has been used to help digestion, reduce nausea and help fight the flu and common cold, to name a few [1]. Ginger can be used fresh, dried, powdered, or as an oil or juice, and is sometimes added to processed foods and cosmetics. It is a very common ingredient in recipes. Research has shown that taking ginger extract lower cholesterol level, "bad" low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol in people with high cholesterol level, Kidney problem, bladder problems, prostrate problems, asthma, arthritis, diabetes, upset stomach, other conditions [2].

High levels of LDL lipoproteins (the "bad" cholesterol) are linked to an increased risk of heart disease. The foods you eat can have a strong influence on LDL levels. Chemical structures of the four main forms of lipids present in plasma are fatty acids triacylglycerols phospholipids and cholesterol. According to Pilai et al [2], the liver helps in synthesis of cholesterol phospholipids, endogenous triglycerides and lipoproteins. The liver enzymes include the Amino transferases which are aspartate Amino transferase (AST) and Alanine Aminotraiisferases (ALT). Lipid proliferation affects the activity of these enzymes which shows signs of liver damage [5]. Poloxamer 407 is a nonionic polyoxyethylene-polyoxypropylene (PEO-PPO-PEO) compound, which is unique in that it undergoes reverse gelatination, it changes from a liquid to a gel when it warm at temperatures higher than 25^{o}C including body temperature [3], [4]. Poloxamer 407 induces significant changes in the plasma lipids profile as well as biochemical and morphological changes in the liver and heart.

Hyperlipidemia is a disease condition characterized by abnormal elevated levels lipid or lipoproteins in the blood. It is the most common form of dyslipidemia [5]. It is characterized by early onset of cardiovascular diseases and the peripheral vascular disease which caused potential threat to human life [6].

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Prevention is a more effective strategy, than treatment of chronic diseases. Plant base foods, such as fruits, vegetables, and whole grains, which content significant amount of bioactive phytochemicals, provide desirable health benefits beyond basic nutrition to reducing the risk of chronic diseases [7]. Plant phytochemicals such as tannins, flavonoids, phytosterol, saponins, cardiac glycosides plays a vital role in reducing the risk of cardiovascular diseases and other disease in general [8]. The research was aimed at investigating the effect of methanolic extract of ginger on the lipid profile, liver enzymes activities, kidney function.

Materials and Methods

The plant was purchased from North Bank Market Makurdi, Benue State and the taxonomic identification was done by the department of Botany, Federal university of agriculture Makurdi, Benue State, Nigeria.

Forty (40) albino rats (male only) weighing between 200 – 250g before the experiment were bought from the animal farm of the college of health science, Benue State University, Makurdi and were acclimatized in the animal house college of veterinary medicine, University of Agriculture, Makurdi for 2 weeks while allowing them free access to standard feeds (Pfizer feed PLC, Lagos, Nigeria), and allowed water ad libitum.

The extract was prepared according to [10]. The collected plant sample was washed in clean water and dried at room temperature for two weeks. The dried plant sample was ground into powder using a mortar and pestle, the powder obtained was preserved in moisture-free, airtight laboratory containers for further use. The powered plant material (100 g) was macerated with N-Hexane ethylacetate and methanol each (1000 ml) in ratio of 1:10 and will be agitated intermittently for 48 hr and filtered into a clean glass jar. The extract was evaporated to complete dryness in a stream of air (Fan) [10].

The most potent extract was subjected to column chromatograph to separate the extract into its component fractions. Silica gel was used in packing the column while different solvent combinations based on increasing polarity was used as the mobile phase [11]. The glass column (70 cm/ 30 cm) was packed as thus: A ball of cotton wool was put into a glass column using a metal wire. A slurry of silica gel will be prepared using (60 g) of silica gel with a solvent system (Hexane/ Ethyl acetate) in the ratio of 9.5:0.5 and introduced into the column in one smooth [11].

The animals were grouped as
Group 1. Negative control: The rats were not induced
Group 2. Positive control: The rats were induced with Poloxomer 407 500 mg/ kg body weight without treatment
Group 3. The rats were induced with Poloxomer 407 500 mg/ kg body weight and treated with crude extract of ginger 200 mg/kg body weight
Group 4. The rats were induced with Poloxamer 407 500 mg/ kg body weight and treated with the first purified extract of ginger 200 mg/kg body weight
Group 5. The rats were induced with Poloxamer 407 500 mg/ kg body weight and treated with the second purified extract of ginger of ginger 200mg/kg body weight
Group 6: The rats were induced with Poloxamer 407 500 mg/kg body weight and treated with the third purified extract of ginger 200 mg/kg body weight
Group 7: The rats were induced with Poloxamer 407 500 mg/kg body weight and treated with the fourth purified extract of ginger 200 mg/kg body weight
Group 8: The rats were induce with Poloxamer 407 500 mg/kg body weight and treated with standard drug (atorvastatin) 20 mg/kg body weight.

The animals were made hypercholesterolaemic by an intraperitoneal injection of 500 mg/kg of P 407, followed by 6 hr of fasting. The P-407 solution was prepared for injection by combining the agent with saline, which was followed by refrigeration overnight to facilitate dissolution of the P-407 via the cold method [12]. 2 hr after administration of P-407, the rats were treated with ginger extract once daily for 3 days ad libitum.

The rats were treated with ginger extract once daily for 14 days ad libitum. The rats were starved for 24 hr prior to the sacrifice. The blood samples were stored in a plain bottle (i.e. without anticoagulant) for the hormonal assay.

TG levels was determined according to a method by [12]. About 10 µl of distilled water, triglyceride standard and serum was pipetted into

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tubes labelled blank, standard and sample respectively. And 1000µl of reagent was pipetted into each of the tubes which was mixed and incubated at 37oC for 5minutes. The absorbance of sample (A sample) and standard (A standard) will be measured against the reagent blank within 60minutes at 500nm wavelength.

Method of [13] was adopted for LDL. This is a combination of polyvinyl sulphate precipitation and enzymatic method. About 0.2 ml of subjects' sample was pipetted into the respective tubes and 0.2ml of reagent (A) pipetted into each of the tubes. The tubes will thoroughly be mixed and allowed to stand for 15minutes at room temperature. Centrifugation will be done at 4000rpm for 15minutes and the Supernatant was carefully collected.

About 20 µl of distilled water, cholesterol standard and sample supernatant was pipetted into the tubes labelled reagent blank, standard and sample respectively and 1.0ml of reagent (A) and pipetted into each of the tubes. The tubes will thoroughly be mixed and incubated for 10minutes at 37^{o}C. The absorbance of the standard and sample will be measured at 500nm against the blank.

The estimation of HDL was performed using the method as described by [11]. This is principally a combination of phosphotungstate precipitation and enzymatic method. About 0.2 ml of the subject's sample was pipetted into the respective tubes and 0.5ml of reagent (A) pipetted into each of the tubes. The tubes was thoroughly mixed and allowed to stand for 10minutes. Centrifugation will be done at 4000rpm for 10minutes and the supernatant was carefully collected.

About 50µl of HDL-cholesterol standard, sample supernatant and distilled water was pipetted into the tubes labelled standard, sample and reagent blank respectively and 1.0ml of reagent (B) pipetted into each of the tubes. The tubes was thoroughly be mixed and incubated for 10minutes at 37oC. The absorbance of the standard and sample was measured at 500nm against the blank.

Total cholesterol was determined using the method as described by [16]. About 10 µl of distilled water, cholesterol standard and serum was pipetted into tubes labelled reagent blank, standard and sample respectively and 1000µl of reagent pipetted into each of the tubes. The tubes was mixed and incubated for 5minutes at 37oC. The absorbance of the sample (A sample) and standard (Astandard) were measured against the reagent blank within 60minutes at 500nm wavelength.

ALT was determined based on the method described by [17] using Randox test kits. ALT has almost the same working principle with AST, the difference however is from the substrate used. Instead of using the AST substrate the ALT substrate is being used here and also a temperature difference, instead of incubating for 60minutes as used for AST, 30minutes is being used for ALT.

ALP was determined based on the method by [17] using Randox test kits/ 0.5 ml of buffer substrate solution was incubated at 37ºC for 3minutes. 0.1ml of the serum sample will be added to it, mixed and incubated for 60minutes. After which 0.5 ml of 2,4-DNPH will be added, mixed and removed from the incubator and allowed to stand at room temperature for 20minutes. 5.0 ml of NaOH of 0.4 N NaOH was added and was allowed to stand for 5minutes at room temperature and read colometrically at 505 nm.

Treated as test except that 0.1 ml of serum was added after incubating for 60minutes apart from this all other things are the same. AST was determined based on the method by [17] using Randox test kits. 2.0 ml of alkaline buffer was incubated at 37ºC for 3minutes. Then 0.1ml of serum sample was added into a test tube, mixed and incubated at 37ºC for 15minutes. The tube was later removed and the following will be added.

i. 0.5 ml of 0.5N NaOH
ii. 1.2 ml of 4-aminophenazone
iii. 1.0 ml of potassium ferricyanide

Mixed and read colomtrically at 520 nm zerod with blank.
Carried out as test but serum was added after addition of hydroxide.

Alkaline phosphatase was determined based on the method by [17] using Randox test kits. 2.0 ml of alkaline buffer was incubated at 37ºC for 3 min. Then 0.1 ml of serum sample was added into a test tube, mixed and incubated at 37ºC for 15 min. The tube was later removed and the following will be added.

i. 0.5 ml of 0.5N NaOH

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ii. 1.2 ml of 4 - aminophenazone
iii. 1.0 ml of potassium ferricyanide

Mixed and read colomtrically at 520 nm zerod with blank. Test blank carried out as test but serum was added after addition of hydroxide.

Standard was treated as test except for replacement of 1ml buffer and 1ml substrate with 1ml of buffer and 1ml of phenol standard respectively. Standard Blank Was treated same as test except for the use of 1.2ml of buffer and distilled water in 1.0 ml of buffer and 1.0 ml of substrate respectively.

Urea was determined using Urease-Berthelot as described by [9]. The solution mixture was added, Mix and incubated for 10 min. at 37ºC. The absorbance of the sample (A Sample) and of the standard (A Standard) was measured against the blank at 550 nm, (530 – 570 nm). Color stable for 5 hr.

Alkaline picrate method was used to determine Creatinine according to a method described by [9]. The solution mixture was added and mixed well. The tubes were kept well at room temperature for exactly 20 minutes. The absorbance of the standard and the test sample was measured at 520nm

One way ANOVA (L.S.D test) was used to test for significant difference between the groups and the normal control at $P \leq 0.05$. Student's t – test was be used to compare test for the difference between two groups using Statistical package for social sciences (SPSS). The results were presented in Mean±SD using Duncan's multiple range test.

Results and discussion

Effects of ginger on the lipid profile of ploxamer 407 induced hyperlipidemic rats

Table 1 shows the effect of ginger on the lipid profile of poloxamer 407 induced hyperlipidemic rats. After induction with poloxamer 407 an increase in the lipid profile activity from 133.30±33 to 154.23±4.55, 104.10±1.23, 120.23 ± 3.45C and from 92.34±3.67 to 113.34±5.67 in TG, LDL and Cholesterol level respectively, a significant decrease was observed in the HDL from 123.45 ± 3.4 to 80.01 ± 2.0. The values varied significantly (P>0.05) down the groups.

Table 1. Effects of ginger on the lipid profile of ploxamer 407 induced hyperlipidemic rats

Group	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	CHOLESTEROL (mg/dl)
Group 1	133.30±2.33^{ac}	123.45±3.4^{ad}	104.10±1.23^{c}	92.34±3.67^{c}
Group 2	154.23±4.55^{d}	80.01±2.00^{ba}	120.23±3..45^{e}	113.34±5.67^{d}
Group 3	130.45±5.60^{c}	120.45±6.78^{c}	102.34±1.23^{c}	91.45±4.56^{c}
Group 4	127.56±4.50^{b}	118.12±12.2^{c}	94.34±3.45^{cb}	90.23±5.67^{c}
Group 5	110.23±0.91^{a}	119.23±9.2^{c}	83.34±5.60^{a}	73.34±6.71^{a}
Group 6	110.34±23.2^{a}	94.23±7.90^{b}	86.32±1.23^{a}	73.34±4.51^{a}
Group 7	123.57±3.50^{b}	93.23±6.50^{b}	96.67±9.80^{b}	75.77±8.00^{a}
Group 8	130.23±5.6^{c}	120.12±12.33^{c}	110.34±12.34^{d}	84.34±6.78^{b}

Key: Group 1=Negative control, Group 2= Positive control (untreated), Group 3= Poloxamer 407 + crude extract 200 mg, Group 4= Poloxamer 407 + first purified fraction of ginger 200 mg, Group 5= Polaxamer 407 + second purified fraction of ginger 200 mg ginger, Group 6= Polaxamer 407 + third purified fraction of ginger 200 mg Group 7= poloxamer 407+ fourth purified fraction of ginger200 mg, Group 8= poloxamer 407+ standard drug(atorvastatin) 20 mg

The result of the lipid profile in the present study revealed a significant decrease in total Cholesterol, and triglycerides in ginger supplemented group and isolated bioactive compound, when compared to that of the control. However, high-density lipoprotein cholesterol level were significantly lower in other treated

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group compared to the control, this suggests the ameorative effect of ginger. This observed positive effect may be associated with decrease risk of cardiovascular disease and result from enhancing rate of hepatic lipid metabolism or decreasing lipid absorption in the gastrointestinal tract. Result of high-density lipoprotein (HDL) cholesterol showed a significant decrease in all supplement groups. This result is similar with the work of [21]. Both reported a significant decrease effect of clove and ginger peel in total serum cholesterol, triglycerides, and low-density lipoprotein. Abnormal lipid content is usually associated with high plasma triglyceride, low HDL cholesterol and increased LDL cholesterol particle concentrations which attribute to increased free fatty acid flux (lipogenesis). The efficacy of ginger may be due to the presence of compound that was isolated from ginger, which lowered plasma cholesterol levels in rats and mice by cholesterol biosynthesis blockage.

Effects of ginger on the liver enzymes of ploxamer 407 induced hyperlipidemic rats

Table 2 shows the effect of ginger on the liver enzymes of p.loxamer 407 induced hyperlipidemic rats. After induction with poloxamer 407 an increase in the liver enzymes activity from 15.23±2.22 to 110.30±3.22 and from 20.45±4.5 to 40.11±6.7 in AST and ALT enzymes respectively. However a decrease was observed from 120.12±1.22 to 92.12±5.6 in the ALP enzymes acitivity. The values varied significantly (P>0.05) down the groups.

Table 2. Effects of ginger on the liver enzymes of ploxamer 407 induced hyperlipidemic rats

Group	AST (mg/dl)	ALP (mg/dl)	ALT (mg/dl)
Group 1	15.23 ± 2.22^{a}	120.12 ± 1.22^{c}	20.45 ± 4.5^{a}
Group 2	110.30 ± 3.22^{d}	92.12 ± 5.6^{b}	40.11 ± 6.7^{c}
Group 3	100.01 ± 2.11^{d}	88.11 ± 3.4^{b}	29.12 ± 3.4^{b}
Group 4	80.45 ± 3.34^{c}	88.09 ± 6.7^{b}	29.45 ± 2.3^{b}
Group 5	70.44 ± 4.55^{b}	80.11 ± 8.9^{a}	29.33 ± 5.6^{b}
Group 6	70.40 ± 6.70^{b}	90.12 ± 7.8^{b}	20.39 ± 3.4^{a}
Group 7	75.11 ± 3.22^{b}	85.34 ± 8.9^{a}	23.34 ± 5.6^{a}
Group 8	20.12 ± 1.23^{a}	90.03 ± 6.7^{b}	19.99 ± 4.6^{a}

Key: Group 1=Negative control, Group 2= Positive control (untreated), Group 3= Poloxamer 407 + crude extract 200 mg, Group 4= Poloxamer 407 + first purified fraction of ginger 200mg, Group 5= Polaxamer 407 + second purified fraction of ginger 200 mg ginger, Group 6= Polaxamer 407 + third purified fraction of ginger 200 mg Group 7= poloxamer 407+ fourth purified fraction of ginger200mg, Group 8= poloxamer 407+ standard drug (atorvastatin) 20 mg

Poloxamer 407 (P-407) has been utilized as a model to induce hyperlipidaemia in rats due to its convenience, reproducibility, low cost and the lack of undesirable underlying pathological conditions [18], [15] the induction of poloxamer 407 caused hyperlipidemedia thereby causing a change in activity in various organs, changes in liver enzyme activity as a decrease in the enzyme activity was observed. Research carried out by [19] showed elevation of all the three liver enzymes (AST, ALP and ALT) after induction of Wistar rats with Polaxamer 407. The discrepancy in the serum levels of the enzymes was attributed to the levels and duration of hyperlipidaemia [20]. In the present study, there was significant elevation in the serum ALT and AST but not ALP of hyperlipidaemic control group when compared to the normal control group. This may be due to injuries inflicted to the liver secondary to the accumulation of triglycerides and other fats in the liver cells.

Effects of ginger on the kidneys of ploxamer 407 induced hyperlipidemic rats

Table 3 shows the effect of ginger on the kidney indices of poloxamer 407 induced hyperlipidemic rats. An induction with poloxamer 407 led to an increase from 35.34±3.35 to 55.56±1.34 in Urea and from 1.33±0.45 to 4.54±0.50 in Creatinine level respectively. The values varied significantly (p>0.05) down the groups. The observed creatinine, major kidney function parameter, high level might have result from the decrease synthesis or increased functional capacity of tubular excretion [15]. P407 increase the kidney function as seen in the urea and creatinine accumulation, it may be used in hyperlipidemic nephropathy because nephropathy is

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accompanied by oxidative stress, advanced glycation end products, abnormal lipid metabolism and renal accumulation of lipids [21-22].

Table 3. Effects of ginger on the kidneys of ploxamer 407 induced hyperlipidemic rats

Group	Urea (mg/L)	Creatinine (mg/L)
Group 1	36.34±3.35[a]	1.33±0.45[a]
Group 2	55.56±1.34[c]	4.54±0.50[b]
Group 3	41.34±6.45[b]	1.33±0.33[a]
Group 4	40.89±3.45[b]	1.35±0.12[a]
Group 5	39.67±2.23[b]	1.28±0.34[a]
Group 6	37.58±4.46[a]	1.18±0.34[a]
Group 7	38.78±12.45[a]	1.29±0.57[a]
Group 8	43.96±4.55[b]	1.47±0.18[a]

Key: Group 1=Negative control, Group 2= Positive control (untreated), Group 3= Poloxamer 407 + crude extract 200 mg, Group 4= Poloxamer 407 + first purified fraction of ginger 200 mg, Group 5= Polaxamer 407 + second purified fraction of ginger 200 mg ginger, Group 6= Polaxamer 407 + third purified fraction of ginger 200mgmGroup 7= poloxamer 407+ fourth purified fraction of ginger 600mg, Group 8= poloxamer407+ standard drug(atorvastatin) 20 mg

Conclusions

In conclusion, the findings of the present study demonstrated that poloxamer 407 induced hyperlipidemia is associated with elevated liver enzymes and kidney disturbance, which were ameliorated by treatment with ginger. The effect observed could be as a result of interactions between supplements biological active constituents and endogenous antioxidant system in the experimental animal model.

Conflict of interest

The authors declare that there is no competing interest.

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