Effects of Irvingia grandifolia, Urena lobata and Carica papaya on the Oxidative Status of Normal Rabbits

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Citation

Abstract
The hypoglycemic effects of Irvingia grandifolia, Urena lobata and Carica papaya are documented. While medicinal plants may have recognizable therapeutic effects, they may also have toxic side-effects. In this study, the sub-chronic effects of aqueous extracts of I. grandifolia 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 tissues, by measuring activities of superoxide dismutase and catalase, and the levels of malondialdehyde. The plants studied did not alter the serum MDA levels, liver and pancreatic MDA levels were significantly (p<0.05) lower. For all plants, serum and tissue, SOD and catalase activities were generally statistical similar to control. I grandifolia, U. lobata and C. papaya, did not appear to exert any form of oxidative damage on normal rabbits, with respect to liver and pancreatic MDA levels; they even seemed to be protective against lipid peroxidation.

INTRODUCTION
Focus on plant research has increased worldwide in recent times and a large body of evidence has been collected to show the immense potentials of medicinal plants used in various traditional systems. Various medicinal plants have been studied using modern scientific approaches. Results from these plants have revealed the potentials of medicinal plants in the area of pharmacology.

Carica papaya, popularly known as pawpaw and more commonly known as the papaya, is common in tropical and sub-tropical countries. Its biologically active constituents include chymopapain and papain, which are used in the treatment of arthritis and digestive disorders. In folkloric medicine, extracts of the fruits are used for a variety of medicinal purposes ranging from treatment of ringworm, malaria and hypertension, while extracts of unripe fruit have been used in treatment of diabetes.

Urena lobata Linn (Malvaceae), otherwise called Caesar weed, is a shrub that grows between 0.6-3 m tall and up to 7 cm in basal diameter. Various extracts of leaves and roots are used in herbal medicine to treat such diverse ailments as colic, malaria, gonorrhea, fever, wounds, toothache, and rheumatism. The methanol extract of Urena lobata root and various crude extracts of the leaves and roots, as well as the solvent fractions have been reported to show a broad spectrum of antibacterial activity. C. papaya and U. lobata have been used in many traditional systems to treat diabetes mellitus.

Irvingia species, including Irvingia gabonensis, Irvingia wombolu and Irvingia grandifolia, are commonly found in most parts of tropical Africa most especially in the forest, it serves as food and is used in the treatment of ailments such as dysentery, diabetes mellitus and it is also used as analgesic.

The hypoglycemic effects of I. grandifolia and U. lobata on normal rabbits and streptozotocin-induced diabetic rats have been reported. Fakeye et al also reported that ethanolic leave extract of C. papaya significantly reduced the blood glucose levels of alloxan-induced diabetic rats.

Oxidative stress is the imbalance between production and removal of reactive oxygen species (ROS). Increased oxidative stress, which contributes substantially to the pathogenesis of diabetic complications, is the consequences of either enhanced ROS production or attenuated ROS scavenging capacity. Several reports have shown the alterations in the antioxidant enzymes during diabetic condition. The antioxidative defense system, like superoxide dismutase and catalase, showed lower activities in diabetic subjects. The decreased activities of SOD and Catalase may be a response to increased production of
hydrogen peroxide and superoxide ion the auto oxidation of excess glucose and non-enzymatic glycation of proteins. 

Millions of people in various traditional systems, including Nigeria, have resorted to the use of medicinal plants to treat their ailments; this could be as a result of the high cost of orthodox health care, or lack of faith in it, or maybe as a result of the global shift towards the use of natural, rather than synthetic products. While the craze for natural products has its merits, care must be taken not to consume plants or plant extracts that could have deleterious effects on the body, either on the short term or on the long term. It therefore means that these plants must be studied for their biochemical/toxicological effects.

In this study, the sub-chronic effects of the aqueous extracts of I. grandifolia bark, U. lobata root and C. papaya leaves on the oxidative status of normal rabbits was monitored at predetermined intervals in the serum for 24 weeks, and in the liver, kidney, heart, and pancreas by measuring activities of superoxide dismutase and catalase, and the levels of malondialdehyde (lipid peroxidation).

MATERIALS AND METHODS

CHEMICAL AND REAGENTS

Adrenaline, trichloroacetic acid and thiobarbituric acid (Sigma, London), other analytical grade chemicals were products of BDH Chemical Limited, Poole, England. Randox kits for blood glucose assay was obtained from C.C. Obi Nigeria Limited, Ashogbon Street, Idumota Lagos.

EXPERIMENTAL ANIMALS

Twenty-four weaned male and female rabbits were obtained from local breeders in Oke Aro, 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; they weighed between 500 g and 1100 g. The rabbits were examined by a veterinary doctor and allowed to acclimatize for two 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 root of Urena lobata, bark of Irvingia grandifolia and the leaves of Carica papaya were obtained locally from open forest at Akungba Akoko, Ondo State, Nigeria and identified by Dr. O.A. Obembe of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-AKoko.

PREPARATION OF PLANT EXTRACT

The aqueous plant extracts were prepared by the method of Onoagbe et al (21), and then quantified.

ADMINISTRATION OF THE PLANT EXTRACT

The plant extracts were orally administered to the rabbits at 200 mg/kg body weight daily for 24 weeks.

GROUP I: Distilled water GROUP II: Irvingia grandifolia GROUP III: Urena lobata GROUP VI: Carica papaya.

The rabbits were weighed weekly.

BLOOD COLLECTION

During the monitoring phase, blood was collected from the ventral vein of the rabbits’ ear, at the end of the monitoring phase, 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 for glucose assay was collected in fluoride bottles while sterile bottles were used for other biochemical assays.

CENTRIFUGATION OF SAMPLE

Blood samples were allowed to clot and centrifuge at 5,000 rpm 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 at 4°C until analysis.

BIOCHEMICAL ANALYSIS

BLOOD GLUCOSE ESTIMATION

Fasting blood glucose was measured by the glucose oxidase method, as described in the manual of the Randox glucose kit.

LIPID PEROXIDATION

The malondialdehyde (MDA) levels were 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.

CATALASE

Catalase activity was determined in the serum, liver, kidney, heart and pancreas by the method of Sinha (24).
SUPEROXIDE DISMUTASE (SOD)

Superoxide dismutase activity was determined in the serum, liver, kidney, heart and pancreas by the method of Misra and Fridovich, (25).

STATISTICAL ANALYSIS

The data are expressed as mean of 4-6 determinations ± S.E.M. The differences between means were analyzed by the Independent Samples T-test on SPSS 11.0, SPSS Inc., Chicago, Illinois, USA. A value of P < 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

More than 400 plant species having hypoglycemic activity are available in literature, (26,27) however, searching for new anti-diabetic drugs from natural plants is still attractive because they contain substances that have alternative and perhaps safer effects on diabetes mellitus. (28) The pathogenesis of diabetes mellitus and the possibility of its management by the oral administration of hypoglycemic agents have stimulated greater interest in recent years. (29) Oxidative stress is suggested to play a prominent role in the pathogenesis of diabetes mellitus, since most of these plants contain glycosides, alkaloids, terpenoids, flavonoids, cartenoids, etc., that are frequently implicated as having anti-diabetic, (28) as well as anti-oxidant effects, it is plausible that some of these medicinal plants exert their hypoglycemic effects by protecting pancreatic cells from oxidative damage. Herbal remedies from medicinal plants have been used traditionally in many parts of the world where access to formal healthcare is limited. There are several reasons why the use of medicinal plants should be studied: herbal remedies may have recognizable therapeutic effects; (30) they may also have toxic side-effect. (31)

This study was designed to evaluate the sub-chronic effect of three hypoglycemic plants on the oxidative status of normal rabbits, in order to ascertain their safety.

EFFECTS OF , AND ON BODY WEIGHT AND RELATIVE ORGAN WEIGHT

The overall effects of sub-chronic administration of I. grandifolia, U. lobata and C. papaya to normal rabbits, were assessed by monitoring body weight and organ-body weight ratio. Figures 1.1, 1.2 and 1.3, show the effects of I. grandifolia, U. lobata and C. papaya respectively on the body weight of rabbits. All three medicinal plants had weight lowering effects on the animals, however only I. grandifolia showed statistically significant (p<0.05) decreases in weeks 10, 13, 14 and 18. The dissected test animals showed prominent reduction of subcutaneous fat compared to control animals. Jyoti et al, (32) reported no significant difference in the body weight of Ocimum sanctum treated rabbits (30 days) compared to control. The weight reducing effects of the medicinal plants in our study may be an enhancement of their hypoglycemic effect, since obesity is a predisposing condition in some types of diabetes.

Figure 1

Figure 1.1: Plotted values are means of 4-6 determinations. Values carrying alphabets are statistically (p
Evaluating oxidative status

Oxidative stress is characterized by increased lipid peroxidation and/or altered non-enzymatic and enzymatic antioxidant systems. To ascertain the oxidative status of the experimental animals exposed to I. grandifolia, U. lobata and C. papaya, serum and tissue SOD, catalase and MDA levels were measured.
EFFECTS OF , AND ON SERUM SOD ACTIVITY

The results of serum SOD for I. grandifolia, U. lobata and C. papaya groups are shown in Figures 3.1, 3.2 and 3.3 respectively. All three medicinal plants showed initial significant (p<0.05) decreases in serum SOD (week 1), indicating an oxidative response. For U. lobata and C. papaya, in week 2, significant (p<0.05) increases were observed, implying that at that point the medicinal plants were enhancing the oxidative status of the rabbits. Thereafter, the serum SOD activities for all three medicinal plants were comparable to control. Mahdi et al, (14) reported that the 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, the oxidative status of normal rabbits was subsequently enhanced and then restored to normal. The fact 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 the oxidative status of the test animals.

Figure 7
Figure 3.1: Plotted values are means of 4-6 determinations. Values carrying alphabets are statistically (p

EFFECTS OF , AND ON TISSUE SOD ACTIVITY

Figures 4.1, 4.2 and 4.3 show the results for tissue SOD for I. grandifolia, U. lobata and C. papaya respectively. In the I. grandifolia group, the increase in liver and kidney, and the decrease in heart SOD activity are not statistically (p<0.05) significant. Pancreas SOD levels were comparable to control. Thus SOD levels of the liver and kidney were most enhanced by I. grandifolia. In the U. lobata group, only liver SOD activity is lower than control (the decrease is not statistically (p<0.05) significant, implying that U. lobata improved the levels of SOD in the kidney, heart and pancreas. The C. papaya group had higher SOD activities for liver and kidney (not statistically significant) while heart and pancreas levels were comparable to control. Panda and Kar,(35) reported significantly increased activity of two antioxidant enzymes in liver i.e. SOD and catalase following treatment with aqueous extract of Ocimum sanctum. Although most of the increases in tissue SOD activity observed in our study were not statistically significant, no significantly lower values were recorded, implying that while the oxidative status may have been enhanced in some

Figure 8
Figure 3.2: Plotted values are means of 4-6 determinations. Values carrying alphabets are statistically (p

Figure 9
Figure 3.3: Plotted values are means of 4-6 determinations. Values carrying alphabets are statistically (p
tissues, it was certainly not depreciated in any.

Figure 10
Figure 4.1: Plotted values are means of 4-6 determinations. Values carrying alphabets are statistically (p

![Figure 10](image)

Figure 11
Figure 4.2: Plotted values are means of 4-6 determinations. Values carrying alphabets are statistically (p

![Figure 11](image)

Figure 12
Figure 4.3: Plotted values are means of 4-6 determinations. Values carrying alphabets are statistically (p

![Figure 12](image)

EFFECTS OF , AND ON SERUM CATALASE ACTIVITY

Results for serum catalase activity for I. grandifolia, U. lobata and C. papaya are shown respectively in figures 5.1, 5.2 and 5.3. I. grandifolia group showed an initial (week 1) significant decrease, there after the catalase activity normalized until weeks 8 and 10 where significant increases were recorded, again the levels dropped to normal. U. lobata and C. papaya groups showed significant (p<0.05) initial (Week 1) decreases, and thereafter serum catalase activity were comparable to control. The serum catalase activity of the C. papaya group significantly (p<0.05) increased in week 1 and then normalized through out the monitoring phase. Adewole and Caxton-Martins (33), reported that the hypoglycemic plant they studied significantly (p<0.05) enhanced the activities of the anti-oxidant enzymes catalase, glutathione peroxidase and SOD compared to diabetic control. While I. grandifolia appeared to have enhanced the catalase activity of the test animal, U. lobata and C. papaya groups recorded values that were mostly similar to control i.e. they did not negatively alter the oxidative status.

Figure 13
Figure 5.1: Plotted values are means of 4-6 determinations. Values carrying alphabets are statistically (p

![Figure 13](image)

Figure 14
Figure 5.2: Plotted values are means of 4-6 determinations. Values carrying alphabets are statistically (p

![Figure 14](image)
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Figure 15
Figure 5.3: Plotted values are means of 4-6 determinations. Values carrying alphabets are statistically (p

Figure 17
Figure 6.2: Plotted values are means of 4-6 determinations. Values carrying alphabets are statistically (p

EFFECTS OF , AND ON TISSUE CATALASE ACTIVITY

The results for the tissue catalase activities are shown in figures 6.1, 6.2 and 6.3. I. grandifolia did not significantly alter the tissue catalase activity, however, it is pertinent to note that pancreatic catalase activity for this group was higher (though not statistically significant) than control. The U. lobata and C. papaya showed the same trend. Jyoti et al, (32) reported increased anti oxidants and antioxidant enzymes in Ocimum sanctum treated normal rabbits. Our study indicate that while the medicinal plants did not alter the liver, kidney and heart catalase activity of test animals, the pancreatic catalase activity of all three medicinal plants were higher (not statistically significant at p<0.05) than control, suggesting an enhancement of the oxidative status.

Figure 16
Figure 6.1: Plotted values are means of 4-6 determinations. Values carrying alphabets are statistically (p

Figure 18
Figure 6.3: Plotted values are means of 4-6 determinations. Values carrying alphabets are statistically (p

EFFECTS OF , AND ON SERUM MALONDIALDEHYDE LEVELS

As shown in figures 7.1, 7.2 and 7.3, for all three plants extracts the serum MDA levels of test animal were comparable to control through out the monitoring period. Jyoti et al (32) reported that Ocimum sanctum extracts administered to normal rabbits for 30 days significantly reduced serum MDA levels. Several report also indicate that hypoglycemic plants reduced MDA levels of streptozotocin/alloxan-induced diabetic rats. (33,34,36) This study revealed that I. grandifolia, U. lobata and C. papaya extracts did not cause any alteration in the serum MDA levels through out the experiment, indicating that the administration of these plant extracts did not exert lipid peroxidation.
Effects of Irvingia grandifolia, Urena lobata and Carica papaya on the Oxidative Status of Normal Rabbits

Figure 19
Figure 7.1: Plotted values are means of 4-6 determinations. Values carrying alphabets are statistically (p

![Figure 19](image)

Figure 20
Figure 7.2: Plotted values are means of 4-6 determinations. Values carrying alphabets are statistically (p

![Figure 20](image)

Figure 21
Figure 7.3: Plotted values are means of 4-6 determinations. Values carrying alphabets are statistically (p

![Figure 21](image)

Figure 22
Figure 8.1: Plotted values are means of 4-6 determinations. Values carrying alphabets are statistically (p

![Figure 22](image)

Figure 23
Figure 8.2: Plotted values are means of 4-6 determinations. Values carrying alphabets are statistically (p

![Figure 23](image)

EFFECTS OF , AND ON TISSUE MALONDIALDEHYDE LEVELS

Figures 8.1, 8.2 and 8.3 show the results for MDA levels for I. grandifolia, U. lobata and C. papaya respectively. For all three herbs, kidney MDA levels were significantly (p<0.05) elevated, on the other hand, liver and pancreatic MDA levels were significantly (p<0.05) reduced for all three plant extracts, heart MDA values were statistically comparable to control. Prakash et al (37) 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 not be unrelated to the dose, frequency and duration of the administration of the medicinal plants which may have over burdened the kidneys. The anti-oxidant nature 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.
CONCLUSION

Most hypoglycemic plants also have anti-oxidant properties, it is thus not surprising that for most part, our study showed that sub-chronic exposure of normal rabbits to these hypoglycemic plants did not exert any form of 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, the plants were even protective against oxidative damage.

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