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Effects of Fenugreek Supplement on Liver Enzymes Level in P-407 Induced Hyperlipidemia in Wistar RatsAbdulrazak, A.¹, Tanko, Y.², Kabiru, A.² and Sada, M.N.¹

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Abstract

The global incident of hyperlipidemia associated with cardiovascular disorders leading to increased morbidity among individuals is alarming. Challenges of adverse effect of certain antihyperlipidemic drugs (statins, fibrates, bile acid sequestrant and cholesterol absorption inhibitors) their high cost and unavailability to the population particularly to those at the grass root. There is need to search for an alternative remedy with lesser side effect and more accessible among masses. Twenty (20) Wistar rats were divided into four groups n=5. Hyperlipidemia was induced using a single dose of P407 (1.5g/Kg) IP at the interval of three days. Group 1 served as the normal control, group 2 served as disease control (hyperlipidemic rats placed on normal diet), group 3 comprises of hyperlipidemic rats placed on Cholestyramine (0.26g/Kg) and group 4 comprises of hyperlipidemic rats placed on Fenugreek seed supplement (2.5%). After three weeks of treatment blood sample were collected via cardiac puncture from which serum was used for assessment of liver enzymes: alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST). Data generated were statistically analyzed. Results generated revealed a significant increase in AST and ALP levels in group 2 animals compared to their levels in group 1 and a significant decreased in the enzymes levels in group 4 animals when compared to the levels in group 2. These results revealed a disarray of liver enzymes in the disease control group (group 2) while the pattern seen in the supplement- treated group was similar to that observed in the normal

control group. These indicate that the supplement has a reversal like activity on distorted liver enzymes in P407 induced hyperlipidemic rats. The activity noticed could be associated with the actions of secondary metabolites of bioactive compounds content of the supplement as observed in the results of preliminary phytochemical screenings. Therefore, the supplement sub-served a hepatoprotective-like activity in the treated experimental animals. With further studies these observed effects may be refined to the applicable format in humans to manage liver disorder due to hyperlipidemia.

Keywords: Fenugreek seed, liver enzymes, ALP, AST, ALP, hyperlipidemia

Introduction

Hyperlipidemia (HL) is a medical condition characterized by an elevation of any or all lipid profile and/or lipoproteins in the blood. It is also called hypercholesterolemia / hyperlipoproteinemia because these excess lipids travel in the blood attached to proteins (Roger *et al.*, 2016). Although elevated low-density lipoprotein cholesterol (LDL) is thought to be the best indicator of vascular disorders such as atherosclerosis while dyslipidemia describe an abnormal amount of lipids in the blood associated with elevated total cholesterol (TC) or triglycerides (TG), or low levels of high-density lipoprotein cholesterol (HDL). Human body is complex system for maintaining the homeostasis of various organ and organ system. Any undesirable change will disturb the balance resulting in diseased state (Ansar *et al.*, 2011;

Thompson, 2004). Lipids are fats in the blood stream, commonly divided into cholesterol and triglycerides. Cholesterol circulates in the bloodstream and is involved in the structure and function of cells. Triglycerides (TG) are best viewed as energy that is either used immediately or stored in fat cells. TG is manufactured in the liver from the foods or by being absorbed from the intestine (Ankur *et al.*, 2012).

The development of medicinal plants in the domain of nutrition has unveiled their additional therapeutic potentials (Ramawat *et al.*, 2009). In the same era, terms like functional foods, nutraceutical and farm foods have taken hold of

the nutrition market, mainly aiming to provide nutrition and healthy diets. Many plants and their components have been extensively investigated for their health-promoting benefits, including antioxidant, cardio-protective, anti-hyperlipidemia, anti-cancer, antimicrobial and immune-modulatory activities (Sharma and Seth, 2004).

Fenugreek is regarded as one of the oldest known medicinal plant in recorded history (Goyal, *et al.*, 2016). Its seed and leaves have medicinal value, and have been used to reduce blood glucose and lower blood cholesterol in humans and animals (Dahanukar *et al.* 2000).



Plate 1: Fenugreek (*Trigonella foenum-graceum* C.F) (Adopted by Author, 2017).

Fenugreek contains three important chemical constituents (steroidal saponins, galactomannans and isoleucine) with medicinal value. These constituents have placed fenugreek among the most commonly recognized "nutraceutical" or health food products. Legume consumption is known to have a beneficial or protective effect in diabetes, hypercholesterolemia and coronary heart disease, as well as protecting against obesity and menopause (Iyer *et al.*, 2009).

Various animal models have been used to study the pathophysiological effects of hyperlipidemia. Genetic variants of hyperlipidemia, such as the obese Zucker rat are

available. Alternatively, hyperlipidemia can be induced by dietary means (e.g. chronic feeding of a high fat diet) (Jimoh *et al.*, 2015) or by treatment with compounds such as Triton or poloxamer 407 (P407) (Johnston and Palmer, 1993).

Poloxamer 407, a non-ionic synthetic copolymer surfactant, provides an attractive means of inducing hyperlipidemia because of its rapid onset and seeming lack of overt toxicity. Within 24 hours of its intraperitoneal (i.p.) injection a profound hyperlipidemia state is achieved (Cogger *et al.*, 2006). It has been used to induce experimental hyperlipidemia in several rodent species including rat (Johnston and Palmer,

1993), mouse and rabbit (Blonder *et al.*, 1999). With chronic administration it has been shown to induce atherosclerosis in the mouse, a species which is quite resistant to its onset (Johnston, 2004). Poloxamer 407 increases serum lipoproteins via its actions at various levels in lipid metabolism, largely by inhibiting lipoprotein lipase, which facilitates the hydrolysis of triglycerides (TG) (Johnston and Palmer, 1993). Liver enzymes are intrahepatocyte- specialized proteins (enzymes), they have a pivotal role in metabolic activities of the liver. Their physiological level in circulation is associated with hepatocyte firm structural integrity while elevated levels indicate a link towards hepatotoxic action of ingested or administered agents or chemicals (Mathur *et al.*, 2016). In prolonged hyperlipidemia or dyslipidemia they are seen to elevate in circulation, this may lead to non-alcoholic fatty liver diseases associated with lots of complications. Drugs used in treatment of lipidemic disorders are majorly linked to elevating liver enzymes in patients with previously normal liver enzymes levels (Elizabeth *et al.*, 2018), there is short of literature on the scientific- based effects of fenugreek seeds supplement on liver enzymes level and many other herbs with therapeutic potential. The aim of this study was to determine the effects Fenugreek (*trigonella foenum-graceum*) supplements on liver enzymes in poloxamer 407 induced hyperlipidemia Wistar rats.

Materials and Methodology

Materials

Plant Materials

- Fenugreek (*Trigonella foenum-graceum*) Seeds

Chemicals/Drugs

- Cholestyrimine
- Chloroform
- Ice block
- Methylated spirit
- Poloxamer 407
- Vitalyte

Equipment

- Digital Glucometer and Strips
- 1ml and 5ml syringes

- Dissecting Kits
- EDTA and Plain bottles
- Scissors
- Gloves
- Cotton wool

Methods

Collection and Preparation of Plant

The Fenugreek seed was purchased from Terminus Jos, Plateau State, and Sabon Gari market in Zaria Nigeria respectively. The Fenugreek seeds were presented to the herbarium unit of the Department of Botany, Faculty of Life Science, Ahmadu Bello University, Zaria, where it was identified by a Taxonomist Malam Namadi Sunusi, with a deposited voucher number 12034. The Fenugreek seeds were ground into fine powder using laboratory mortar and pestle. The powder was kept in an air-tight container until use.

Diet formulation

Fenugreek supplemented Diet: -

2.5% of fenugreek (*Trigonella foenum-graceum*) seeds supplement was combined to the 97.5% standard animal diet making the total feed formulation (100%) and fed to P407 induced hyperlipidemic Wistar rats for 21 days (Johnston, 2004).

Management of Experimental Animals

Thirty (20) Wistar rats were purchased from the animal house of Human Physiology Department, College of Medicine Ahmadu Bello University Zaria. The animals were kept in well aerated laboratory cages in the animal house of the Department of Human Physiology, Ahmadu Bello University, Zaria. The rats were given access to drinking water *ad libitum* during the experimental period.

Animal Grouping and Experimental Design

Twenty (20) Wistar rats were divided into four groups $n=5$, hyperlipidemia was induced using a single dose of P407 (1.5g/Kg) IP at the interval of three days.

Group 1: Normolipidemic feed with normal diet.

Group 2: Served as disease control were administered with Poloxamer 407 1.5g/kg (IP) body weight and normal feed.

Group 3: Hyperlipidemic rat and received Cholesteramine 0.26g/kg body weight and normal feed.

Group 4: Hyperlipidemic rat and received P407-1.5g/kg body weight and Fenugreek (2.5%) supplemented diet.

Induction of hyperlipidemia

The experimental animals were intraperitoneally injected with 1.5g/kg of poloxamer 407.

Collection and Preparation of Serum Samples for Biochemical Assays

Three weeks after the treatment period, all animals were fasted for 12 hours and sacrificed after using mild anaesthesia with chloroform. Blood sample (about 4 ml) was collected by cardiac puncture. Blood samples were collected in plain tubes and allowed to clot. The serum was separated by centrifugation at $2000 \times g$ for 10 minutes. The serum collected was used for laboratory assay of liver enzymes.

Quantitative Analysis of the Phytochemical Constituents

Preparation of Fat Free Sample: About 2 g of sample was weighed and defatted with 100 mL of diethyl ether using a Soxhlet apparatus for 2 hours. Alkaloid determination was carried out using Harborne (1973) method. About 5g of the sample was weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 hours. This was then filtered and the extract was then concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was then allowed to settle and the precipitate was then collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid which was dried and weighed (Harborne, 1998).

Flavonoid determination by the method of Boham and Kocipai-Abyazan (1994): About 10g of the supplement was extracted repeatedly with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm).

The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight (Boham and Kocipai-Abyazan, 1994).

Tannin determination by Van-Burden and Robinson (1981) method: About 500 mg of the sample was weighed into a 50 mL plastic bottle and 50 mL of distilled water was added and shaken for 1 hour on a mechanical shaker. This was then filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filtrate was pipetted out into a test tube and mixed with 2 mL of 0.1 M FeCl_3 in 0.1 N HCl and 0.008 M potassium ferrocyanide ($\text{C}_6\text{N}_6\text{FeK}_4$). The absorbance was measured at 120 nm within 10 minutes (Van-Burden and Robinson, 1981).

Determination of total phenols by spectrophotometric method: The fat free sample was boiled with 50 mL of ether for the extraction of the phenolic component for 15 minutes. About 5 mL of the extract was pipetted into a 50 mL flask then 10 mL of distilled water was added. About 2 mL of ammonium hydroxide solution and 5 mL of concentrated amyl alcohol were added. The sample was made up to the mark and left to react for about 30 minutes for color development. The absorbance was measured at a wave length of 505 nm.

Saponin determination by the method of Obadoni and Ochuko (2001): Twenty grams (20g) of the sample was weighed and put into a conical flask then 100 mL of 20% aqueous ethanol was added. The sample was heated over a hot water bath at 100°C for four hours with continuous stirring at about 55°C . The mixture was filtered and the residue was re-extracted with another 200 mL of 20% ethanol. The combined extract was reduced to 40 mL over water bath at about 90°C . The concentrate was transferred into a 250 mL separatory funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 mL of n-butanol was added. The combined n-butanol extract was washed twice with 10 mL of 5% of aqueous sodium chloride. The remaining solution was heated in a water bath. After

evaporation, the sample was dried in the oven to a constant weight, the saponin content was calculated as percentage (Obadoni and Ochuko, 2001).

Oxalate determination by Day and Underwood (1996) method: About 1g of the sample was weighed into 100ml conical flask and 75ml of 3N H₂SO₄ was added and stirred for 1hour. It was then filtered using Whatman No. 1 filter paper. From the filtrate, 25ml was taken and filter while hot (80°-90°C) against 0.1N KMnO₄ solution, faint pink colour persists for 30 seconds. The oxalate content was calculated.

$$\text{Oxalate (mg/100mg)} = \frac{T \times V \times D \times 100}{M \times M(g)}$$

Where T is the titre of KMnO₄ (ml)
 V is the volume mass equivalent
 D is the dilution factor
 M is the molar equivalent of KMnO₄ redox reaction and
 M is the mass of sample used

Statistical Analysis

Data collected were expressed as mean + SEM. It was analyzed using one-way analysis of variance, ANOVA, and Tukey's post-hoc test was used to compare the level of significance between the control and treatment groups, using SPSS version 20.0. Values of p<0.05 were considered significant (Duncan et al., 1977).

Results and Discussion

Table 1. Fenugreek (*Trigonella foenum-graceum*) seed phytochemical analysis

Phytoconstituents	Percentage Composition
Alkaloid	2.05%
Flavonoids	4.15%
Saponins	0.55%
Tannins	0.1%
Phytate	0.14%
Oxalate	2.0%
Total Phenol	0.7%
Trysin	6.5%

Table 1 shows the results of the phytochemical analysis. It revealed that the supplement (*Trigonella foenum-graceum*) has 6.5% of trypsin, flavonoids 4.1%, oxalate 2.0%, alkaloids 2.05%, tannins 0.1%, saponins 0.55%, phytate 0.14%, and 0.7% of total phenol among other bioactive compounds.

Table 2: Liver Enzymes Levels in P-407 Induced Hyperlipidemia in Wistar Rats Treated with Fenugreek Seeds Supplements

Groups	Treatments	AST (U/L)	ALT (U/L)	ALP (U/L)
1	Normal Control	28.90 ± 1.35*	21.40 ± 0.71*	25.58 ± 1.95*
2	Disease Control P407 (1.5g/kg)	54.32 ± 2.54	8.06 ± 0.50	33.40 ± 1.46
3	P407 (1.5g/kg) + Cholesteramine (0.26 g/kg)	6.42 ± 0.91*	7.12 ± 0.97	6.96 ± 0.46*
4	P407 (1.5g/kg) + Fenugreek (2.5 %)	21.78 ± 1.59*	14.06 ± 0.57*	20.70 ± 1.03*

Significant values: p<0.05= * in comparison to group 2; p<0.01



Table 2 revealed the results of liver enzymes (alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST)) levels in all the experimental groups, These results displayed shows a significant increased in AST and ALP levels in the disease control group (group 2) while the pattern seen in the supplement treated group was similar to that observed in the normal control group, both groups shows a significant decrease in AST and ALP levels when compared to the levels in group 2.

Hyperlipidemia and dyslipidemia are important signs that indicates an individual's risk for many health complications ranging from cardiovascular diseases down to metabolic disorders such as NAFLD and diabetes mellitus, a single intraperitoneal administration of 1.5g/kg of P-407 lead to defenestration of the liver sinusoidal endothelium within 24 hours and a sustained hyperlipidemia is observed to last for about 48 hours after (Cogger *et al.*, 2006), chronic hyperlipidemia is associated with distorted liver enzymes profile. Physiologically disproportionate liver enzymes level apart from serving as indicator for progress of degenerative diseases, is another key indicator used for observing hepatic responses to drugs and prognosis as well (Mohamed *et al.*, 2016). The results of the present study (Table 2) revealed a significant increase in AST and ALP levels in group 2 animals when compared to their levels in group 1 and a significant decreased in the enzymes levels in group 4 animals when compared to the levels in group 2. These results revealed a disarray of liver levels enzymes in the disease control group (group 2), the abnormal liver enzymes spectrum in the diseased untreated group observed is probably the evidence of P407 action at the hepatic level. This may be due to accumulated level of unprocessed lipids that leads to circulatory lipotoxicity, thereby leading to incompetent cell membrane integrity, thus cytoplasmic content leaks in to extracellular compartments (Wout *et al.*, 2017), while the pattern seen in the supplement treated group were not statistically different from that observed in the normal control group. These indicate that the supplement has a reversal like activity on distorted liver enzymes in P407 induced hyperlipidemic rats seen in group 2. The activity noticed could be due to stabilization of biological membrane or directly do to the

supplement hyperlipidemic activity which is associated with the actions of secondary metabolites of bioactive compounds content of the supplement as observed in the results of preliminary phytochemical screenings (Table 1), phenolic, flavonoids saponins and alkaloid appears positive in appreciated amounts in the supplement this is in line with previous reports (Gong *et al.*, 2016) on the phytochemicals. These phytochemicals have been proven with antioxidant property, anti-cholesteremic as well as anti-inflammatory activities (Sharma and Choudhary, 2017; Sharma and Choudhary, 2014) which is the under lying pathogenesis of the liver induced responses in the experimental animals (Mohamed *et al.*, 2016). Therefore, the supplement sub-served a hepatoprotective-like activity in the treated experimental animals. With further studies these observed effects of the study supplement may be refined to the applicable and suitable form to manage liver disorders due to hyperlipidemia.

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