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Ethanolic extracts of Vitex doniana possesses hepatocuractive property in Poloxamer induced hyperlipidemia

Victor Duniya Sheneni*, Dorcas Bolanle James, Sunday Eneojo Atawodi

Department of Biochemistry, Ahmadu Bello University, Samaru Zaria, Nigeria

Email address:
shenenivictor@gmail.com (V. D. Sheneni), dbjams16187@yahoo.com (D. B James), atawodi.se@gmail.com (S. E Atawodi)

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Abstract: The hypolipidemic effect of *Vitex doniana* ethanolic leaves, stem bark and root bark extracts on some biochemical parameters in Poloxamer 407 (P407) induced hyperlipidemic rats was studied for a period of 21 days. Fifty four mixed sex rats weighing 100-200g were divided into nine groups comprising six animals per group: group given feed and water only, group induced by an intra-peritoneal injection of P407 every 48 hours without treatment, groups induced and treated with atorvastatin, leaves, stem bark, root bark extracts and groups of normal rats treated with leaves, stem bark and root bark extracts without induction. In all the groups, P407, atorvastatin, leaves, stem bark and root bark extracts were administered at a dose of 1000mg/kg, 20mg/kg, 100mg/kg, 100mg/kg and 30mg/kg body weight respectively. Blood samples were collected from the rats in all the groups at the end of the 21 days of treatment for determination of serum levels of; aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP), total bilirubin (TB), indirect bilirubin (IB) and direct bilirubin (DB) using Randox kits. Serum concentration of liver marker enzymes (AST, ALT and ALP) was significantly ($p<0.05$) higher in hyperlipidemic control group when compared to normal control and all treated groups. However there was a significant ($p<0.05$) decrease and non-significant ($p>0.05$) change in the levels of these marker enzymes in hyperlipidemic groups treated with the extracts and normal treated groups when compared with the hyperlipidemic and normal control group respectively. There was a significantly ($p<0.05$) lower level of TP, DB and a significantly ($p<0.05$) higher level of TB, ID in hyperlipidemic group when compared with rats in the normal control and all treated groups. Normal groups treated with the extracts without induction shows a non-significant ($p>0.05$) difference in TP, DB, TB and ID when compared with normal control group. These results highlight the efficacy of *Vitex doniana* (leaves, stem bark and root bark) ethanolic extracts in the amelioration of some undesirable effects of hyperlipidemia.

Keywords: *Vitex Doniana*, Hyperlipidemia, Poloxamer 407, Biochemical Parameters

1. Introduction

The Liver, the largest gland is a vital organ. It has a pivotal role in regulation of physiological processes. It is involved in several vital functions such as metabolism, secretion and storage. Furthermore, detoxification of a variety of drugs and xenobiotics occurs in liver. The bile secreted by the liver has, among other things, an important role in digestion. Liver diseases are among the most serious ailment. They may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non-inflammatory diseases) and cirrhosis (degenerative disorder resulting in fibrosis of the liver). Liver diseases are mainly caused by infections, excess consumption of alcohol, autoimmune/disorder and toxic chemicals (certain antibiotics, chemotherapeutics, peroxidised oil, aflatoxin, carbon-tetrachloride, chlorinated hydrocarbons, etc.). In recent years, researchers have used scientific methods to evaluate the effects of medicinal plants used in traditional medicine for the treatment of liver ailments [1, 2, 3, 4]. In many cases, the mechanisms and modes of action of these plants as well as their therapeutic effectiveness have been confirmed in clinical studies. Several hundred plants have been examined so far, but only a handful has been studied thoroughly. Among these are *Silybummarianum* (milk thistle), *Picrorhizakurroa* (kutki), *Curcuma longa* (turmeric), *Camellia sinensis* (green tea), and *Glycyrrhizaglabra* (licorice) [5, 6,].

A medicinal plant according to world health organization

[7] is a plant that one or more of its organ contains substances that may be used for therapeutic purposes or as precursor for the synthesis of useful drugs. About 80% of the world population relies on the use of traditional medicine which is predominantly based on the plant materials. The traditional medicine refers to a broad range of ancient natural health care practices including folk/tribal practices as well as Ayurveda, Siddha, Amchi and Unani. These medical practices originated from time immemorial and developed gradually, to a large extent, by relying or based on practical experiences without significant references to modern scientific principles. These practices incorporated ancient beliefs and were passed on from one generation to another by oral tradition and/or guarded literature. Although herbal medicines are effective in the treatment of various ailments very often these drugs are unscientifically exploited and/or improperly used. Therefore, these plant drugs deserve detailed studies in the light of modern science. The major active substance found in many medicinal plants is polyphenol [8].

Polyphenols are natural organic chemicals characterized by the presence of large number of phenol structural units [8]. Researchers and food manufacturers have become increasingly interested in polyphenols due to the recognition of their antioxidant property, abundance in our diet and their probable role in the prevention of various diseases associated with oxidative stress such as cardiovascular, cancer and neurodegenerative diseases. Polyphenols can be extracted from fresh, frozen or dried plant samples. Usually before extraction plant samples are treated by milling, grinding and homogenization, which may be preceded by air-drying or freeze-drying. Generally, freeze-drying retains higher levels of phenolics content in plant samples than air-drying. Solvent extractions are the most commonly used procedures to prepare extracts from plant materials due to their ease of use, efficiency, and wide applicability. It is generally known that the yield of chemical extraction depends on the type of solvents with varying polarities, extraction time and temperature, sample-to-solvent ratio as well as on the chemical composition and physical characteristics of the samples. The solubility of phenolics is governed by the chemical nature of the plant sample, as well as the polarity of the solvents used. Depending on the solvent system used during exaction, a mixture of phenolics soluble in the solvent will be extracted from plant materials. Epidemiological studies and associated meta-analyses strongly suggested that long term consumption of diets rich in plant polyphenols offered some protection against development of cardiovascular diseases, cancers, diabetes, osteoporosis and neurodegenerative diseases [9, 10]. Polyphenols and other food phenolics is the subject of increasing scientific interest because of their possible beneficial effects on human health.

Hyperlipidemia is the abnormal elevation of lipids in the blood, largely cholesterol and triglycerides. It is also known hyperlipoproteinemia due to abnormal elevations of lipoproteins that transports lipids in the blood. It is characterized by elevated lipid level in the blood, and is one of the disease conditions that are injurious to the liver. It sometimes results in fatty infiltration of the liver leading to a condition known as non-alcoholic fatty liver [11] which damages the liver.

Vitex doniana (*V. doniana*) is a common medicinal plant in south western Nigeria. It is commonly known as black plum or African olive, Dinya in Hausa, Oori-nlain Yoruba, Ucha coro in Igbo. The plant has been used as medication for liver disease, anodyne, stiffness, leprosy, backache, hemiplegia, conjunctivity, rash, measles, rachitis, febrifuge, as tonic galactagogue to aid milk production in lactating mothers, sedative, digestive regulator and treatment of eye troubles, kidney troubles and as supplement for lack of vitamin A and B [12]. Therefore, this study was conducted to determine the effect of ethanolic extracts of different parts (leaves, stem bark and root bark) of *Vitex doniana*on some biochemical parameters of P407 induced hyperlipidemic rats.

2. Materials and Methods

2.1. Experimental Animals

A total of 54 apparently healthy rats of both sexes of two weeks of age weighing between 100 – 200g were obtained from National Institute for Trypanosomiasis Research, Kaduna, Nigeria, and kept according to sexes in well aerated laboratory cages in the Animal house, Department of Pharmacology, Ahmadu Bello University, Zaria, Nigeria. The animals were allowed to acclimatize to the laboratory environment for a period of two weeks before the commencement of the experiment which lasted for 21 days. They were fed with water and grower mash *ad libitum.*

2.2. Collection of Plant Material and Identification

The fresh leaves, stem bark and root bark of *V. doniana* were collected from the Institute of Agricultural Research, Ahmadu Bello University, Zaria Kaduna State, Nigeria in April 2012. The plant was identified at the herbarium unit in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria where a voucher specimen (1162) was deposited.

2.3. Plant Preparation and Extraction

One hundred gram of each of the grounded leaves, stem bark and root bark were weighed into three sterilized conical flasks and 500 ml of distilled water was poured into each of the flasks. The contents of the three flasks were shaken and the tops were covered with aluminium foil and kept at ambient temperature for 48 h after which the extracts were obtained by filtering using clean cloth with fine pore. The extracts were then concentrated in crucibles using water bath set at a temperature of 45 °C. The weight of the concentrated extracts were taken and then stored in an air-tight sample bottles in a refrigerator until required for analysis [13].

2.4. Acute Toxicity Study (LD_{50})

The mean lethal dose (LD_{50}) of *Vitex doniana* (leaves, stem bark and root bark) ethanolic was determined by a method described by Lorke [14].

2.5. Preparation of Standard Drug

Atorvastatin (Pfizer Ireland pharmaceuticals, Ireland) was purchased in a tablet form at strength 20 mg. Tablets were dissolved in distilled water and administered orally.

2.6. Induction of Hyperlipidemia

Poloxamer 407 (Lutrol F127; BASF, Ludwigshafen, Germany) was used as the inducing agent. Prior to the administration, Poloxamer 407 was dissolved in distilled water and refrigerated overnight to facilitate its dissolution. Needles and syringes to be used for administration were also cooled to prevent gelation within the syringe during injection [15].

2.7. Animal Grouping and Treatment

A total of 54 rats were used. The rats were randomly divided into 9 groups of 6 rats each.

- Group I: were fed normal chow and distilled water only for 21 days (NC).
- Group II: were induced without treatment (HC).
- Group III: were induced animals and treated with Atorvastatin (ATV) at 20mg/kg body weight/day for 21 days
- Group IV: were induced animals and treated with leaves extract (HLE) at 100mg/kg body weight/day for 21 days
- Group V: were induced animals and treated with stem bark extract (HSE) at 100mg/kg body weight/day for 21 days
- Group VI: were induced animals and treated with root bark extract (HRE) at 30mg/kg body weight/day for 21 days
- Group VII: were normal animals treated with leaves extract (LE) at 100mg/kg body weight/day for 21 days
- Group VIII: were normal animals treated with stem bark extract (SE) at 100mg/kg body weight/day for 21 days
- Group IX: were normal animals treated with root bark extract (RE) at 30mg/kg body weight/day for 21 days

The dose regimens were administered once daily for the period of the study. The rats were monitored for clinical signs and death.

2.8. Collection and Preparation of Sera Samples and Biochemical Analysis

At the end of the 21-day experimental period, the chloroform-inhalation anesthesia was performed on all experimental animals. The anesthetized animals were bled by cardiac puncture. The blood samples were collected into anticoagulant free tubes, centrifuged at a speed of 3000 r/m for 15 minutes and the resultant serum harvested into plain sample bottles for biochemical analysis. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP), total Bilirubin (TB), indirect bilirubin (IB) and direct bilirubin (DB) concentrations were determined using Randox kits.

2.9. Data Analysis

Data were expressed as mean ± standard deviation (SD) and were analyzed by the analysis of variance (ANOVA) with the aid of the Statistical Package for Social Science version 17.0. The difference between the various extracts and animal groups were compared using the Duncan Multiple Range Test. p value less than 0.05 was considered significant ($p<0.05$).

3. Results

3.1. Serum Liver Marker Parameters

The result of the studies presented in Table 1 showed that Poloxamer 407 (P407) significantly ($p<0.05$) increased the serum level of AST, ALT and ALP of hyperlipidemic rats when compared to normal rats. All the induced treated groups significantly ($p<0.05$) lowered the serum levels of AST, ALT and ALP when compared with the hyperlipidemic group (Table 1). AST, ALT and ALP levels of all normal treated rats were not significantly ($p<0.05$) changed when compared to normal control rats (Table 1).

Table 1. *Effect of ethanolic Vitex doniana on liver marker parameters in P (407) induced hyperlipidemic and normal rats in the liver.*

Group	ALT (U/I)	AST(U/I)	ALP(U/I)
NC	53.01 ± 2.09^{a}	13.33 ± 2.58^{a}	36.80 ± 14.25^{a}
HC	90.50 ± 1.01^{e}	41.25 ± 2.50^{d}	158.70 ± 13.80^{c}
H+SD	57.66 ± 1.50^{b}	24.16 ± 3.76^{c}	96.60 ± 15.11^{b}
H+LE	57.33 ± 1.63^{b}	19.16 ± 2.04^{b}	78.20 ± 11.26^{b}
H+SE	63.50 ± 1.02^{c}	26.25 ± 2.50^{c}	92.00 ± 15.93^{b}
H+RE	70.03 ± 0.90^{d}	26.66 ± 2.58^{c}	94.62 ± 14.75^{b}
N+LE	52.66 ± 1.03^{a}	12.89 ± 2.25^{a}	32.20 ± 11.26^{a}
N+SE	54.40 ± 2.01^{a}	13.00 ± 2.44^{a}	36.80 ± 14.25^{a}
N+RE	54.33 ± 1.50^{a}	13.16 ± 2.13^{a}	36.82 ± 14.27^{a}

Values are mean ± SD n=6 with different superscript down the column are significantly different ($p<0.05$).

NC: Normal Control rat. HC: Lipid control rats. HSD: Hyperlipidemic rats + 20mg/kg Atorvastatin. HLE: Hyperlipidemic rats + 100mg/kg ethanolic leaf extract. HSE: Hyperlipidemic rats + 100mg/kg ethanolic stem bark extract. HRE: Hyperlipidemic rats + 30mg/kg ethanolic root bark extract. NLE: Normal rats +100mg/kg ethanolic leave extract. NSE: Normal rats + 100mg/kg ethanolic stem extract. NRE: Normal rats + 30mg/kg ethanolic root bark extract.

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase.

3.2. Liver Function Parameters

Table 2 show serum concentrations of total protein (TP), total bilirubin (TB), indirect bilirubin (IB) and direct bilirubin (DB) of hyperlipidemic and normal rats. The studies shows that there is a significant (p>0.05) reduction in TP and DB of hyperlipidemic rats when compared with normal rats (Table 2). All the induced treated groups shows a significant (p>0.05) increase in TP and DB when compared with hyperlipidemic rats (Table 2). TP and DB levels of all normal treated rats were not significantly (p<0.05) changed when compared to normal control rats (Table 2). There was a significantly (p<0.05) increased in TB and IB of hyperlipidemic rats when compared to normal rats (Table 2). All the induced treated groups significantly (p<0.05) lowered the TB and IB when compared with the hyperlipidemic group (Table 2). TB and IB levels of all normal treated rats were not significantly (p<0.05) changed when compared to normal control rats (Table 2).

Table 2. *Effect of ethanolic extract of vitex doniana on liver function parameters.*

Group (n=6)	Total protein (g/dl)	Total bilirubin (mg/dl)	Indirect bilirubin (mg/dl)	Direct bilirubin (mg/dl)
NC	5.95 ± 0.10^{f}	2.44 ± 0.05^{a}	2.41 ± 0.05^{a}	0.03 ± 0.01^{d}
HC	2.95 ± 0.13^{a}	5.75 ± 0.16^{e}	5.73 ± 0.16^{d}	0.01 ± 0.03^{a}
H+SD	5.05 ± 0.11^{c}	4.01 ± 0.08^{d}	3.98 ± 0.08^{c}	0.02 ± 0.01^{b}
H+LE	5.56 ± 0.08^{e}	3.42 ± 0.11^{b}	3.39 ± 0.11^{b}	0.02 ± 0.01^{b}
H+SE	5.21 ± 0.24^{d}	4.02 ± 0.10^{d}	3.99 ± 0.10^{c}	0.02 ± 0.02^{b}
H+RE	4.82 ± 0.14^{b}	3.87 ± 0.08^{c}	3.63 ± 0.57^{b}	0.02 ± 0.01^{b}
N+LE	5.84 ± 0.12^{f}	2.37 ± 0.06^{a}	2.36 ± 0.08^{a}	0.03 ± 0.02^{d}
N+SE	5.88 ± 0.11^{f}	3.39 ± 0.08^{a}	2.34 ± 0.68^{a}	0.03 ± 0.01^{d}
N	5.34 ± 0.82^{f}	2.39 ± 0.08^{a}	2.36 ± 0.90^{a}	0.03 ± 0.01^{d}

Values are mean ± SD n=6 with different superscript down the column are significantly different (p<0.05)
NC: Normal Control rat. HC: Lipid control rats. HSD: Hyperlipidemic rats + 20mg/kg Atorvastatin. HLE: Hyperlipidemic rats + 100mg/kg ethanolic leaf extract. HSE: Hyperlipidemic rats + 100mg/kg ethanolic stem bark extract. HRE: Hyperlipidemic rats + 30mg/kg ethanolic root bark extract. NLE: Normal rats +100mg/kg ethanolic leave extract. NSE: Normal rats + 100mg/kg ethanolic stem extract. NRE: Normal rats + 30mg/kg ethanolic root bark extract.

4. Discussion

P-407 is a hydrophilic non-ionic surface-active agent with a low degree of toxicity and an LD_{50} greater than 15g/kg and 1.8 g/kg following intraperitoneal (i.p.) administration in rabbits and mice, respectively [16, 17, 18]. It has been demonstrated that P-407 causes inhibition of plasma lipoprotein lipase (LPL), both *in vitro* and *in vivo* accompanied by an increase in tissue LPL activity [15]. The accumulation of plasma triglycerides following the administration of P-407 has, therefore, been suggested to be due to inhibition of capillary-bound LPL activity. Other studies have indicated that, in rats, the increase in plasma cholesterol following P-407 i.p. administration may be due to inhibition of cholesterol *a*-hydroxylase, but not stimulation of 3-hydroxy-3-methylglutaryl coenzyme A (HMG Co A) reductase [19].

There has been conflicting reports on the effect of poloxamer 407 induced hyperlipidemia on biochemical parameters related to hepatic functions (ALP, AST and ALT). Report on the effects of poloxamer 407 induced hyperlipidemiaon serum levels of the above enzymes showed that hyperlipidemia elevated serum levels of ALT and AST [20]. Ameh et al., [21] found no effect on ALT except on AST, while Johnston et al., [22] reported that P407 does not cause hepatic injury or damage. The discrepancy in the serum levels of the enzymes could be attributed to the levels and duration of hyperlipidemia [23].

Hyperlipidemia is one of the disease conditions that are injurious to the liver; it sometimes results into fatty infiltration of the liver leading to a condition known as non-alcoholic fatty live [11]. Fatty liver is an accumulation of triglycerides and other fats in the liver cells, if not treated leads to inflammation of the liver. It is characterized by varying degree of liver injury from steatosis to tratohepatitis, fibrosis and necrosis [24]. In these studies, the elevated levels of AST, ALT and ALP observed in the serum of induced not treated group may be due to injuries inflicted to the liver due to the accumulation of triglycerides and other fats in the liver cells and these is conform with the work of Hyeung et al , [20]. There was a significant (P<0.05) restoration of these liver marker enzyme levels in all the induced treated groups on administration of the extracts and atorvastatin (Table 1). The reversal of this liver marker enzymes towards normalcy by the extracts observed in this study may be due to the prevention of the leakage of intracellular enzymes by the presence of polyphenol in the extracts and their membrane stabilizing activity [25, 26]. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [26]. It is therefore, a clear manifestation of hepatocurative effects of the extracts especially the leaves and stem bark extracts.

Serum levels of total protein (TP), total bilirubin (TB) and indirect bilirubin (IB) and direct bilirubin (DB) are indices used to assess liver function as well as disease progression [27, 28]. The levels of these liver function parameters were affected as a result of fatty liver injuries inflicted by hyperlipidemia.

Total protein and bilirubin levels are also affected as a result of these fatty liver injuries inflicted by hyperlipidemia. Total protein and bilirubin are indices for liver function; bilirubin is excreted by the liver and as such interference with the normal liver function which in turn affects its rate of conjugation or excretion. Thus a high level of total protein and bilirubin is used as indices for liver function and bile excretion status [29]. The present studies showed a significant (p<0.05) decrease in the levels

of total protein and direct bilirubin and increase in the levels of total bilirubin and indirect bilirubin in hyperlipidemic control when compared to normal control. These changes could be as a result of fatty liver injuries inflicted by hyperlipidemia. Although, the levels are however restored towards normalcy by treatment with the plant extracts, with the leaves extract having the highest effect. Thus, suggesting the enhancement of liver functions by the extracts. All the animals treated with the extracts without induction showed no significant ($P>0.05$) difference in the total protein, total bilirubin, direct bilirubin and indirect bilirubin when compared with animals in the normal control group.

5. Conclusion

The findings of this study have shown potential efficacy of *Vitex doniana* (leaves, stem bark and root bark) extract in ameliorating some negative effects of hyperlipidemia on the liver validating their use in ethno-medicine. Further studies are also required to elucidate the principles responsible for these ameliorative effects and the ability of these extract to protect against the damaging effect of hyperlipidemia on the liver (in non-poloxamer models).

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