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Original Article

Long-term effects of three hypoglycaemic plants (*Irvingia gabonensis*, *Urena lobata* and *Carica papaya*) on the oxidative status of normal rabbits

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ABSTRACT: Medicinal plants have been recognized to have therapeutic effects and they may also have toxic side effects. Our previous studies have shown that *Irvingia gabonensis*, *Urena lobata* and *Carica papaya*, locally used in Nigeria to treat diabetes, possess long term hypoglycaemic and anti-obesity effects on normal rabbits. In this study, the long term effects of aqueous extracts of *I. gabonensis* bark, *U. lobata* root and *C. papaya* leaves on the oxidative status of normal rabbits were monitored at specific intervals in the serum for 24 weeks, and in the tissues. Oxidative status was determined by measuring activities of superoxide dismutase (SOD) and catalase (CAT), and the concentration of malondialdehyde (MDA). Significant ($p < 0.05$) decreases were observed in some weeks in the serum MDA levels; also, liver and pancreatic MDA levels were significantly ($p < 0.05$) lower for all treated rabbits. SOD and catalase activities in the serum and tissue of the rabbits treated with the medicinal plants were generally higher or statistical similar to control. Findings in this study showed that these hypoglycemic medicinal plants did not exert oxidative damage; in some instances, particularly in the pancreas, they were found to be protective against oxidative damage.

KEYWORDS: Medicinal plants, *Irvingia gabonensis*, *Urena lobata*, *Carica papaya*, Hypoglycaemic plants, oxidative damage

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INTRODUCTION

Focus on medicinal plant research has increased worldwide. Evidence abounds on the immense potentials of medicinal plants used in various traditional systems. Various medicinal plants have been studied using scientific approaches; results from these studies have revealed the potentials of medicinal plants in the area of pharmacology (Dahanukar *et al.*, 2000; Auddy *et al.*, 2003; Fatahi *et al.*, 2003; Somova *et al.*, 2003). *Carica papaya*, *Urena lobata* and *Irvingia gabonensis* are used in folkloric medicine for the treatment of many diseases including diabetes mellitus (Oloyede, 2005; Oyelola, 2005; Lans, 2006). The hypoglycemic and anti-diabetic effects of the plant extracts have also been reported (Adamson *et al.*, 1990; Olagunju *et al.*, 1995; Onoagbe *et al.*, 1999a; Ngondi *et al.*, 2005; Ngondi *et al.*, 2006; Onoagbe *et al.*, 2010). Recent studies have also shown that *C. papaya* (Omonkhua and Onoagbe, 2011a), *U. lobata* (Omonkhua and Onoagbe, 2011b) and *I. gabonensis* (Omonkhua and Onoagbe, 2012) possess long term hypoglycaemic and anti-obesity effects on normal rabbits for a period of 24 weeks.

Oxidative stress, which is the imbalance between production and removal of reactive oxygen species (ROS) and free radicals, has been reported to contribute substantially to the pathogenesis of diabetic complications (Nishikawa *et al.*, 2000; Schmidt and Stern, 2000). Several reports have shown that there are alterations in the endogenous antioxidant enzymes in diabetic condition (Genet *et al.*, 2002; Preet *et al.*, 2005), especially the anti-oxidative defense system, like superoxide dismutase and catalase, which are lowered in diabetic subjects.

In this study, the long term effects of the aqueous extracts of *I. gabonensis* bark, *U. lobata* root and *C. papaya* leaves on the oxidative status of normal rabbits were monitored at pre-determined intervals in the serum for 24 weeks; and in the liver, kidney, heart, and pancreas after 24 weeks by measuring activities of superoxide dismutase (SOD) and catalase, and the concentration of malondialdehyde (MDA) (lipid peroxidation). This investigation is part of a biochemical evaluation of these medicinal plants, in order to ascertain their efficacy and safety in managing diabetes mellitus.

MATERIALS AND METHODS

Chemicals and reagents

Adrenaline, trichloroacetic acid and thiobarbituric acid (Sigma, London), and other analytical grade chemicals were products of BDH Chemical Limited, Poole, England.

Experimental animals

Twenty-four weaned rabbits of the New Zealand strain, weighing between 800-1200g, were obtained from Animal Unit of Federal University of Technology, Akure, Ondo State. The animals were placed on commercial feed (Ewu growers from the Bendel Feed and Flour Mill Ewu, Nigeria) and allowed to drink water freely. The rabbits were allowed to acclimatize for three weeks before the commencement of the experiments. Treatment of the animals was in accordance with the Principles of Laboratory Animal Care (NIH Publication 85-93, revised 1985).

Medicinal plants

The roots of *U. lobata*, bark of *I. gabonensis* and the leaves of *C. papaya* were obtained locally from open forest at Akungba-Akoko, Ondo State, Nigeria and identified in the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko. Herbarium specimen with voucher numbers UIH 22286 (*Irvingia gabonensis*), UIH 22287 (*Urena lobata*) and UIH 22288 (*Carica papaya*) were deposited at the Herbarium of the University of Ibadan, Nigeria.

Preparation of plant extract

The aqueous plant extracts were prepared and quantified by the method of Onoagbe *et al.* (1999b).

Administration of plant extracts to experimental animals

The plant extracts were administered orally to the rabbits at a concentration of 200 mg/kg body weight daily for 24 weeks; GROUP I: Control; GROUP II: *Irvingia gabonensis* treated rabbits; GROUP III: *Urena lobata* treated rabbits; GROUP IV: *Carica papaya* treated rabbits.

Blood collection

During the monitoring phase (Weeks 1, 2, 3, 4, 6, 8, 10, 12, 15, 18 and 21), blood was collected from the ventral vein of the rabbits' ear, at the end of the monitoring phase (Week 24), the rabbits were stunned and in this unconscious state, the thoracic and abdominal regions were opened to expose the heart and other organs. Blood was obtained through heart puncture, the liver, kidneys, heart and pancreas, were also collected. Blood samples were allowed to clot and centrifuged at 1000 X g for 5 minutes; the serum was then separated for analysis. Tissues were homogenized in ice cold normal saline (1:4 w/v), centrifuged and the supernatant stored in the freezer until analysis.

Biochemical Analysis

The malondialdehyde (MDA) level was used to estimate the level of lipid peroxidation. MDA levels were determined in the serum, liver, kidney, heart and pancreas by the thiobarbituric acid reactive

substances (TBARS) method (Varshney and Kale, 1990). Catalase activity was determined in the serum, liver, kidney, heart and pancreas by the method of Sinha (1972). Superoxide dismutase activity was assayed in the serum, liver, kidney, heart and pancreas by the method of Misra and Fridovich (1972).

Statistical analysis

The data are expressed as mean of 4 to 6 determinations \pm S.E.M. The differences among groups were analyzed by one-way analysis of variance (ANOVA). Inter-group comparisons were done using Duncan's Multiple Range Test (DMRT) with 95% confidence intervals. The SPSS 11.0, SPSS Inc., Chicago, Illinois, USA, was used for this analysis.

RESULTS

The results of serum SOD for *I. gabonensis*, *U. lobata* and *C. papaya* treated rabbits (Figure 1) show that all three medicinal plants caused initial significant ($p < 0.05$) decreases in SOD activities (week 1). At week 2, significant ($p < 0.05$) increases were observed for all test groups, thereafter, the serum SOD activities of rabbits treated with the medicinal plants were comparable to control. Figure 2 shows the results for tissue SOD for *I. gabonensis*, *U. lobata* and *C. papaya* treated rabbits. Liver and pancreatic SOD activities for all tests animals were comparable to control, while kidney levels for *I. gabonensis* and *U. lobata*, as well as, heart levels for *U. lobata* treated rabbits, were significantly ($p < 0.05$) higher than control.

Significant ($p < 0.05$) decreases were observed in week 4 for serum catalase activities of *I. gabonensis*, *U. lobata* and *C. papaya* treated normal rabbits (Figure 3), weeks 8 and 10 recorded significantly ($p < 0.05$) higher catalase activities, other values were mostly comparable to control. The result for the tissue catalase activities (Figure 4) shows that *I. gabonensis*, *U. lobata* and *C. papaya* did not significantly alter liver, kidney and heart catalase activities of treated animals, however, it is pertinent to note that pancreatic catalase activities for all groups were statistically ($p < 0.05$) higher than control.

As shown in Figure 5, for all three plants extracts, the serum MDA levels of test animals decreased in weeks 1 and 6, most of the other values were comparable to control. Figure 6 shows the results of tissue MDA levels for control and test animals. For all test groups, kidney MDA levels were significantly ($p < 0.05$) elevated, on the other hand, liver and pancreatic MDA levels were significantly ($p < 0.05$) reduced, heart MDA values were statistically comparable to control.

DISCUSSION

More than 400 plant species have been reported to have hypoglycaemic activities (Oliver-Bever, 1986; Rai, 1995), however, the search for new anti-diabetic drugs from natural plants is still attractive as they contain substances that have alternative and perhaps safer effects on diabetes mellitus (Loew and Kaszkin, 2002). Oxidative stress is characterized by increased lipid peroxidation and/or altered non-enzymatic and enzymatic antioxidant systems (Adewole and Caxton-Martins, 2006).

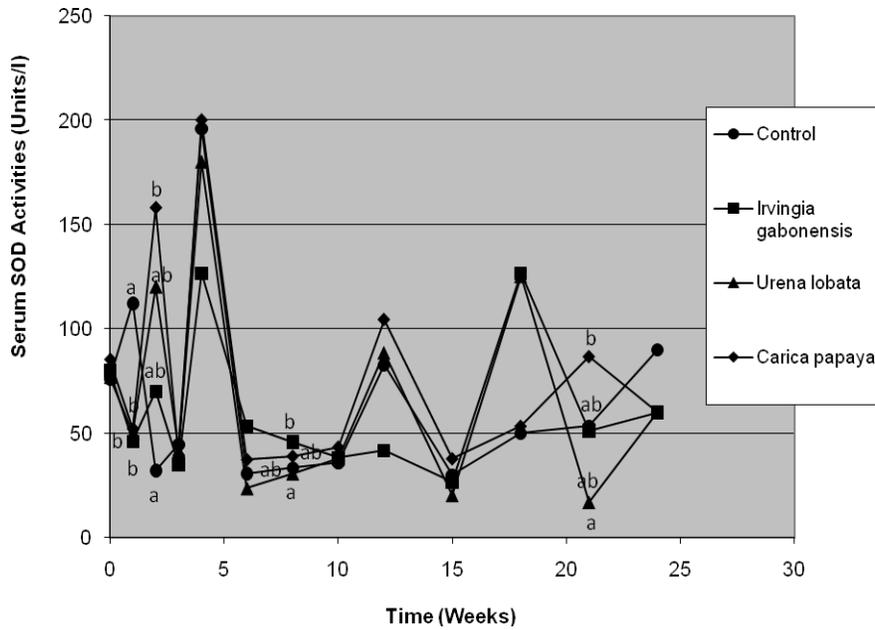


FIGURE 1 Effects of repeated daily oral administration of aqueous extracts of *I. gabonensis* bark, *U. lobata* root and *C. papaya* leaves for 24 weeks at 200 mg/kg body weight on serum (U/L) SOD activities of normal rabbits. Data were obtained from serum at pre-determined intervals. Values are means of 4-6 determinations ± SEM. Values carrying different notations are statistically different at p < 0.05.

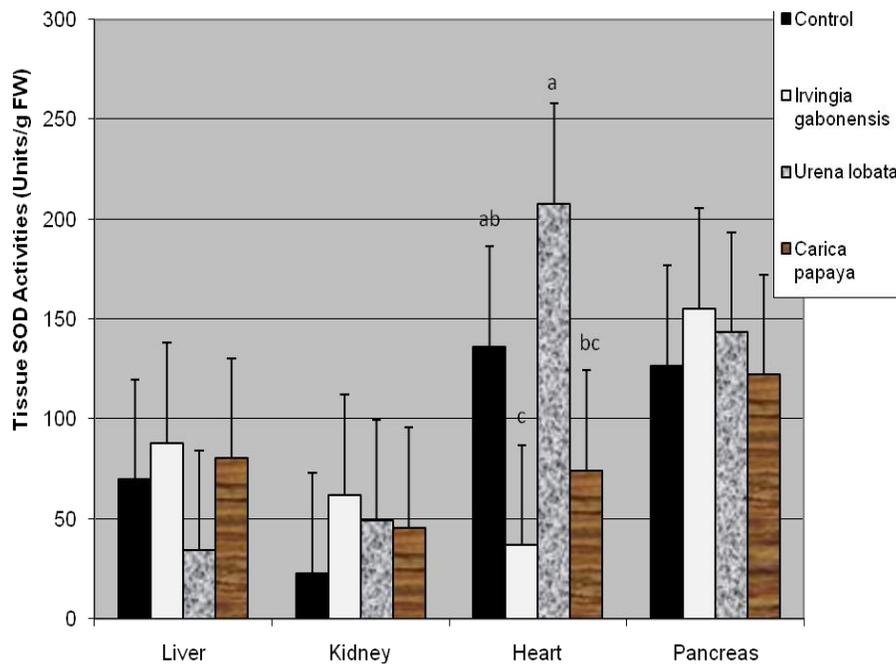


FIGURE 2 Effects of repeated daily oral administration of aqueous extracts of *I. gabonensis* bark, *U. lobata* root and *C. papaya* leaves for 24 weeks at 200 mg/kg body weight on tissue (U/gFW) SOD activities of normal rabbits. Data were obtained from tissue homogenates at the end of 24 week of monitoring. Values are means of 4-6 determinations ± SEM. Values carrying different notations are statistically different at p < 0.05.

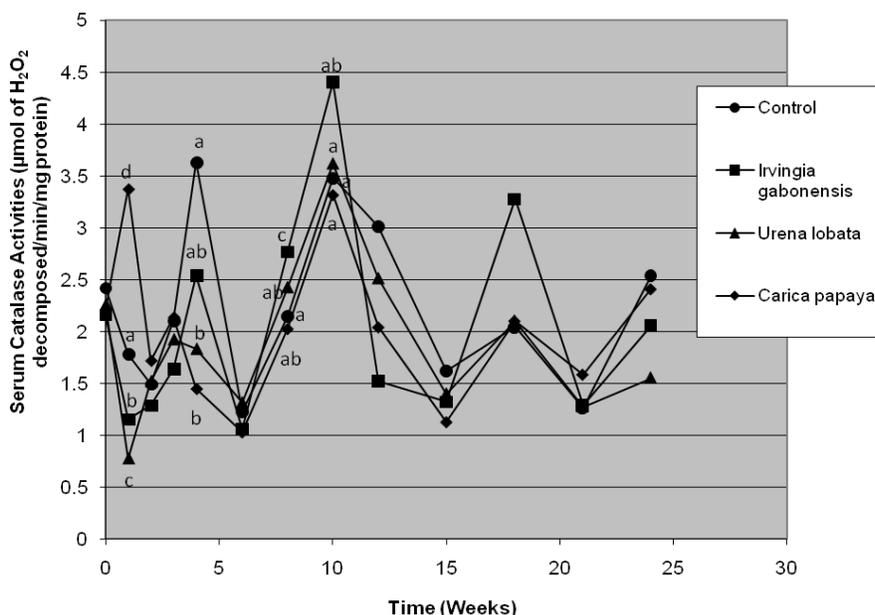


FIGURE 3 Effects of repeated daily oral administration of aqueous extracts of *I. gabonensis* bark, *U. lobata* root and *C. papaya* leaves for 24 weeks at 200 mg/kg body weight on serum ($\mu\text{g H}_2\text{O}_2$ decomposed/min/mg protein) catalase activities of normal rabbits. Data were obtained from serum at pre-determined intervals. Values are means of 4-6 determinations \pm SEM. Values carrying different notations are statistically different at $p < 0.05$.

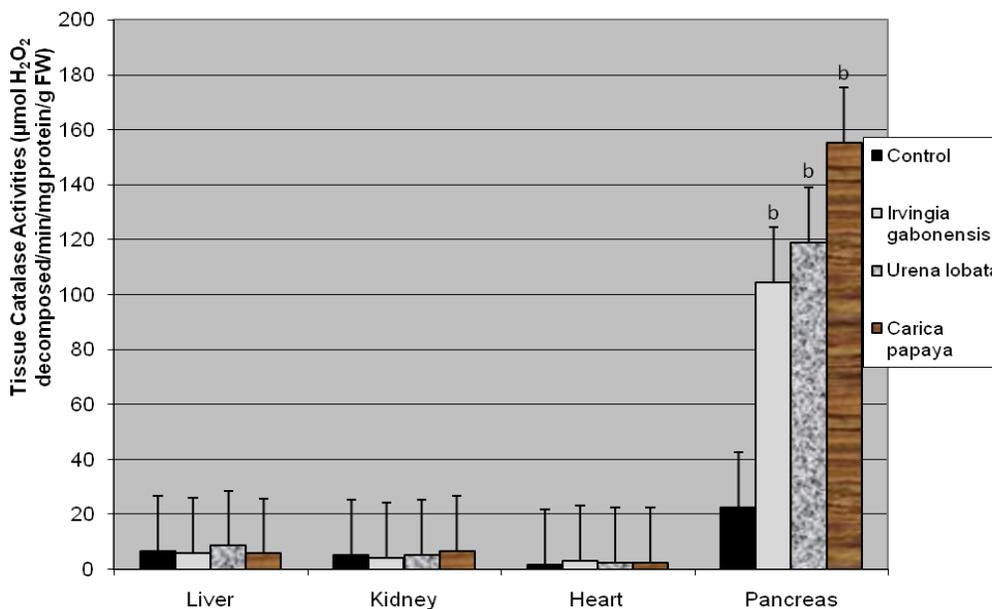


FIGURE 4 Effects of repeated daily oral administration of aqueous extracts of *I. gabonensis* bark, *U. lobata* root and *C. papaya* leaves for 24 weeks at 200 mg/kg body weight on tissue ($\mu\text{g H}_2\text{O}_2$ decomposed/min/mg protein/gFW) catalase activities of normal rabbits. Data were obtained from tissue homogenates at the end of 24 week of monitoring. Values are means of 4-6 determinations \pm SEM. Values carrying different notations are statistically different at $p < 0.05$.

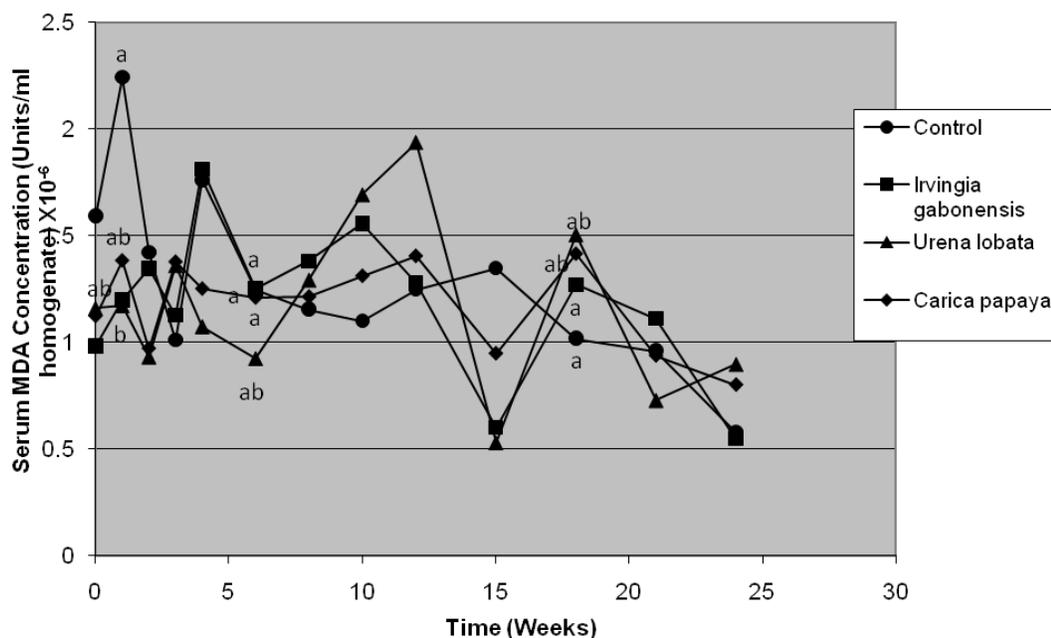


FIGURE 5 Effects of repeated daily oral administration of aqueous extracts of *I. gabonensis* bark, *U. lobata* root and *C. papaya* leaves for 24 weeks at 200 mg/kg body weight on serum (Units/ml homogenate X 10⁻⁶) MDA concentration of normal rabbits. Data were obtained from serum at pre-determined intervals. Values are means of 4-6 determinations ± SEM. Values carrying different notations are statistically different at p < 0.05.

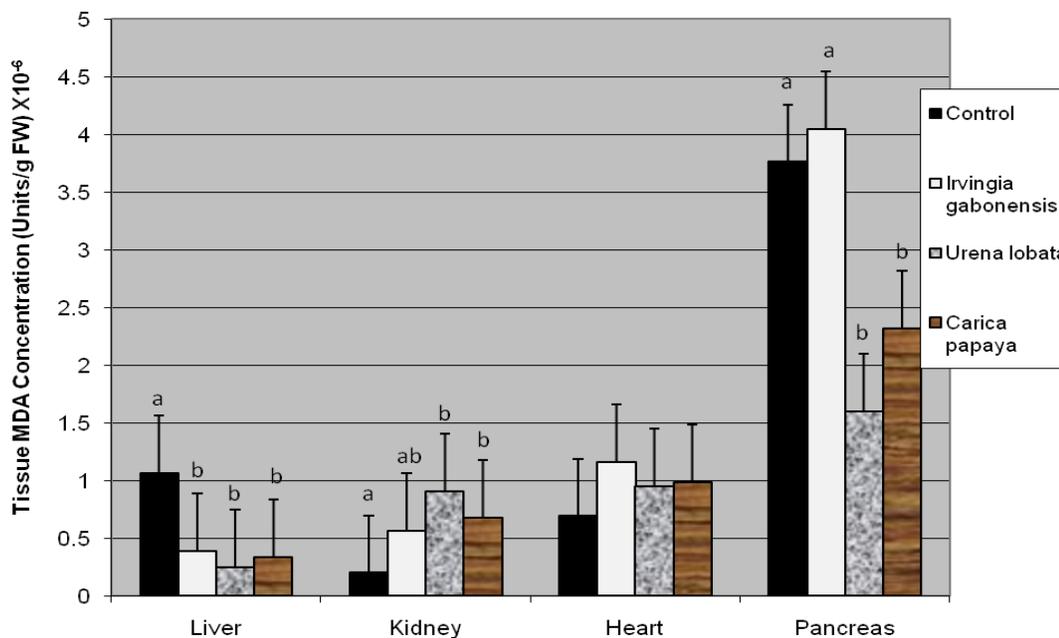


FIGURE 6 Effects of repeated daily oral administration of aqueous extracts of *I. gabonensis* bark, *U. lobata* root and *C. papaya* leaves for 24 weeks at 200 mg/kg body weight on tissue (Units/g FW X 10⁻⁶) MDA concentration of normal rabbits. Data were obtained from tissue homogenates at the end of 24 week of monitoring. Values are means of 4-6 determinations ± SEM. Values carrying different notations are statistically different at p < 0.05.

To ascertain the oxidative status of the experimental animals treated with *I. gabonensis*, *U. lobata* and *C. papaya*, serum and tissue SOD, catalase and MDA levels were assessed.

Mahdi *et al.* (2003) reported that three of the four hypoglycemic plants they studied significantly increased superoxide dismutase activity compared to diabetic control, implying that the plants improved the oxidative status of the diabetic subjects; the fourth hypoglycemic plant did not have any effect on SOD activity. The results obtained in our study indicate that though an initial oxidative response was observed (Week 1), the oxidative status of normal rabbits was subsequently enhanced (Week 2) and then restored to normal. The observation that the tests animals had serum SOD activities that were comparable to control for most of the monitoring period implies that the medicinal plants did not negatively alter their oxidative status. Apart from kidney SOD levels for *I. gabonensis* and *U. lobata*, as well as, heart levels for *U. lobata* treated rabbits that were increased, most of the tissue SOD activities recorded were similar to control. Panda and Kar (1998) reported significantly increased activity of two antioxidant enzymes in liver i.e. SOD and catalase following treatment with aqueous extract of *Ocimum sanctum*. The increases observed in this study correlates well with their observation, implying that the medicinal plants used in this study enhanced the oxidative status of some tissues.

For catalase activities, a decrease was observed in the first few weeks of monitoring, thereafter catalase activities was boosted and then returned to control levels. Adewole and Caxton-Martins (2006), reported that the hypoglycemic plant they studied, *Annona muricata* Linn, significantly ($p < 0.05$) enhanced the activities of the anti-oxidant enzymes catalase, glutathione peroxidase and SOD compared to diabetic control. The increases observed in this study agree with their findings, suggesting again the all three medicinal plants, at some point, enhanced the oxidative status of test animals. Chronic oxidative stress due to hyperglycemia may play an important role in progressive β -cell dysfunction (Tiwari and Rao, 2002; Robertson, 2004), since pancreatic islets have low expression of antioxidant enzymes (Lenzen *et al.*, 1996; Tiedge *et al.*, 1997), the significant increase in pancreas catalase activities seen in this study is particularly advantageous as it may enhance the pancreas' ability to combat destructive oxidants and improve pancreatic function. Indeed studies have shown that antioxidants can ameliorate β -cell dysfunction (Matsuoka *et al.*, 1997; Tanaka *et al.*, 1999).

Jyoti *et al.* (2004) reported that *Ocimum sanctum* extracts administered to normal rabbits for 30 days significantly reduced serum MDA levels. Several reports also indicate that hypoglycemic plants reduced MDA levels of streptozotocin/alloxan-induced diabetic rats (Adewole and Caxton-Martins, 2006; Mahdi *et al.*, 2003; Pari and Umamaheswari, 2000), the reductions observed in our study agree with this. Taken together the results for serum MDA levels indicate that the administration of these plant extracts did not exert lipid peroxidation, in some instances they were even protective against it. Prakasam *et al.* (2005) reported that *Casearia esculenta* root extract restored the increased liver and kidney MDA levels in streptozotocin-induced diabetic rats to non-diabetic

control values. Keeping in mind that most of the results obtained in this study favored the enhancement of the oxidative status of the experimental animal, the reason for the elevated kidney MDA levels was quite puzzling, but may be related to the dose, frequency and duration of the administration of the medicinal plants that may have overburdened the kidneys. The anti-oxidant effect of the medicinal plants examined in this study was more clearly seen in the significant reductions in the liver and pancreatic MDA levels, indicating that all three plants were protective against lipid peroxidation in these tissues.

This study showed that long term treatment of normal rabbits with these hypoglycemic plants did not exert oxidative damage, indeed in some instances, such as liver and pancreatic MDA levels, as well as, the increases observed in serum and tissue anti-oxidant enzymes (particularly in the pancreas), the plants were even protective against oxidative damage. Oxidative stress is suggested to play a prominent role in the pathogenesis of diabetes mellitus, the presence of polyphenolics in the plants examined in this study (Omonkhua and Onoagbe, 2010), which are frequently implicated as having anti-diabetic (Loew and Kaszkin, 2002), as well as anti-oxidant effects, makes it likely that these medicinal plants may reduce oxidative damage to tissues, especially pancreatic cells, thus enhancing their functions and also protect against long term diabetic complications.

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