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*Full Length Research Paper*

# Anti-hyperlipidemic and biochemical effect of extract of *Tulbaghia violacea* rhizomes on high cholesterol diet fed rats

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**Anti-hyperlipidemic and biochemical effect of methanolic extract of *Tulbaghia violacea* rhizomes were investigated in cholesterol rich diet-induced hypercholesterolemia Wistar albino rats. Hypercholesterolemia was induced in rats by feeding with 2% cholesterol rich diet (high cholesterol diet, HCD) for four weeks. Some biochemical parameters and histology of the liver were assessed following 28 days oral treatment. Co-administration of extract of *T. violacea* rhizomes at 250 and 500 mg/kg respectively, significantly ($p<0.05$) protected against hypercholesterolemia induced alanine amino transferase (ALT) alterations in activities of serum aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), lactate dehydrogenase (LDH), gamma glutamyl transferase (γGT), total cholesterol, triglycerides, albumin and total protein in a dose dependent manner. The extract also caused significant ($p<0.05$) increase in high density lipoprotein (HDL)-cholesterol level. Anti-cholesterolemic and hepatoprotective effect of the extract at both doses were comparable and similar to that of the standard treatment of atorvastatin (30 mg/kg body weight). Histological examination of the liver showed that the extract markedly protected against hypercholesterolemia induced micro-vesicular steatosis. This study suggests that *T. violacea* rhizome extract may protect against hypercholesterolemic induced diseases and this may account for its folklore usage.**

**Key words:** Anti-hyperlipidemic, *Tulbaghia violacea* rhizome, biochemical, lipid profile.

## INTRODUCTION

Cardiovascular disease (CVD) is one of the major causes of mortality and morbidity in Europe, United States, most parts of Asia (Ashraf et al., 2005; Sans et al., 1997) and recently in developing countries (Karen and Ana, 2009). It encompasses conditions that range from hypertension, acute coronary syndrome, stroke and chronic heart failure (Karen and Ana, 2009). The high burdens of CDV in the developing countries are attributable to the increasing incidence of atherosclerotic diseases, urbanization and higher risk factor levels such as obesity, diabetes, dyslipidamia and hypertension (Salim et al., 2001). A large body of studies in experimental animals has clearly demonstrated that the common risk factor for cardiovascular disease is hypercholesterolemia (Law, 1999; Shaten et al., 1991; Castelli et al., 1992). Therefore, treatment of hypercholesterolemia may reduce the risk or development of cardiovascular disease (Doha et al., 2010). Presently, the existing drugs use in the

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**Abbreviations: TVR,** *Tulbaghia violacea* rhizome; **HCD,** High cholesterol diet, **ALT,** alanine amino transferase; **AST,** aspartate amino transferase; **ALT,** alanine amino transferase; **ALP,** alkaline phosphatase; **TB,** total bilirubin; **LDH,** lactate dehydrogenase; **γGT,** gamma glutamyl transferase.

management of cardiovascular disease is associated with side effects such as abnormal liver disease, diarrheal, gastric irritation and nausea (Doha et al., 2010). Several herbs have been reported to reduce high blood cholesterol without negative side effect and they are relatively affordable (William and John, 2010). These herbs include members of the *Allium* sp. (garlic, onions and chives), members of the *Labiatae* (mint) family.

One of the frequently used plants in the Nkonkobe Municipality, Eastern Cape, South Africa, in the management of heart diseases, is *Tulbaghia violacae* (Joanna and Thompson, 2003). *Tulbaghia violacea* belongs to the family of *Alliacea* and is believed to possess biological activities similar to garlic (Allium sativum) (Olorunnisola et al., 2011). Various scientific publication (Bungu et al., 2006; Joanna and Thompson, 2003) have reported that the leaves, root, stem and rhizomes of *T. violacea* possess pharmacological properties. Bungu et al. (2006) reported that the plant is used in the treatment of fever and colds, asthma, tuberculosis, stomach problems and oesophageal cancer. Recently, Olorunnisola et al. (2011) reported that methanolic extracts of rhizomes of *T. violacea* (RTV) exhibited potent antioxidant activities in a concentration dependent manner. However, despite the reported use of the plant in the management of cardiovascular disease and as an anti-hypercholesterolemia agent, to the best of our knowledge there is no scientific information on its anti-lipidemic activities in the literature. The present study was designed to investigate antilipidemia and biochemical effect of methanolic extracts of *T. violacea* rhizome in high fat diet fed rats.

## MATERIALS AND METHODS

### Plant material and preparation

Plant collection and extract preparation was as earlier described by Mohammad and Woodward (1986) and modified by Olorunnisola et al. (2011).

### Animals

Healthy eight-week old female Wister albino rats (130 to 160 g) were randomly assigned to control and treated groups (six animals per group/cage). They were maintained in standard environmental conditions (22 ± 2°C, 12:12 h dark/light cycle, humidity: 45 to 50%) frequent air change and had free access to tap water and food. All animals were obtained from the animal house of the laboratory of School of Biological Sciences, University of Fort Hare, Alice 5700, South Africa. All procedures used in the present study followed the "Principles of Laboratory Animal Care" from NIH Publication No.85-23 and were approved by the Animal Ethics Committee of our University.

### Cholesterol supplemented diet

Hypercholesterolemia was induced using earlier modified method of Onody et al., (2003). Briefly, cholesterol (2% w/w) powder was thoroughly mixed with crushed pellet diet and reconstituted with water and allowed to dry properly to prevent microbial contamination.

### Experimental designs

Experimental animals were divided into the following groups after two weeks of acclimatization. Each group comprised of 6 animals.

Group 1: Control rats fed with normal pellet diet for 4 weeks by orally gavage.
Group 2: Rats fed with cholesterol mixed pellet diet for 4 weeks by orally gavage.
Group 3: Rats fed with cholesterol (2% w/w) mixed pellet diet plus *T. violacea* rhizome (TVR, 250 mg/kg b.wt./day) for 4 weeks by orally gavage.
Group 4: Rats fed with cholesterol mixed pellet diet plus TVR (500 mg/kg b.wt/day) for 4 weeks by orally gavage.
Group 5: Rats fed with cholesterol (2% w/w) mixed pellet diet together with atorvastatinan antihypercholestrolemia standard drug (30 mg/kg b.wt/day) for 4 weeks by orally gavage.

### Biochemical determinations

#### *Assessment of lipid profile and biochemical parameters*

Blood samples were collected from overnight fasted rats using the method described by Yakubu et al. (2005). Briefly, under ether anaesthesia, the neck was quickly cleared of fur and skin to expose the jugular veins. These animals were thereafter made to bleed through their cut jugular vein and their blood was collected with lithium heparinized tubes. Total cholesterol, triglycerides, low density lipoprotein (LDL), very low density lipoprotein (VLDL), high-density lipoprotein-cholesterol (HDL-C) levels, aspartate amino transferase, alanine amino transferase, alkaline phosphatase, total bilirubin, lactate dehydrogenase, gamma glutamyl transferase (γGT) and glucose were determined in the blood using piccolo automated chemistry analyser (Abaxis, Inc., Union City, CA, USA).

### Statistical analysis

Values were given as means ± standard deviation (mean ± SD). Data was statistically analysed by using one-way analysis of variance (ANOVA)

## RESULTS AND DISCUSSION

### Effect of TVR on serum lipid profile

Derangements in cholesterol metabolism have been associated with the etiology of most human diseases. It is widely reported that hypercholesterolemia occasioned by a defect in cholesterol transportation, biosynthesis or catabolism is a risk factor in coronary heart disease (CAD) and atherosclerosis (Yogendrasinh et al., 2010; Mohammed et al., 2011). Hence, prevention of hypercholesterolemia will make a positive contribution to the management and treatment of cardiovascular diseases. The results of the present investigation show that rats fed with cholesterol rich diet developed

**Table 1.** Effect of extracts of *T. violacea* rhizomes on serum lipid profile and glucose in diet induced hypercholesterolemic rats.

| Plasma parameter (mg/dl) | Normal | HCD only | HCD + 30 mg/kg Atorvastatin | HCD + dosage of *T. violacea* in mg/kg | |
|---|---|---|---|---|---|
| | | | | 250 | 500 |
| TC | 76.21 ± 0.14$^{a}$ | 164.55 ± 0.15$^{b}$ | 108.21 ± 0.11$^{c}$ | 116 ± 0.19$^{c}$ | 98.98 ± 0.17$^{d}$ |
| HDL-C | 31.23 ± 0.16$^{a}$ | 15.17 ± 0.12$^{b}$ | 60.01 ± 0.19$^{c}$ | 53.20 ± 0.15$^{c}$ | 56.02 ± 0.15$^{c}$ |
| LDL-C | 15.02 ± 0.18$^{a}$ | 35.21 ± 0.14$^{b}$ | 21.05 ± 0.18$^{c}$ | 28.12 ± 0.13$^{c}$ | 23.18 ± 0.17$^{c}$ |
| VLDL-C | 14.32 ± 0.16$^{a}$ | 29.01 ± 0.11$^{b}$ | 12.14 ± 1.18$^{c}$ | 11.45 ± 0.14$^{c}$ | 12.01 ± 0.15$^{c}$ |
| TG | 58.13 ± 0.17$^{a}$ | 261.2 ± 0.14$^{b}$ | 103.20 ± 0.19$^{c}$ | 110.01 ± 0.12$^{c}$ | 54.18 ± 0.16$^{d}$ |
| GLU | 73.53 ± 0.19$^{a}$ | 89.10 ± 0.11$^{b}$ | 100.01 ± 0.15 | 85.02 ± 0.15 | 90.03 ± 0.19 |

TC, Total cholesterol; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein cholesterol; VLDL-C, very low density lipoprotein-cholesterol; TG, triglycerides; GLU, glucose. Values are mean ± SD triplicate determinations (n = 3). Values in the same row with different alphabet (a, b, c or d) are significantly different ($p < 0.05$).

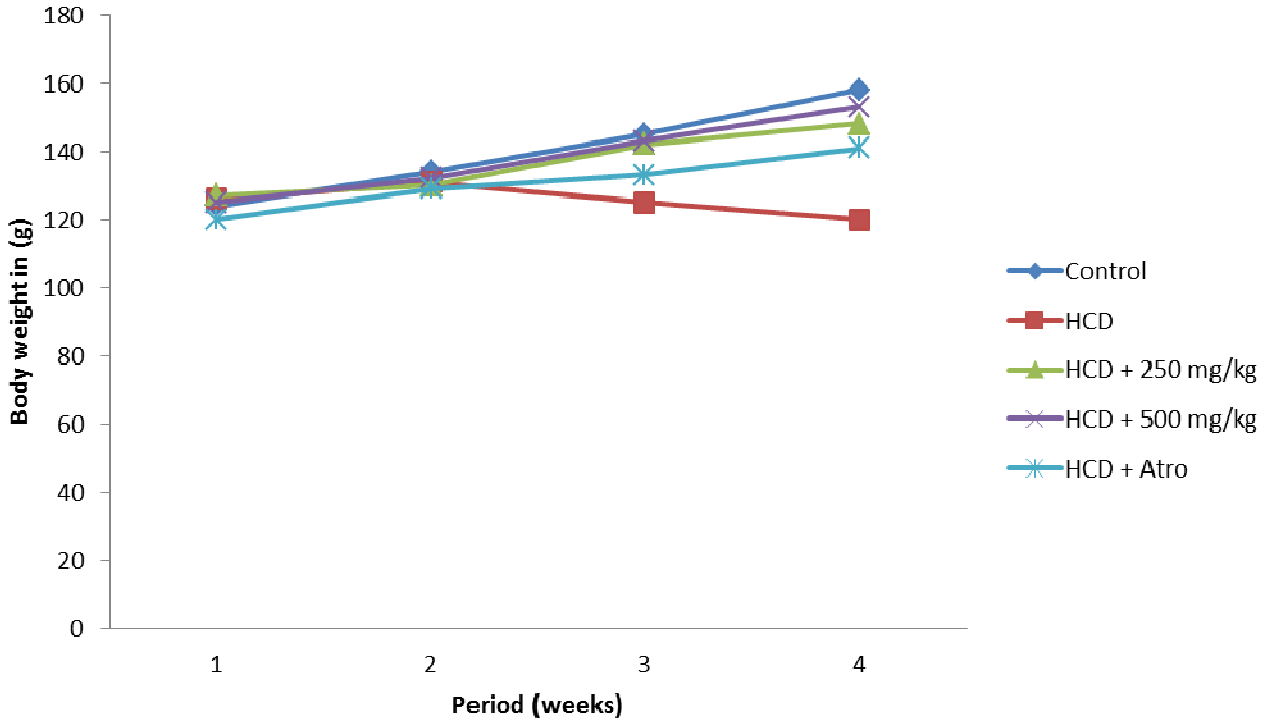


**Figure 1.** The growth response of Wistar rats fed with control, hypercholesterolemic (HCD) diet and extracts.

hypercholesterolemia with a significant ($P < 0.05$) increase in total cholesterol (TC), LDL, VLDL, triglyceride levels (TG), and a significant ($P < 0.05$) decrease in HDL-C levels as compared to the control rats (Table 1). Increased fat deposition was also observed in the liver of hyperlipidemic control rats (Figure 1). These results are in agreement with earlier reports on dietary hyperlipidemia (Qadir, 2005; Majeed, 2006; Abdulazeez, 2011). However, co-administration with TVR (250 and 500 mg/kg b.wt) and standard drug atorvastatin to high cholesterol fed rats significantly ($P<0.05$) decrease the level TC, TG, LDL, VLDL and cause a significant increase in the level of HDL-C when compared with a hypercholesterolemic group (Table 1). It also prevented the micro-vesicular steatosis (Figure 1). The activity of the extract was dose dependent. At 500 mg/kg.bwt, the *T. violacea* demonstrated higher percentage reduction in serum triglyceride (79.3/43.6%) and cholesterol (39.8/34.2%) when compared with the standard drug. Atorvastatin is a standard drug usually employed in the treatment of elevated total cholesterol, LDL and triglycerides. It also increased HDL cholesterol level in

the liver possibly be blocking 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (Ginter and Simko, 2010). Although, the mechanism(s) of anti-hyperlipidemia activities of the plant is still unknown, it may be suggested to be similar to atorvastatin or it may have a stimulating effect on lipoprotein lipase activity. This theory may explain the significant percentage reduction (79.3%) in triglyceride, LDL and VLDL concentration.

The strong anti-hypertriglyceridemia property of the extract may be employed in the management of hypertriglyceridemia induced atherogenesis, ischemic heart disease, obesity and cholesterol deposition in body tissue.

Also, the observed low level of HDL in the hypercholesterolemic rats is consistent with earlier studies (Mohammed et al., 2011). In the current investigation, TVR treatment decreased the levels of total cholesterol and triglycerides, and increased the levels of HDL-C, thereby suggesting a cardioprotective and lipid lowering potential. The lipid lowering potential of the plant may be due to polysulfide (Sanjay et al., 2002), flavonoids and/or saponins (Hostettman, 1995; Ramachandran et al., 2003) which we found to be part of the main constituents of TVR (Olorunnisola et al., 2011). This lipid lowering ability of the plant is consistent with other members of the family alliance such as garlic. Several studies have shown that garlic possesses hypercholesterolemic properties (Yu-Yan and Lijuan, 2001).

**Effect of extract of TVR on body weight gain**

Figure 1 shows that there were significant differences ($p < 0.05$) in the weight gain pattern of the animals fed with high cholesterol diet (HCD) as compared to normal control. There is a controversy about the effect of high cholesterol diet on weight of rats. Ramachandran et al. (2003) and Harnafi et al. (2009) reported that there were no significant weight gain or there was a linear weight increase between the control and hypercholesterolemic animals while Matos et al. (2005) and Otunola et al. (2010) revealed a significant reduction in weight gain in animals on high cholesterol diet. In this present study, a consistent weight gain was observed in animals on standard diet throughout the period, while those on the HCD only gained weight up till the 2$^{nd}$ week, before a steady decline in weight gain till the end of the experiment. The resultant weight loss could be due to reduction in nutrient intake caused by high cholesterol content of the diet which might have impaired the absorption of protein and other nutrients (Matos et al., 2005; Woo and Henry, 1996).

Co-treatment with TVR (250 and 500 mg/kg b.wt) and standard drug to rats on high cholesterol diet significantly ($P < 0.05$) restored the weight gain pattern to near normal (Figure 2). The extract activity is dose dependant and it compared favourably with the standard drug.

This result agrees with other investigators who noticed an increase in body weight gain upon the improvement of hyperlipidemia status (Lamiaa, 2011). The mechanism of action is unknown but it may be due to improvement in nutritional status of the animals (Prasad, 2010).

**Effect of the extracts on biochemical parameters**

There has been conflicting reports on the effect of high cholesterol diet on serum biochemical parameters related to hepatic functions (ALP, AST, ALT and γ-GT). Some reported on the effects of hypercholesterolemia on serum levels of the above enzymes (Prasad, 2010). Lu et al. (2007) and Nader et al. (2011) showed that a high cholesterol diet moderately elevated serum levels of ALT, AST and ALP in rats, while Molgaard et al. (1989) reported that there was no change in the serum AST, ALT and ALP or AST and ALP (Mabuchi et al., 2007; Arafa, 2005), and Assy et al. (2000) reported no change in the ALP in hypercholesterolemia rats. The discrepancy in the serum levels of the enzymes could be attributed to the levels and duration of hypercholesterolemia (Lu et al., 2007).

The results of the present study indicate that animals on high cholesterol diet had about 3.2 total bilirubin (TB), 1.33 (ALT), 1.17 (AST), 2.61 (ALP), 1.15 (LDH) and 1.80 (γ-GT) folds increase, respectively, in their enzymatic activities when compared with normal control rats. The observed increase in the enzyme activities is in agreement with Nader et al. (2011) and Lu et al. (2007) report. Co-administration of methanolic extract of *T. violacea* rhizomes to rats lead to reduction ($p<0.05$) in elevated serum level of ALT, AST, LDH and γ-GT. The effect of the extract was dose dependent and it compared favorably with the activity of the standard drug (atorvastatin) at the two doses. It is worth mentioning that these enzymes play critical roles in ischemic liver injury, myocardial or pulmonary infarction, hepatobilliary dysfunction and alcohol abuse, cardiovascular and atherosclerotic diseases.

ALT, AST and LDH are notable markers of hepatocyte injury; however, LDH is less specific (Nader et al., 2011). LDH activity has also been reported to increase in ischemic liver injury (Al Hamedan, 2010), myocardial or pulmonary infarction, kidney, heart, liver, lungs and skeletal muscle damage (Nader et al., 2011). Hence, the reduction of ALT, AST and LDH activities in the extracts treated group suggested that the extracts may protect against ischemic liver or hepatocyte injury. Also, the reduction in serum γ-GT activity, an enzyme which is an independent risk factor in cardiovascular mortality (Sakuta et al., 2007) or prognostic index in chronic forms of coronary heart disease, congestive heart failure and ischemic or hemorrhagic stroke (Nader et al., 2011) suggested that the extract may protect against

**Table 2.** Effect of extracts of *T. violacea* rhizomes on serum concentration in hypercholesterolemic rats.

| Parameter | Control | HCD | HCD + 30 mg/kg$^{-1}$ Atro | Extract of TVR | |
|---|---|---|---|---|---|
| | | | | HCD + 250 mg/kg | HCD + 500 mg/kg |
| TP | 7.19 ± 0.20$^{a}$ | 6.10 ± 0.17$^{b}$ | 6.83 ± 0.22$^{b}$ | 6. 67 ± 0.15$^{b}$ | 6.79 ± 0.11$^{b}$ |
| AL | 3.42 ± 0.51$^{a}$ | 2.10 ± 0.11$^{b}$ | 3.35 ± 0.15 $^{a}$ | 3.25 ± 0.13$^{a}$ | 3.30 ± 0.14$^{a}$ |
| Tb (µmolL$^{-}$) | 10.21 ± 1.21$^{a}$ | 20.78. 1.00$^{b}$ | 8.89 ± 1.10$^{c}$ | 9.34 ± 1.11$^{c}$ | 8.01 ± 1.01$^{c}$ |
| Alanine amino transferase (ALT) ( U/L) | 38.5 ± 1.22$^{a}$ | 51.3 ± 1.05$^{b}$ | 42.31 ± 0.23$^{c}$ | 45.11 ± 1.21$^{c}$ | 40.12 ± 1.23$^{c}$ |
| AST (U/L) | 65.2 ± 2.11$^{a}$ | 76.4 ± 1.12$^{b}$ | 68.21 ± 0.40 $^{a}$ | 70.10 ± 0.14 $^{b}$ | 68.21 ± 0.31$^{a}$ |
| ALP (U/L) | 11.22 ±1. 29$^{a}$ | 29.2 ± 1.21$^{b}$ | 14.2 ± 1.42$^{a}$ | 17.12 ± 1.21$^{c}$ | 16.4 ± 0.17$^{c}$ |
| LDH | 128.3 ± 0.10$^{a}$ | 147.5 ± 0.11$^{b}$ | 130.4 ± 0.20$^{c}$ | 133.3 ± 0.15$^{c}$ | 131.1 ± 0.32$^{c}$ |
| ɣGT (µ/L) | 31.30 ± 1.46$^{a}$ | 56.34 ± 1.23$^{b}$ | 32.20 ± 1.62$^{c}$ | 39.10 ± 0.25$^{c}$ | 34.23 ± 0.13$^{c}$ |

AST, Aspartate amino transferase; ALT, alanine amino transferase; ALP, alkaline phosphatise; Tb, total bilirubin; LDH, lactate dehydrogenase; ɣGT, gamma glutamyl transferase; TP, total protein; TVR, *Tulbaghia violacea* rhizome. Values are Mean + standard deviation (SD) triplicate determinations (n = 6). Values in the same row with different alphabet are significantly different ($p < 0.05$).

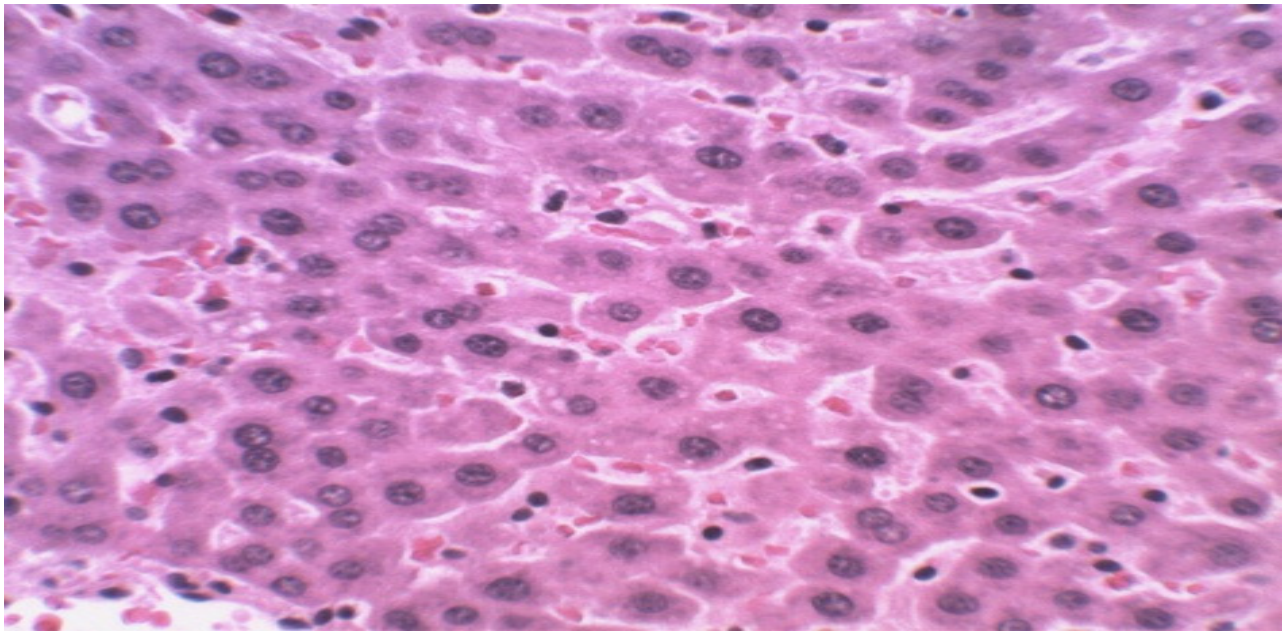

**Figure 2a.** Photomicrograph of liver of rat fed with normal diet (control) (400x).

cardiovascular diseases. The exact mechanism of action(s) of the extract is unknown but it may be hypo-cholesterolemic effect or the presence of phytochemicals such as flavonoids (Myron, 2004) in the plant (Olorunnisola et al., 2011).

Table 2 also shows the effect of hypercholesterolemia on total protein, albumin and total bilirubin. The results reveal an insignificant reduction in the serum concentration of total protein, significant ($p<0.05$) reduction in serum albumin and a significant increase in serum total bilirubin in HCD fed rats as compared to animals on standard diet. Co-treatment of rats with TVR significantly prevented a reduction in total protein, albumin and significantly reduced elevated bilirubin at both doses used. The activity of the extract was similar to the standard drug (atorvastatin). The reduction in serum albumin concentration and elevated bilirubin may be due to hypercholesterolemia-induced liver damage (Al Hamedan,

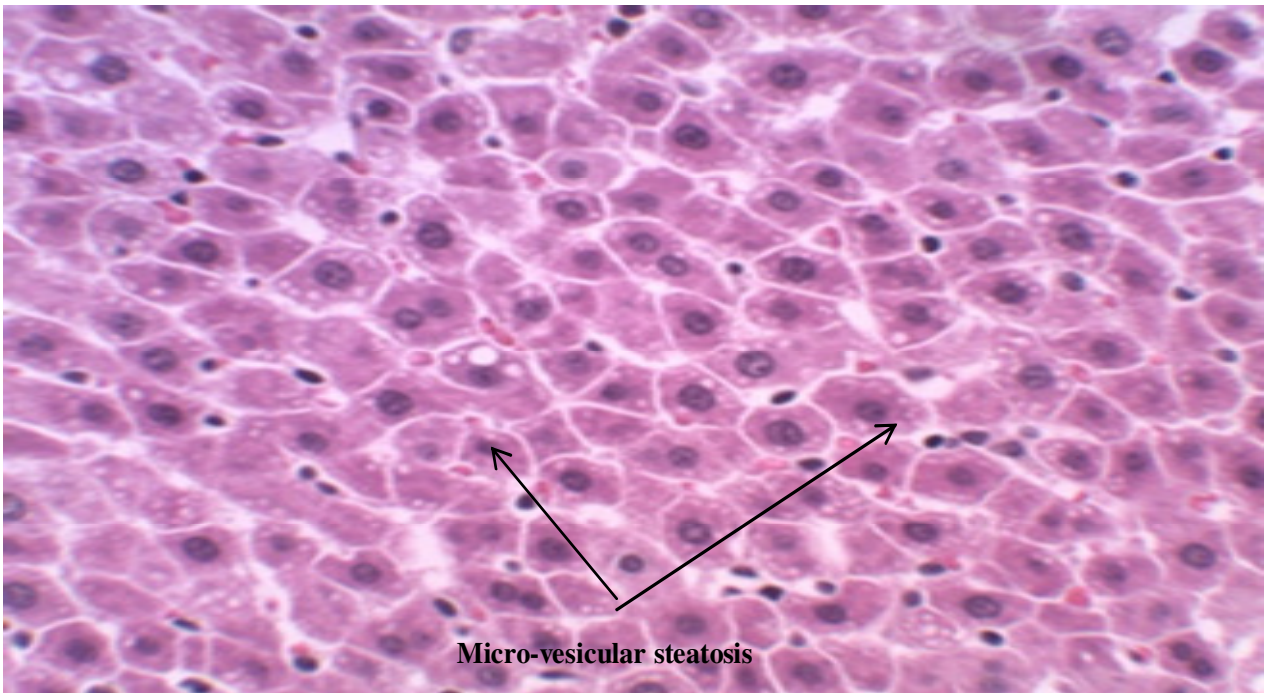


**Figure 2b.** Photomicrograph of liver of rat fed with high cholesterol diet showing micro-vesicular steatosis (400x).

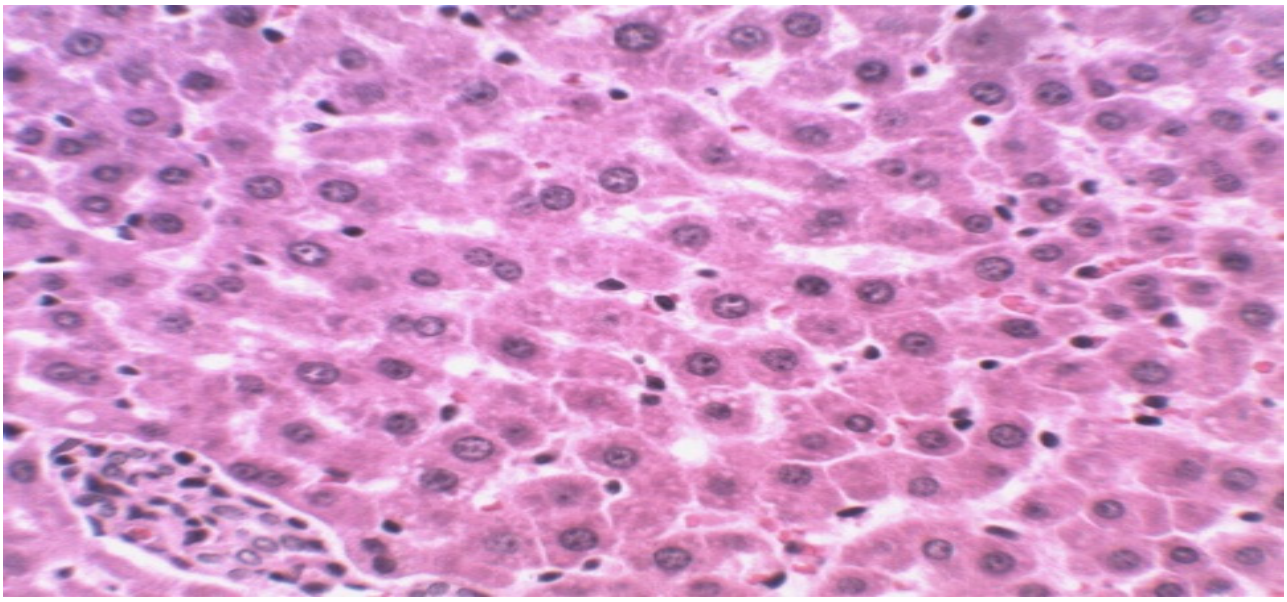

**Figure 2c.** Photomicrograph of liver of rat fed with high cholesterol diet and TVR showing no lesion (500 mg/kg) (400x).

2010) as evident in the increase in activities of marker enzymes of liver damage (Table 2). Co administration of the extracts or drug might have prevented liver damage resulting in the improvement of the status of albumin and bilirubin. Histological examination of the liver showed that the extract markedly protected against hypercholesterolemia induced micro-vesicular steatosis (Figure 2).

In conclusion, extract of *T. violacea* has demonstrated strong anti-lipidemic and hepatoprotective tendency which suggested that the plant should be investigated further to determine its possible mechanism(s) of action(s).

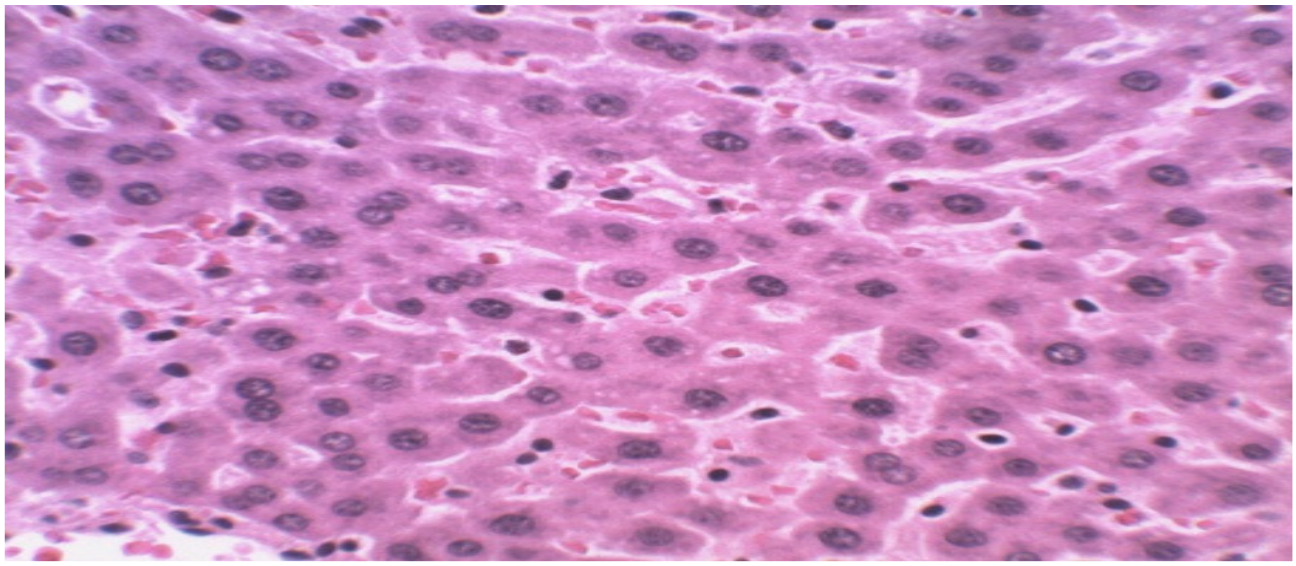

**Figure 2d.** Photomicrograph of liver of rat fed with high cholesterol diet and atorvastatin (30 mg/kg) showing no lesion (400x).

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