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Research Article

EFFECT OF *CARICA PAPAYA* SEED POWDER ON LIVER AND MUSCLE OF COMMONLY CULTURED CATFISH, *CLARIUS BATRACHUS*

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ABSTRACT
Papaya plants are extensively used in integrated aquaculture because of the high ability of the roots of this plant to hold the dick of the ponds. Apart from its use in aquaculture, the toxic seeds of papaya may find their way into the natural aquatic environment through effluents from industries that use *carica papaya* fruits as raw materials for the production of juice. In spite of the wide uses of papaya seeds as biocides in aquaculture little toxicological studies have been done on the culture fishes. The present study therefore is directed towards finding some select antioxidant parameters of one of the common cultured catfish, *Clarius batrachus* in response to papaya seed powder exposure. The enzymatic antioxidants like catalase, and superoxide dismutase was estimated in liver and muscle of catfish and the activity of these enzymes were found to decrease significantly in papaya seed powder extract exposed fish. Correspondingly the lipid peroxidase activity in these tissues was enhanced considerably. The non-enzymatic antioxidants ascorbic acid and reduced glutathione were very significantly reduced in liver and muscle of the treated fish. From these toxicity tests it is clear that *Carica papaya* seed powder concentration as low as 5mgl^{-}1 in the medium can be potentially hazardous to freshwater fish species.

Keywords: *Papaya Seed, Antioxidant, Catfish*

INTRODUCTION
The use of toxic plants for catching fish is a common practice worldwide. The ichtyotoxic characteristics of some of these plants make them potent tools for catching or stupefying fish all over the world. Local fishermen in Nigeria have reportedly used specific biocides derived from plants for fishing (Reed *et al.,* 1967). Fisher folks of various African countries extensively use many plants and plant products for capturing fish (Neuwinger, 2004; Fafioye *et al.,* 2004). Barks of ethanobotanical origin and their application in capturing fish have also been reported from other regions of the world such as South America (Schultes and Raffauf, 1990), Nepal (Kulakkatolickal and Kramer, 1988) and India (Singh and Singh, 2002). In addition to their use as traditional piscicidal agents for catching wild fish, plant derived fish toxicants are also used in aquaculture management for controlling the predatory and wild fishes. The eradication of wild fishes in the culture ponds before the stocking of desired species is an important step in pond management as the former compete and/or prey upon the latter. The use of plant origin ichthyotoxicant as a fisheries management tool has been practiced in at least 30 countries (Murphy and Willis, 1996; Sanger and Koehn, 1997; Lintermans, 2000).

Different species of plants employed as piscicides have different effects, depending on the species of fish targeted (Van Andel, 2002). The active principles in the plant part used (seeds, kernel and bark) have varying potencies and modes of action depending on whether it is applied directly and the forms of extracts, aqueous or alcohol (Sambasivam *et al.,* 2003).

Papaya Seed
Papaya seed is known to contain piscicidal and antifertility properties and have been wildly used as fish poison for controlling the excessive breeding of tilapia in aquaculture in Nigeria. The major active ingredients (carpine, chymopapain, papain, bactericidal a glycone of lucotropaeolin benzyl isothiocyanate, aglycoside, sinigrin, the enzyme myrosin and carpasemine) are in the black seeds (Akah *et al.,* 1997). A piscicidal substance called carpine is present in traces in black seeds of papaya. Carpine in large quantities is said to lower the pulse rate and depress the nervous system. Papaya seed also contains

antifertility properties (Lohiya *et al.,* 1999). A complete loss of fertility has been reported in male Nile tilapia (Ekanemm and Okronko, 2003) and female Nile Tilapia (Ekanemm and Bassey, 2003), as well as rats and monkeys fed with an extract of papaya seeds (Pathak *et al.,* 2000; Lohiya *et al.,* 1999).

Papaya plants are extensively used in integrated aquaculture because of the high ability of the roots of this plant to hold the dick of the ponds. Apart from its use in aquaculture, the toxic seeds of papaya may find their way into the natural aquatic environment through effluents from industries that use *Carica papaya* fruits as raw materials for the production of juice.

In spite of the wide uses of papaya seeds as biocides in aquaculture little toxicological studies have been done on the culture fishes. We therefore chose to study some select antioxidant parameters of one of the common cultured catfish, *Clarius batrachus* in response to papaya seed powder exposure.

Any environmental disturbances can be considered a potential source of stress, as it prompts a number of responses in the animal to deal with the physiological changes triggered by exterior influences. As a consequence of metabolic activity, reactive oxygen species (ROS) are continuously produced and act as strong oxidants. Large repertories of antioxidant enzymes, in addition to small antioxidant molecules, acting as a defence mechanism, are produced by the cell. Superoxide dismutase (SOD), which hastens the dismutation of O_2^- to H_2O_2, catalase (CAT), and glutathione peroxidise (GPx), which converts H_2O_2 to H_2O, are the most important antioxidant enzymes found in all vertebrates. When the antioxidant defences are inadequate to combat the action of the ROS, oxidative stress results. The formation of ROS can be increased in response to different variations in the internal or external medium, whereupon oxidative alterations occur in the cellular constituents.

MATERIALS AND METHODS

Extraction of the seeds

The seeds of *C. papaya L.* were procured from local vendor and seeds were soaked overnight and sundried. Finally they were grounded in a food grinder and the powder was used for experimentation.

Experimental Design

Twenty four adult Catfish (40 ±5 cm) irrespective of sex weighing 250 ± 20 g were procured from Local Fishes market, Vadodara. Fishes were housed in Tubs (180 liter capacity) filled with 100 liters of aerated water on a 12-h light/dark cycle and fed *ad libitium*, with small sized Tilapia. Fishes were acclimatized for period of 5 days. The fishes were then divided in three groups, one served as control while two groups were exposed to 5mg/L and 15mg/L of *Carica papaya* seed powder respectively, for 28 days for its toxicity assessment. Fishes were observed each day for any behavioral and physical changes.

Biochemical Analysis

Fishes were euthanized by severe head blow and tissues were collected for measuring Enzymatic and non-enzymatic antioxidant parameters.

Enzymatic Antioxidant Milieu

Catalase activity was measured using method described by Sinha (1972). The activity of catalase was read at 590nm and expressed as units/mg protein (1 unit is the amount of enzyme that utilizes 1 micromol of H_2O_2 consumed/min).

The degree of lipid peroxidation was estimated by the rate of malonaldehyde (MDA) production using the thiobarbuteric acid (TBA) method as previously described by Beuge and Aust, 1978. The absorbance was recorded at 535 nm against reagent blank and the values expressed as MDA (nmol/g of heart tissue)

Superoxide dismutase (SOD) was estimated by the method of Kakkar *et al.,* (1984). This method is based on the ability of SOD to inhibit oxidation of reduced PMS under specific conditions. Reading was taken at 560 nm and the values expressed as U/mg tissue.

Non Enzymatic Antioxidants

Ascorbic acid levels were measured using Roe and Kuether (1943), briefly tissue was digested in 6% TCA , reduced with activated charcoal, filtrate is incubated with 2,4 DNPH and thiourea. Finally treated with Sulphuric acid and read at 540 nm against control.

Reduced Glutathione was measured using method described by Beutler *et al.*, (1963). Homogenate was treated with precipitating reagent in phosphate buffer (0.1 M) and DTNB. After incubation the tubes were read at 412 nm against blank within 1 min of addition of DTNB.

RESULTS

Catalase: Fishes Treated with low (5mg/L) dose of *Carica papaya seed powder* showed decrease in hepatic ($p< 0.05$) and muscle ($p< 0.05$) catalase activity. Similarly high dose (15mg/L) treated fish showed further decrease in Catalase activity in both the tissues. However the effect was more prominent in liver ($p< 0.01$) as compared to muscle.

LPO: The LPO activity increased significantly in liver in fish exposed to low concentration ($p< 0.05$) and high concentration ($p< 0.01$) of *Carica papaya seed powder* . In contrast the muscle showed very highly significant increase in LPO activity in both low and high concentration of *Carica papaya seed powder*.

SOD: Similarly SOD showed significant increase ($p< 0.01$) in activity in both muscle & liver in fish exposed to low as well as high concentration of *Carica papaya seed powder*.

The non enzymic antioxidant ascorbic acid and glutathione reductase both decreased significantly in low and high concentration exposed fish.

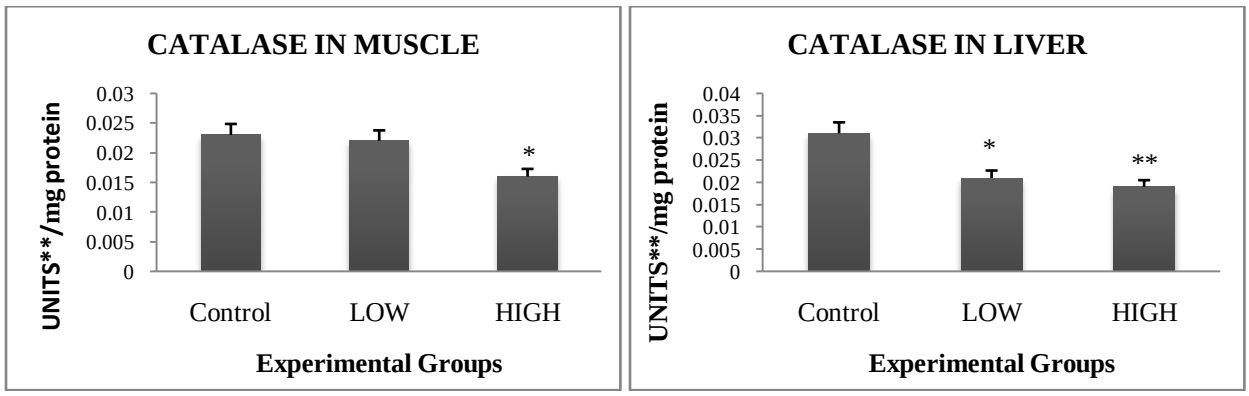

Figure 1: Catalase activity in muscle and liver of *Clarius batrachus* treated with *Carica papaya* seed Powder

Values are expressed as Mean ± SE.(n=8)

Control is compared with 5mg/L (Low) Carica papaya seed powder & 15mg/L(High) Carica papaya seed powder resp. $^{*}P<0.05, ^{**}P<0.01$

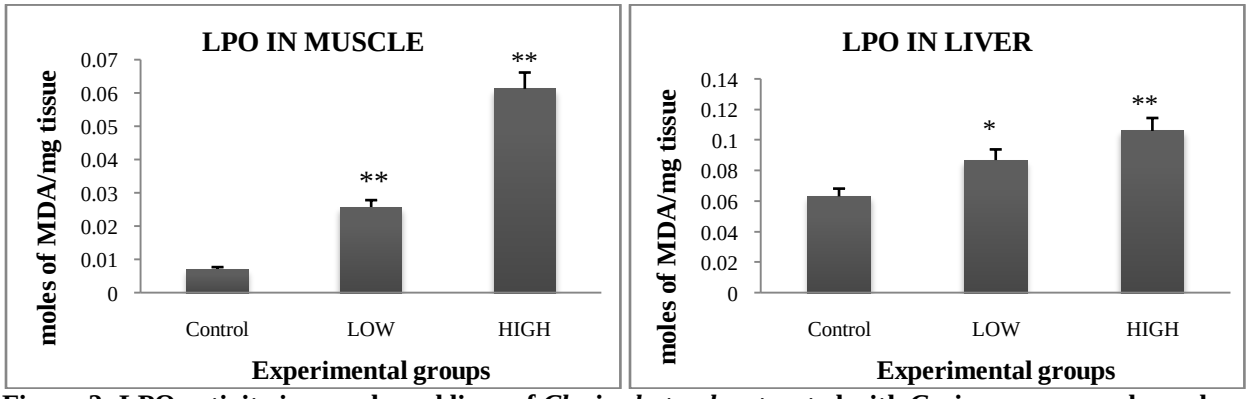

Figure 2: LPO activity in muscle and liver of *Clarius batrachus* treated with *Carica papaya* seed powder

Values are expressed as Mean ± SE.(n=8)

Control is compared with 5mg/L (Low) Carica papaya seed powder & 15mg/L (High) Carica papaya seed powder resp. $^{*}P<0.05, ^{**}P<0.01$

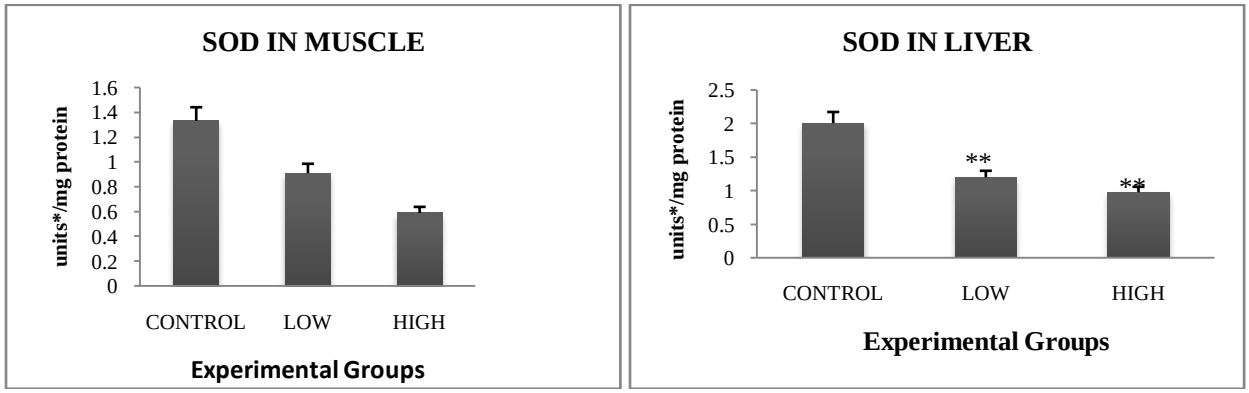

Figure 3: SOD activity in muscle and liver of *Clarius batrachus* treated with *Carica papaya* seed powder
Values are expressed as Mean ± SE.(n=8)
*Control is compared with 5mg/L(Low) Carica papaya seed powder & 15mg/L(High) Carica papaya seed powder resp. *P<0.05,**P<0.01*

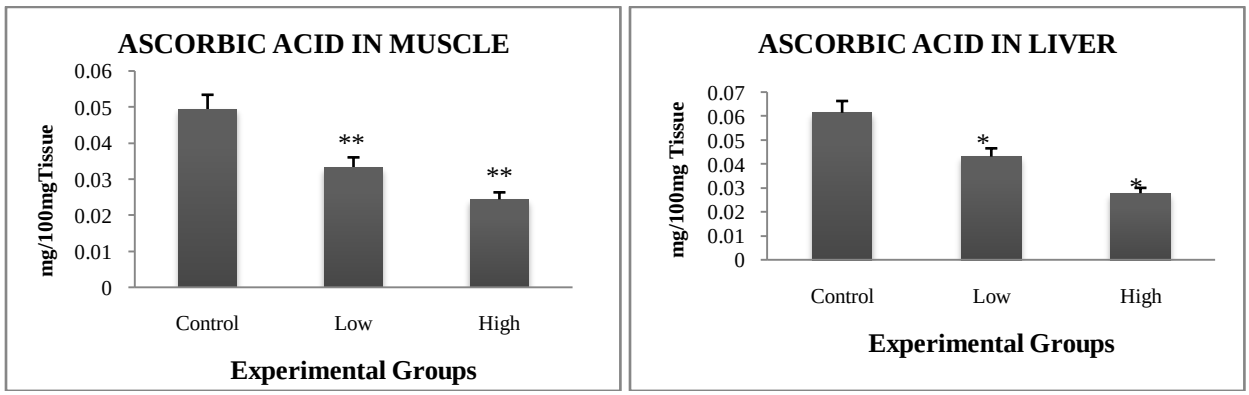

Figure 4: Ascorbic Acid levels in Muscle and Liver of *Clarius batrachus* treated with *Carica papaya* seed powder
Values are expressed as Mean ± SE.(n=8)
*Control is compared with 5mg/L(Low) Carica papaya seed powder & 15mg/L(High) Carica papaya seed powder resp. *P<0.05,**P<0.01*

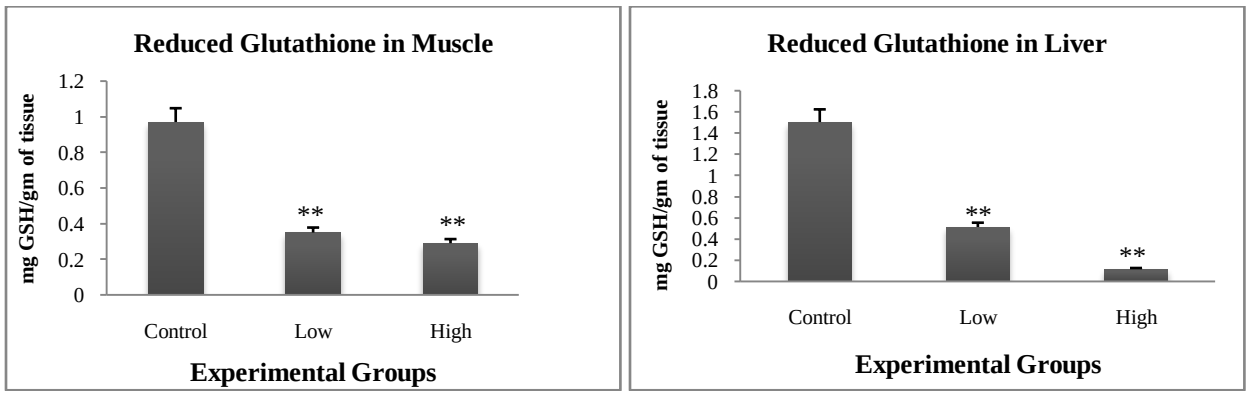

Figure 5: Reduced Glutathione levels in Muscle and Liver of *Clarius batrachus* treated with *Carica papaya* seed powder
Values are expressed as Mean ± SE.(n=8)
*Control is compared with 5mg/L(Low) Carica papaya seed powder & 15mg/L(High) Carica papaya seed powder resp. *P<0.05,**P<0.01*

DISCUSSION

Adverse response may be defined in terms of a measurement that is outside the "normal" range for healthy organisms, such as abnormal mortality, reproduction or growth. The presence of *Carica papaya* seeds in water bodies has been reported (Lohiya *et al.,* 2002) and the negative effects on aquatic life have been proven (Ayotunde and Ofem, 2008). However, despite their widespread use, little is known about their toxicity to fish. In the present study we have studied the effect of papaya seed powder on antioxidant parameters of *Clarius batrachus* muscle and liver. We observed a significant decrease in oxidative enzymes SOD and Catalase while the LPO showed corresponding increase. Non-enzymatic antioxidants, GSH and Ascorbic acid also showed a significant decrease.

Oxygen is a necessity of all living organisms but it is also toxic molecule that leads to the generation of free radicals. Free radicals are molecular marauders that have an unpaired valence electron. As such, these species are highly reactive and often destructive to other molecules in the vicinity of their production. Molecules that have the capability of detoxifying radical species are referred to antioxidants, and their role allows them to prevent macromolecular damage and the overt dysfunction that results from that mutilation (Reiter, 2000). Under normal physiological conditions, there appears to be four key sources for the generation of free radicals: mitochondrial electron transport, peroxisomal fatty acid metabolism, cytochrome P-450 reactions and the respiratory burst (phagocytic cells) (Beckman and Ames, 1997; Frie, 1999; Bandyopadhyay *et al.,* 1999). The full reduction of oxygen to water by cytochrome oxidase is the key step in the mechanism of aerobic ATP formation. Transference of electrons between oxygen species (cellular respiration) allows each of us to survive on this planet; not only at the cellular level but also as an organism.

Living in an oxygenated environment has required the evolution of effective cellular strategies to detect and detoxify the reactive oxygen species, which are metabolites of molecular O_2. The burden of ROS is largely counteracted by an intricate antioxidant defense system including enzymatic scavengers (SOD, GPx, GR and catalase) and non-enzymatic antioxidant defense (pyruvate, gluthione, flavanoids). An alteration in this balance will give rise to oxidative stress that may damage bio-molecules like DNA, proteins and lipids.

The primary defense against ROS is by SODs which is a metalloprotein found in both prokaryotic and eukaryotic cells. The SODs are thought to dismutate O_2^- via a ping-pong mechanism whereby the transition metal prosthetic group is reduced by O_2. The metal in the prosthetic group is then immediately re-oxidized by another O_2 molecule resulting in the production of H_2O_2 (Fridovich, 1974). The production of H_2O_2 within the cell may lead to the production of $^{\cdot}OH$ and subsequent cellular damage via the metal-catalysed Haber-Weiss reaction. Catalase, a manganese or heme-containing enzyme, functions to rapidly dismutate H_2O_2 to H_2O and O_2 (Krinsky, 1992). Catalase is mainly found in peroxisomes, perhaps because of the large number of H_2O_2 producing oxidases found in these organelles, while lower levels are also found in the mitochondria and the cytosol.

Thus SOD and Catalase maintain a balance between ROS production and detoxification. Exogenous sources trigger excess ROS production and thereby disturbs this homeostasis which leads to ROS accumulation, predominantly (O_2^-) and OH-) species. Apart from the hydroxyl radical, H202 which is generated by the action of SOD, is highly toxic by itself and can generate hydroxyl radicals by reacting with ferrous ions. Hydroxyl radicals are highly toxic and induce lipid peroxidation of cell membranes. H_2O_2 is neutralized by the enzyme Catalase (Halliwell B, 1994). In the present study, the generation of such free radicals may have occurred thereby decreasing the SOD and Catalase activity significantly and increasing the lipid peroxidase (LPO) activity. Decreased SOD and Catalase activity lead to the generation of more and more ROS, which causes the peroxidation of membrane lipids, that is evident from increased activity of LPO.

Glutathione (GSH) dependent system plays a vital role in the antioxidant defence mechanism in animals. Glutathione is considered to be one of the most important components of the antioxidant defence of living cells. The reduced tripeptide GSH is a hydroxyl radical and a singlet oxygen scavenger and participates in a wide range of cellular functions such as protein and DNA synthesis, intermediary metabolism, and

transport (Halliwell and Gutteridge, 1989; Meister and Anderson, 1983; Deneke and Fanburg, 1989). Since glutathione is present in high intracellular concentrations, there is a high probability that reactive oxygen species (such as superoxide, singlet oxygen and hydrogen peroxide) will be quenched by reaction with glutathione, before they can initiate their chain reaction-damaging effects (Jones *et al.*, 1981). Reduced glutathione acts as a hydrogen donor and as such is a substrate of key antioxidant enzymes including the Se-dependent glutathione peroxidase (GPx) and glutathione-S-transferase (GSTs). Glutathione peroxidase removes hydrogen peroxide and organic hydroperoxides (Ahmad, 1995; Fridovich, 1998; Hermes-Lima *et al.*, 1998) while GSTs catalyze conjugation reactions between glutathione and ROS-damaged cellular components. GSH synthetase is a key enzyme in the formation of GSH (Willmore and Storey, 1997) and thiol-disulfide oxidoreductase enzymes (e.g. thioltransferase, thiredoxin) catalyze the removal of thiol compounds (usually GSH) from a sulfhydral protein mixed disulfide. Relatively high ratios of GSH/GSSG are maintained intracellularly through the action of GR in an NADPH-dependent reaction (Andeson, 1996; Akerboom and Sies, 1981). GSH also acts as a substrate or co-substrate in many essential reactions, such as with the antioxidant enzyme GPx. Thus, the depletion of GSH during oxidative stress could have significant impact within a cell.

From these toxicity tests it is clear that *Carica papaya* seed powder concentration as low as 5 mgL-1 in the medium can be potentially hazardous to freshwater fish species. The acute toxicity data of the present study provide baseline information needed to develop models of *Carica papaya* seed powder effects, however more information is needed to assess their potential impacts on aquatic environment.

REFERENCES

Ahmad S (1995). *Antioxidant Mechanisms of Enzymes and Proteins* (Newyork: Champman and Hall) 238-272

Akah PA, Oli AN and Enwenem NM (1997). Preliminary studies on purgative effect of Carica papaya root extract. *Fitoterapia* **68** 327-331.

Akerboom TM and Sies H (1981). Assay of glutathione, glutathione disulphide, and glutathione mixed di-sulphides in bilogical samples. *Detoxication and Drug Metabolism: Conjugation and Related Systems* **77** 373-382.

Anderson ME (1996). *Free Radicles: A- Practical Approach* (Oxford IRL).

Ayotunde BE and Ofem EO (2008). Acute and chronic toxicity of pawpaw (Carica papaya) seed powder to adult Nile tilapia (Oreochromis niloticus Linne 1757). *African Journal of Biotechnology* **7** 2267-2274.

Bandyopadhyaya U, Das D and Banerjee RK (1999). Reactive Oxygen species: Oxidative damage and pathogenesis. *Current Science* **77**(5) 658-666.

Beckman KB and Ames BN (1997). *Oxidants, Antioxidants and Aging* (Cold Srping Laboratory Press).

Deneke SM and Fandurg BL (1989). Regulation of cellular Glutathione. *American Journal of Physiology* **257** 163-173.

Ekanemm ES and Okoronkwo BT (2003). Pawpaw seed as a fertility control agent on male Nile tilapia. NAGA. *World Fish Center Q* **26** 8-10.

Ekanemm SB and Bassey PO (2003). Pawpaw seed as a fertility control agent on female Nile tilapia. *Journal of Fish Research in Tropics* **18** 181-188.

Fafioye OO, Adebisi OA and Fagade AS (2004). Toxicity of Parkia biglobosa and Raphia vinifera extracts on Clarias gariepinus juveniles. *African Journal of Biotechnology* **3** 627-630.

Frei B (1999). Molecular and biological mechanisms of antioxidant action. *The FASEB Journal* **13**(9) 963-4.

Fridovich I (1974). Superoxide dismutase. *Advances in Enzymology and Related Areas of Molecular Biology* **41** 35-97.

Fridovich I (1998). Oxygen toxicity: radicle explaination. *The Journal of Experimental Biology* **201** 1203-9.

Habig WH and Jakoby WB (1981). Glutathione S-transferases: (Rat and Human). *Methods in Enzymology* **77** 218-231.

Halliwell B (1994). Free radicles, antioxidants and human disease: curiosity, cause or cconsequences. *Lancet* **344**(8924) 721-724.
Halliwell B and Gutteridge JM (1989). *Free Radicles in Bilogy and Medicine* 2 edition (Clarendon Press, Oxford).
Hermes-Lima M and Storey KB (1998). Role of antioxidant defences in the tolerance of severe dehydration by anurans. *Molecular and Cellular Biochemistry* **189** 79-89.
Jones DP, Eklow L, Thor H and Orrenius S (1981). Metabolism of Hydrogen Peroxides in isolated hepatocytes: relative contribution of catalase and Gpx in decomposition of endogenously generated H2O2. *Archives of Biochemistry and Biophysics* **210**(2) 505-516.
Krinsky NI (1992). Mechanism of action of biological antioxidants. *Proceedings of the Society for Experimental Biology and Medicine* **200**(2) 248-254.
Kulakkatolickal LA and Kramer TD (1988). The role of air breathing in the resistance of bimodally respiring fish to waterborne toxins. *Journal of Fish Biology* **32** 119-127.
Lintermans M (2000). Recolonization by the mountain galaxias Galaxias didus of a mountain stream after the eradication of rainbow trout Oncorhynchus mykiss. *Marine and Freshwater Research* **51** 799-804.
Lohiya NK, Mishra PK, Pathak N, Manivannan B and Jain SC (1999). Reversible zoospermia by oral administration of the benzene chromatographic fraction of the chloroform extract of the seeds of Carica papaya in rabbits. *Advances in Contraception* **15** 141-161.
Meister A and Anderson ME (1983). Glutathione. *Annual Review of Biochemistry* **52** 711-760.
Murphy RB and Willis RD (1996). Sampling with Toxicants. In: *Fisheries Techniques* 2 edition (Bethseda MD: American Fisheries Society).
Neuwinger HH (2004). Plants used for poison fishing in tropical Africa. *Toxicon* **44** 417-430.
Pathak NN, Mishra PK, Manivannan B and Lohiya NK (2000). Sterility due to inhibition of sperm motility by oral administration of benzene chromatographic fraction of the chloroform extract of the seeds of Carica papaya in rats. *Phytomedicine* **7** 325-333.
Reed J, Burchad W, Hopson JA and Jenness J (1967). *Fish and Fisheries of Northern Nigeria* 1st edition (Ministry of Agriculture, Northern Nigeria, Nigeria).
Sambasivam S, Chandran S and Karpagam RG (2003). Toxicity of leaf extracts of oleander, Thevetia neriflora on tilapia. *Journal of Environmental Biology* **24** 201-204.
Sanger DA and Koehn JC (1997). Use of chemicals for carp control Proceedings of the Workshop on Controlling Carp. In: *Exploring the Options for Australia* (Australia: CSIRO Albury) 37-57
Schultes FR and Raffauf ER(1990). *The Healing Forest: Medicinal and Toxic Plants of the Northwest Amazonia, Oregon* (Oregon: Dioscorides Press).
Singh DD and Singh A (2002). Piscicidal effect of some common plants of India commonly used in freshwater bodies against target animals. *Chemosphere* **49** 45-49.
Van der O (2002). The diverse uses of fish-poison plants in Northwest Guyana. *Economic Botany* **54** 500-12.
Willmore WG and Storey KB (1997). Glutathione System and anoxia tolerance in turtles. *American Journal of Physiology* **273** R219-R225.