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Long Term Effect of *Urena lobata* Administration on the Serum Lipid Panel and Atherogenic Indices of Normal Rabbits

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Abstract

Urena lobata L. (Malvaceae), commonly called Caesar weed, is used traditionally to treat several ailments including diarrhea, colic, skin diseases, boils, pneumonia, rheumatism, cough, and diabetes. The short and long term antidiabetic effects of the aqueous root extract of *U. lobata* in experimental animal models have been demonstrated. This study was designed to evaluate the effect of daily oral administration of *U. lobata* aqueous root extract at 200 mg/kg body weight (for 24 weeks) on the serum lipid panel and atherogenic indices of normal rabbits. Serum cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol, as well as atherogenic and coronary risk indices, were monitored at pre-determined intervals for 24 weeks. The administration of aqueous root extracts of *U. lobata* to normal rabbits for 24 weeks caused reductions in serum total cholesterol, LDL-cholesterol and triglycerides; serum HDL-cholesterol concentration was not significantly altered. Atherogenic and coronary risk indices were also not altered. The long term hypolipidaemic effect of *U. lobata* observed in this study can serve as basis for further studies to elucidate the phytochemicals responsible for these effects and determine their mechanism of action.

Keywords: *Urena lobata*, Lipid Panel, Atherogenic Indices, Medicinal Plants, Rabbits

Introduction

Medicinal plant and natural product research has provided scientific basis for the integration of herbal remedies into modern therapeutic strategies. Natural products have long been used in the synthesis of drugs for the treatment of different diseases (Bharel *et al.*, 1996; Schwikkard and van Heerden, 2002). Phytochemicals in medicinal plants can work synergistically to modulate different aspects of disease conditions. The growing concern of increase in diseases such as type 2 diabetes mellitus, cardiovascular disease, and the metabolic syndrome in Africa and other developing countries has rekindled the search for natural remedies that can manage or even cure these conditions with little or no side effects. Medicinal plant and natural products research can be further strengthened not only by establishing scientific efficacies, but also by evaluating both the short and long term toxicological effects of consumption of these products in normal and disease states. The medicinal properties of *Urena lobata* L. (Malvaceae),

commonly called Caesar weed (Francis, 2003), have been demonstrated (Arun and Vareishang, 2007; Adeloje *et al.*, 2007). It is used locally to treat malaria, gonorrhoea, fever, wounds, toothache, and rheumatism (De Las Heras *et al.*, 1998; Adeloje *et al.*, 2007). Previous studies have demonstrated its long term anti-diabetic, anti-hyperlipidaemic (Omonkhua and Onoagbe, 2013) and anti-oxidant, (Omonkhua and Onoagbe, 2012) effects in experimental animals. Phytochemical analysis of this plant revealed several pharmacologically active biomolecules, such as steroidal/triterpenoidal saponins (Omonkhua and Onoagbe, 2010), flavonoids (Adeloje *et al.*, 2007) mangiferin, and quercetin (Srinivasan and Subramanian, 1981), that can be exploited for their therapeutic effects.

As part of the toxicological assessment of *U. lobata*, the long term (24 weeks) effect of aqueous root bark administration on normal rabbits' lipid profile and other atherogenic parameters were assessed periodically in serum.

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Materials and Methods

Reagents

Analytical grade reagents as well as assay kits for total cholesterol, total triglycerides and HDL-Cholesterol, products of Randox Laboratory Ltd, Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom, were used for this study.

Extract preparation

U. lobata roots were obtained from a farm in Akungba-Akoko, Ondo State; they were washed, shade dried and pulverised. Aqueous extract of the pulverized plant was prepared as described by Onoagbe *et al.* (1999).

Herbarium specimen of the plant, which was assigned a voucher number UIH 22287, was deposited at the Herbarium of the University of Ibadan, Nigeria.

Animals and experimental protocol

Twelve (12) post weaned male and female rabbits of the New Zealand strain with an average weight of 1000g were used for this study. They were obtained from the Animal Unit of Federal University of Technology, Akure, Ondo State, inoculated, and allowed to acclimatize for three weeks before the commencement of experiment. The animals were allowed free access to food (pelleted growers mash, product of Bendel Feed and Flour Mill, Ewu, Nigeria) and water. The study protocol was approved by the local Institutional Review Board (IRB) and treatment of the animals was in accordance with the guidelines for the Care and Use of Laboratory Animals (The National Academy of Sciences, 2011).

The animals were divided into two groups of six (6) rabbits each i.e. Normal Control and *U. lobata* treated normal rabbits. Extract was orally administered to the rabbits at 200 mg/kg body weight daily for 24 weeks. This dose was selected from a pilot study and reflects a balance between the safe and therapeutic dose.

Results

Table 1: Effect of *U. lobata* on Serum Total Cholesterol Concentration of Normal Rabbits

Weeks	Normal Control	<i>U. lobata</i> Treated Normal Rabbits
0	2.99 ± 0.71	2.11 ± 0.32
1	2.77 ± 0.46	2.31 ± 0.41
2	2.04 ± 0.14	1.27 ± 0.1*
3	2.45 ± 0.19	1.72 ± 0.22*
4	1.61 ± 0.1	1.87 ± 0.49
6	3.37 ± 0.55	1 ± 0.19*
8	2.17 ± 0.25	1.38 ± 0.15*
10	3.09 ± 0.51	1.13 ± 0.14*
12	1.59 ± 0.13	2.28 ± 0.32
15	3.29 ± 0.34	2.31 ± 0.48
18	1.9 ± 0.31	2.13 ± 0.22
21	2.65 ± 0.35	1.04 ± 0.17*
24	0.86 ± 0.4	1.7 ± 0.29

Blood Collection

About 1ml of blood was drawn from the ventral vein of the rabbits' ear at specific intervals (week 1, 2, 3, 4, 6, 8, 10, 12, 15, 18, 21 and 24). Baseline blood samples (week 0) were collected 24 hours before the commencement of extract administration. Blood samples were collect into labelled plain blood bottles, allowed to clot on ice and centrifuged at 1,000 X g for 5 minutes; the serum was then separated for analysis.

Biochemical Analyses

Serum total triglycerides, serum total cholesterol and serum HDL-cholesterol concentrations were analyzed by the methods of Tietz, (1990), Richmond, (1973), and Lopes-Virella *et al.* (1977) respectively. Serum LDL-cholesterol levels were calculated by the Friedewald *et al.* (1972) method while atherogenic index (AI) and coronary risk index (CRI) were calculated by the formulas described by Beyegue *et al.* (2012), and Alladi and Shanmugasundaram, (1989) respectively.

Atherogenic Index (AI) = LDL-cholesterol/HDL-cholesterol

Coronary Risk Index (CRI) = Total cholesterol/HDL-cholesterol

Statistical analysis

The differences between means of control and test groups were analyzed by the Independent Samples t-test. The IBM SPSS Software Version 21.0 was used for this analysis. A value of $P < 0.05$ was considered as statistically significant.

Effect of repeated daily oral administration of aqueous extracts of *U. lobata* root for 24 weeks at 200 mg/kg body weight on serum total cholesterol concentration (mmol/l) of normal rabbits. Values carrying notations are statistically different from control at $p < 0.05$

Significantly ($p < 0.05$) lower serum cholesterol values were obtained in weeks 2, 6, 8, 10 and 21 of the *U. lobata* treated rabbits compared to control, other values were mostly similar to control (Table 1).

Page |
3087

Table 2: Effect of *U. lobata* on Serum Total Triglycerides Concentration of Normal Rabbits

Weeks	Normal Control	<i>U. lobata</i> Treated Normal Rabbits
0	0.77 ± 0.12	0.59 ± 0.16
1	2.75 ± 0.52	1.45 ± 0.5
2	1.19 ± 1.16	1.50 ± 0.23
3	2.12 ± 0.1	1.85 ± 0.45
4	3.23 ± 0.82	2.1 ± 0.77
6	1.03 ± 0.14	1.5 ± 0.43*
8	1 ± 0.17	1.83 ± 0.42*
10	1.36 ± 0.25	1.86 ± 0.44
12	1.19 ± 0.18	1.92 ± 0.08*
15	1.87 ± 0.05	0.81 ± 0.11*
18	0.79 ± 0.15	0.68 ± 0.05
21	0.66 ± 0.07	0.7 ± 0.14
24	2.42 ± 1.3	0.83 ± 0.12*

Effect of repeated daily oral administration of aqueous extracts of *U. lobata* root for 24 weeks at 200 mg/kg body weight on serum total triglyceride concentration (mmol/l) of normal rabbits. Values carrying notations are statistically different from control at $p < 0.05$

Table 2 shows that the triglyceride level of the *U. lobata* treated rabbits were initially lower than control (weeks 1, 3, and 4), then significantly ($p < 0.05$) higher values were recorded in weeks 6, 8, and 12. A significant ($p < 0.05$) drop was recorded in weeks 15 and 24; the other values were similar to control.

Table 3: Effect of *U. lobata* on Serum LDL-Cholesterol Concentration of Normal Rabbits

Weeks	Normal Control	<i>U. lobata</i> Treated Normal Rabbits
0	2.20 ± 0.55	1.24 ± 0.44
1	1.37 ± 0.36	1.51 ± 0.38
2	1.24 ± 0.07	0.67 ± 0.17
3	0.93 ± 0.12	0.7 ± 0.14
4	0.99 ± 0.32	1.14 ± 0.37
6	0.82 ± 0.23	0.73 ± 0.13
8	0.77 ± 0.15	0.44 ± 0.14
10	1.68 ± 0.49	1.12 ± 0.21
12	0.78 ± 0.15	0.56 ± 0.14
15	1.22 ± 0.33	1.32 ± 0.5
18	0.84 ± 0.3	1.27 ± 0.24
21	0.73 ± 0.22	0.55 ± 0.1
24	2.19 ± 0.84	1.19 ± 0.31

Effect of repeated daily oral administration of aqueous extracts of *U. lobata* root for 24 weeks at 200 mg/kg body weight on serum LDL-cholesterol concentration (mmol/l) of normal rabbits.

All LDL-cholesterol levels recorded for *U. lobata* treated rabbits were statistically similar to control. However, most of the values were lower than control (weeks 2, 3, 6, 8, 10, 12, 21 and 24) (Table 3).

Table 4: Effect of *U. lobata* s on Serum HDL-Cholesterol Concentration of Normal Rabbits

Weeks	Normal Control	<i>U. lobata</i> Treated Normal Rabbits
0	0.59 ± 0.09	0.60 ± 0.12
1	0.15 ± 0.02	0.15 ± 0.52
2	0.26 ± 0.06	0.33 ± 0.28
3	0.59 ± 0.07	1.32 ± 0.14*
4	0.76 ± 0.10	0.78 ± 0.05
6	2.48 ± 0.32	1.04 ± 0.22*
8	0.95 ± 0.11	0.50 ± 0.09*
10	0.79 ± 0.11	1.40 ± 0.18*
12	1.36 ± 0.20	1.47 ± 0.15
15	1.21 ± 0.21	0.65 ± 0.01*
18	0.76 ± 0.19	0.67 ± 0.10
21	1.69 ± 0.20	0.97 ± 0.25*
24	1.94 ± 0.84	1.19 ± 0.61

Effect of repeated daily oral administration of aqueous extracts of *U. lobata* root for 24 weeks at 200 mg/kg body weight on serum HDL-cholesterol concentration (mmol/l) of normal rabbits. Values carrying notations are statistically different from control at p<0.05

The *U. lobata* treated rabbits recorded significant (p<0.05) increases in HDL-cholesterol concentration in weeks 3 and 10, significant (p<0.05) decreases in weeks 6, 8, 15 and 21, other values were similar to control (Table 4).

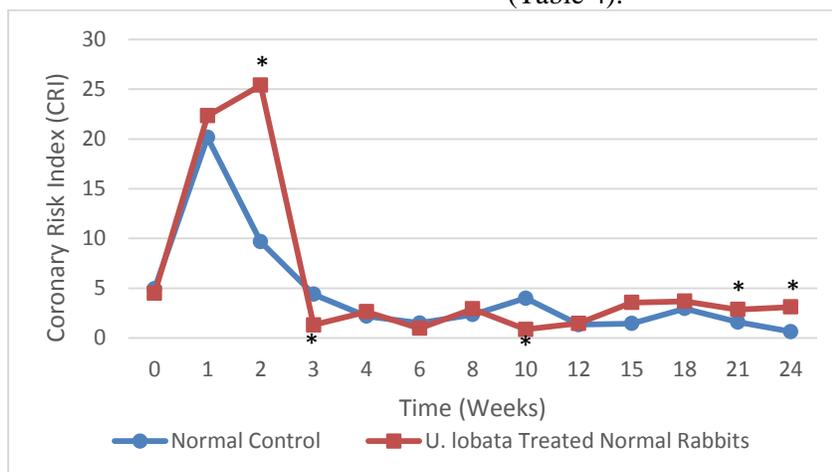


Figure 1: Effect of repeated daily oral administration of aqueous extracts of *U. lobata* root for 24 weeks at 200 mg/kg body weight on the atherogenic index (AI) of normal rabbits. Values carrying notations are statistically different from control at p<0.05

Although initially increased atherogenic index values were obtained in weeks 1 (significant) and 2 (not significant), other values were mostly statistically comparable with control (Figure 1).

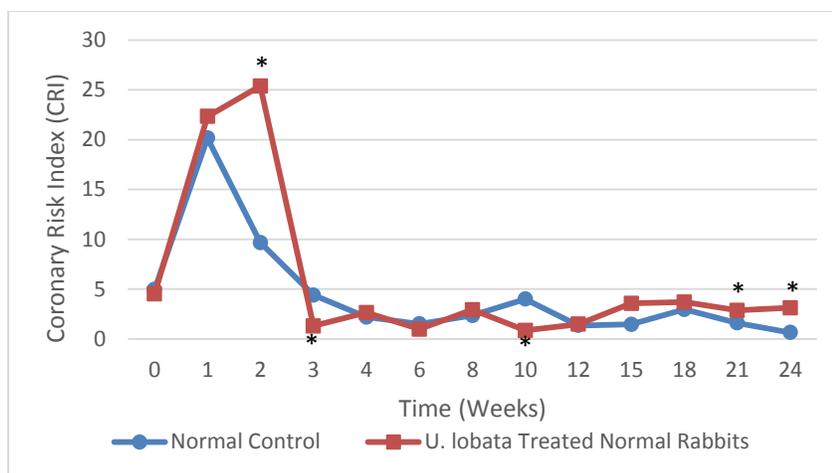


Figure 2: Effect of repeated daily oral administration of aqueous extracts of *U. lobata* root for 24 weeks at 200 mg/kg body weight on the coronary risk index (CRI) of normal rabbits. Values carrying notations are statistically different from control at $p < 0.05$.

An initial increase was observed in the coronary risk index of the *U. lobata* treated rabbits (week 2), this then significantly ($P < 0.05$) reduced at

week 3 and remained relatively similar to control until the end of the experiment (Figure 2).

Discussion

Dyslipidaemia i.e. increased serum concentration of total cholesterol, triglycerides, LDL-cholesterol and decreased serum HDL-cholesterol predisposes to the development of cardio vascular diseases (Farias *et al.*, 1996; Laakso, 1996; Yokozawa *et al.*, 2003). For most of the monitoring period, *U. lobata* administration exerted significant decreases on serum total cholesterol levels of the treated rabbits compared to control. Although higher total triglyceride levels were recorded in some weeks in the medicinal plant treated rabbits, generally, the values obtained were similar or lower than control. Apart from an initial increase observed in the *U. lobata* (week 4) treated rabbits; most of the values obtained for LDL-cholesterol were lower or similar to control. Generally the serum HDL-cholesterol of the *U. lobata* treated rabbits showed higher values for the first part of the monitoring phase of the *U. lobata* treated rabbits; most of the values recorded after week 15 were similar or lower than control. The initial increase observed in AI and CRI in weeks 1 and 2 for the *U. lobata* treated rabbits leveled out from week 3 to give values that were mostly comparable with control. The hypolipidaemic effect of *U. lobata* aqueous root extract was most evident in its ability to lower total cholesterol, LDL-cholesterol and total triglycerides. This property has the potential to protect against cardiovascular diseases as can be seen in

the relatively normal AI and CRI values recorded from week 3. Several medicinal plants have been reported to possess hypolipidaemic effects in non-disease experimental animals (Mansi *et al.*, 2009; Saravanan and Ignacimuthu, 2015). Indeed the hypolipidaemic effect of *U. lobata* roots is documented (Onoagbe *et al.*, 2010; Omonkhua and Onoagbe, 2013). The presence of high proportion of fibre (Omonkhua and Onoagbe, 2010) and various phytochemicals, such as and steroidal/triterpenoidal saponins (Omonkhua and Onoagbe, 2010), flavonoids (Adeloye *et al.*, 2007), mangiferin and quercetin (Srinivasan and Subramanian, 1981), which have established anti-hyperlipidaemic effects may be responsible for the observed effect. Soluble fibers (Arvill and Bodin, 1995), and flavonoids, including quercetin (Liao *et al.*, 2001; Song *et al.*, 2003; Rivera *et al.*, 2008) have been found to possess anti-hyperlipidaemic properties. Plant saponins have been shown to speed up the utilization of cholesterol in the liver; saponins form mixed micelles with bile acids and accelerate its excretion. Cholesterol is thus recruited for bile acids formation; this ultimately leads to the reduction of serum cholesterol concentration (Francis *et al.*, 2002). Other studies have also reported the ability of saponins to decrease the rate of intestinal absorption of dietary cholesterol (Harwood *et al.*, 1993). Saponins have also been reported to reduce serum LDL-cholesterol (Francis

et al., 2002). The presence of fibre, saponins and flavonoids (quercetin) in *U. lobata* could account for its hypolipidaemic effects. The results obtained from this study can serve as a basis for further studies to elucidate the phytochemicals responsible for the long term anti-hyperlipidaemic effects of *U. lobata* roots and determine the mechanism of action.

Conclusion

The administration of aqueous root extracts of *U. lobata* to normal rabbits for 24 weeks caused reductions in serum total cholesterol, LDL-cholesterol and triglycerides; serum HDL-cholesterol concentration was not significantly altered. Atherogenic and coronary risk indices were also not altered. The long term hypolipidaemic effect of *U. lobata* observed in this study can serve as basis for further studies to explain the chemicals and mechanism of this effect.

Conflict of Interest

The authors declare no conflict of interest

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