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Ficus Capensis Modulates Oxidative Stress Parameters in Cyanide Induced Rats

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Abstract
The sub-acute effect of *F. capensis* was assessed on the oxidative stress markers in the kidney of rats following exposure to cyanide. These effects were compared to those of sodium thiosulphate ($Na_2S_2O_3$), a classical antidote of cyanide toxicity. The rats were divided into 9 groups of 5 animals each. Group 1: rats (normal control); Group 2: rats were exposed to 3mg/kg of cyanide; group 3: cyanide induced rats that received 660mg/kg sodium thiosulphate pentahydrate and 6.6 mg/kg sodium nitrite; group 4 and 5: rats exposed to cyanide, pre-treated with 200 and 400mg/kg extract respectively; group 6 and 7: rats were exposed to cyanide, post-treated with 200 and 400 mg/kg extract respectively; group 8 and 9: rats exposed to cyanide, co-treated with 200 and 400mg/kg extract respectively. The study lasted for 28 days after which the rats were sacrificed and kidney homogenates were collected for biochemical assays. The level of malondialdehyde (MDA) in Groups 2 rats (367.99±17.73 Units/g tissue) was significantly increased in the kidney relative to the control (148.92±4.50 Units/g tissue). This was accompanied with a decrease in antioxidant enzymes Superoxide Dismutase (SOD) (7.81±2.45 Units/g of tissue), Catalase (CAT) (34.33±1.73 Units/g of tissue), compared with the control (16.18±0.42 units/g of tissue) and 64.82±8.91 units/g tissue respectively. Pre-administration of *F. capensis* extract significantly increased these antioxidant enzymes and significantly decreased the concentration of malondialdehyde in the kidney better than post and co-treatment. The results indicate that Pre-treatment with *F. capensis* extract reduced lipid peroxidation in the kidney and increased antioxidant status of animals exposed to cyanide and was more effective at 400 mg/kg dose.

Keywords: Cyanide, Ficus capensis, oxidative stress, kidney

Introduction

Cyanide is one of the most potent cytotoxic compounds known to humans and animals. Besides acute cyanide poisoning, its chronic intoxication has often been described and it has been suggested that the majority of cyanide toxicity complications are attributed to chronic exposure of this toxic compound to dietary, industrial, and environmental sources (Mathangi and Namasivayam, 2000). Scientific studies on some animal species have shown that chronic cyanide ingestion causes impaired body growth, neurological and thyroid disorders, as well as pathological effects on different tissues (Kadiri *et al*., 2020; Tulsawani *et al*., 2005; Sousa *et al*., 2002). However, the underlying mechanism by which cyanide acts injuriously on tissues is not clear, although some researchers have suggested that oxidative stress may be implicated in harmful effects of cyanide poisoning (Kadiri and Asagba 2019; Okolie and Asonye, 2004). Cyanide has hitherto been shown to cause oxidative stress and damage in a number of biological systems. Cyanide-induced oxidative stress may be due to increased levels of reactive oxygen species (ROS) and nitric oxide (Mills *et al*., 1996) as well as suppression of antioxidant systems (Ardelt *et al*., 1989) and mitochondrial function (Way, 1984).

The therapeutic use of indigenous plant products for ethno-medicinal and nutritional purposes has increased enormous interest among scientists in the search for bioactive components (Asagba *et al*., 2019; Wangensteen *et al*., 2004) that are useful to the society. Recently, interest and use of natural products from plants has grown greatly, including in areas where traditional medicines are still widely available. Medicinal plants are raw materials for the formulation of pharmaceuticals (WHO, 2014). A large proportion of medicinal plants used by rural people in Africa are affordable compared to the high cost of conventional drugs. In rural areas, the majority of people rely on conventional medicine, which also acknowledges the socio-cultural and religious history of orthodox medicine (Adesina, 2014). Studies indicates that medicinal plants contain abundant active compounds, such as nutrients and phytochemicals, and these compounds have physiological effects on humans (Olowokudejo *et al*., 2008), and these innate active ingredients are used for the treatment of various diseases (Owolabi, 2013).

Ficus capensis commonly referred to as "bush fig tree" belongs to the Moraceae family. In Nigeria, it is called Akokoro by the Igbo, Opoto (Yoruba) and the Hausa (Uwaraya) (Otitoju *et al*., 2014). F. carpensis has broad greenish leaves and fruits all through the year round. It has been used in traditionally for the treatment of epilepsy, dysentery and wound dressing (Igoli *et al*., 2005) while studies has shown that it has the following effects; blood boosting (Otitoju *et al*., 2014) anti-sickling (Umeokoli *et al*., 2013) immune-stimulatory (Daikwo *et al*., 2012) and antioxidant effect (Ramde-Tiendrebeogo *et al*., 2012) Despite all these studies carried out on *F. capensis* leaves there is little or no information on its antioxidant effect on cyanide induced stress. Hence the need to carry out this investigation.

Collection of plant material and identification

Ficus capensis leaves were harvested from natural habitat in Abraka, Delta State. The studied plant was identified and authenticated by Dr. Harrison A. Erhenhi of the Botany department, Delta State University Abraka. The leaves were properly rinsed with clean water and air-dried for about 10 days, and then milled using an automatic electrical blender (Model MS-223, China) to powdered form which was then used for extraction and treatment.

Animals

Forty-five healthy Wistar albino rats weighing between 93-160g were obtained from the Animal House, Faculty of Basic Medical Science, Delta State University, Abraka. They were acclimatized for one week. The rats were maintained on standard pellets, growers mash (Top feed, Premier feed mills Co. Ltd, Ibadan, Oyo State) and water *ad-libitum.*

Experimental design

In the experiment, a total of forty-five (45) female Wistar rats were used. They were randomly divided into 9 groups, containing 5 rats per group. The rats were acclimatized for 7 days before experimental exposure of 28 days. The animals were housed in plastic (polypropylene) cages using paddy husk bedding at room temperature ($25 \pm 1\ ^0C$) in a 12 H light/dark cycle with $50 \pm 5\%$ humility. The rats were provided with starter mash diets and water *ad-libitum*. The experimental animals were grouped as follows:

Group 1: Normal rats that received feed and water (Normal control).

Group 2: Cyanide induced rats. These rats were exposed to 3 mg/kg of cyanide at the last 14 days of exposure (Negative control).

Group 3: Cyanide induced rats that received 660 mg/kg sodium thiosulphate pentahydrate and 6.6 mg/kg sodium nitrite (Positive control).

Group 4: Pre-treatment Group. These rats were given the 200 mg/kg extract of *F. capensis* for first 2 weeks, and later exposed to cyanide for the remaining 2 weeks.

Group 5: Pre-treatment Group. These rats were given 400 mg/kg extract of *F. capensis* for first 2 weeks, and later exposed to cyanide for the remaining 2 weeks.

Group 6: Post-treatment Group. These rats were exposed to 3 mg/kg of cyanide for the first 2 weeks before administering 200 mg/kg extract of *F. capensis* for the remaining 2 weeks.

Group 7: Post-treatment Group. These rats were exposed to 3 mg/kg of cyanide for the first 2 weeks before administering 400 mg/kg extract of *F. capensis* for the remaining 2 weeks.

Group 8: Co-treatment Group. These rats were exposed to 3 mg/kg of cyanide and 200 mg/kg extract of *F. capensis* during the last 2 weeks of administration.

Group 9: Co-treatment Group. These rats were exposed to 3 mg/kg of cyanide and 400 mg/kg extract of *F. capensis* during the last 2 weeks of administration.

Both cyanide and extract administration were done orally three times a week for the consecutive durations of the study model.

Determination of biochemical parameters

Estimation of lipid peroxidation (LPO)

Malondialdehyde and other thiobarbituric acid reactive species were estimated by their reactivity with thiobarbituric acid (TBA) in acidic condition to generate a pink-coloured chromophore which was read at 535 nm in a spectrophotometer (Niehius and Samuelsson, 1968).

Determination of catalase (CAT) activity

Catalase is determined according to the method of Aebi (1974) by the depletion rate of H_2O_2 at 240 nm in a reaction buffer.

Determination of super oxide dismutase (SOD)

Super oxide dismutase (SOD) activity was determined by the method of Misra and Fridovich (1972).

Statistical analysis

All data were subjected to statistical analysis. Values were reported as Mean ± Standard deviation while one way ANOVA was used to test for differences between treatment groups. The results were considered significant at p-values of less than 0.05, that is, at 95% confidence level ($p<0.05$).

Effect of pre, co and post administration of ethanol leaf extract of *Ficus capensis* on body weight and relative kidney weight of cyanide induced female Wistar rats

The results of the effect of pre, co and post administration of ethanol leaf extract of *Ficus capensis* on body weight gain and relative kidney weight in cyanide induced female Wistar rats are shown in table 1. The results showed significant increase ($p<0.05$) in all *F. capensis* extract treated groups when compared to the negative control groups. Also, from the body weight gain, *F. capensis* treated groups recorded no significant difference ($p>0.05$) when compared to the positive control group, excluding group 5 and 9, which showed significant decrease. There were no significant differences ($p>0.05$) on the relative liver weights across all treated groups (excluding group 6 and 8) when compared with the negative control group. Also, the treated groups showed no significant difference ($p>0.05$) on the relative liver weights (excluding group 6 and 8) when compared with the positive control group.

Table 1: Body weight gain and relative kidney weight of cyanide induced female Wistar rats administered with ethanol leaf extract of *Ficus capensis*

Groups	Body Weight Gain (%)	Relative organ Weight (%)
1	21.86 ± 6.21[a]	3.44 ± 1.08[a]
2	8.60 ± 11.04[b]	3.22 ± 0.61[a]
3	28.04 ± 17.18[c]	3.86 ± 0.73[a]
4	34.98 ±1.76[c]	3.72 ± 0.17[a]
5	25.40 ± 8.84[a]	3.29 ± 0.46[a]
6	33.71 ± 10.97[c]	4.51 ± 0.45[b]
7	38.78 ± 17.89[c]	3.68 ± 0.87[a]
8	29.47 ± 11.10[c]	4.08 ± 0.80[b]
9	20.55 ± 5.95[a]	3.49 ± 0.42[a]

Values are presented as Mean ± Standard Deviation of triplicate determinations. Values on the same column with different superscripts differ significantly (p<0.05).

Effect of pre, co and post administration of ethanol leaf extract of *Ficus capensis* on hepatic oxidative stress markers in cyanide induced female Wistar rats

The results of the *in-vivo* study of pre, co and post administration of ethanol leaf extract of *Ficus capensis* on oxidative stress markers in female Wistar rats are shown in table 4.3. The activities of SOD and CAT in cyanide treated group was significantly reduced ($p<0.05$) when compared with the control group. However, a significant increase in SOD and CAT activities was recorded in all treated groups when compared with the untreated group (negative control), excluding group 8 which showed no significant difference in SOD activity, also group 4 and 6 which showed no significant difference in catalase activity. A significant increase in MDA level was recorded in cyanide treated group when compared with the control. However, a significant decrease in MDA level was observed in all treated groups when compared with the negative control.

Table 2: Renal oxidative stress markers in cyanide induced female Wistar rats administered with ethanol leaf extract of *Ficus capensis*

GROUPS	SOD (Units/g tissue)	CAT (Units/g tissue)	MDA (Units/g tissue)
1	16.18 ± 0.42^{a}	64.82 ± 8.91^{a}	148.92 ± 4.50^{a}
2	7.81 ± 2.45^{b}	34.33 ± 1.73^{b}	367.99 ± 17.73^{b}
3	15.81 ± 3.11^{a}	55.56 ± 6.94^{a}	292.63 ± 5.28^{c}
4	7.61 ± 1.76^{b}	37.04 ± 8.91^{b}	248.75 ± 12.55^{d}
5	15.06 ± 3.01^{a}	55.82 ± 10.39^{a}	247.19 ± 2.77^{d}
6	12.11 ± 2.03^{a}	37.04 ± 8.91^{b}	258.94 ± 7.83^{d}
7	16.78 ± 2.94^{a}	47.30 ± 9.28^{c}	313.78 ± 9.67^{e}
8	9.93 ± 1.06^{b}	55.56 ± 0.00^{a}	327.27 ± 15.88^{e}
9	10.51 ± 1.67^{ab}	46.30 ± 8.91^{c}	335.33 ± 17.90^{e}

SOD = Superoxide dismutase, CAT = Catalase, MDA = Malondialdehyde.
Values are presented as Mean ± Standard Deviation of triplicate determinations. Values on the same column with different superscripts differ significantly ($p<0.05$).

Discussion

The present study revealed the subacute effect of *Ficus capensis* on renal oxidative stress markers of Wistar rats exposed to cyanide poisoning. Supplementation with dietary antioxidants is a promising means to strengthen antioxidant defense and oxidative damage repair systems. Currently, phytochemicals play a leading role due to their potency in the reduction of oxidative stress in vivo (Amadi *et al*., 2016). *Ficus capensis* had been shown to possess phytochemicals, most especially flavonoids (Njoku *et al.,* 2016).

Oral administration of 3 mg/kg KCN or more has been shown to result in decreased water and food consumption by rats. This suggests poor palatability (Tulsawani *et al.,* 2005). The study shows that Potassium cyanide solution fed to rats by oral gavage at 3mg/kg body weight decreased food body weights significantly ($p<0.05$) when compared with the control group. Thus, cyanide toxicity, even at this concentration and method of ingestion, may cause reduced appetite or palatability in animals. This study has shown a reduction in weight gain and indeed weight loss in rats given 3mg KCN/kg body weight by gavage for 28 days. The study also shows that weight loss due to cyanide toxicity was reversed by *F. capensis* as shown in all the treated groups. This observation is understandable as it is possible that the plant extracts restored the appetite of the rats for food. Cyanide has been shown from previous studies to induce oxidative stress in different organism such as in birds (Kadiri and Asagba 2019), rats (Kadiri *et al*, 2020) by increasing reactive oxygen species (Kadiri and Ekayode 2019; Mills *et al*., 1996) as well as the inhibition of antioxidant systems (Ardelt *et al*., 1989). Elevated levels of reactive oxygen species give rise to lipid peroxidation (Kadiri *et al*., 2020) that culminates in oxidative stress in tissues (Okoro *et al*., 2019; Liu and Mori, 1994). An increase in lipid peroxidation has been reported for rats exposed to cyanide (Kadiri *et al*., 2020). During the lipid peroxidation process, the activities of different membrane-bound enzymes are altered (Thomas and Poznasky, 1990; Kukreja *et al*., 1988), resulting in the degeneration of cell membrane (Reiter, 1995), which predisposes the affected organisms to a wide array of disease processes (Morris *et al*., 1995). This could possibly explain the mechanism of the plant's action to mop up free radicals produced as a result of oxidative damage caused by cyanide poisoning in all treated groups.

The results of the study had also shown the activities of SOD and catalase were significantly decreased in the tissues of rats exposed to cyanide only after 28 days treatment (Tables 2) which may account for the corresponding increase in lipid peroxidation. Although an increase in the production of reactive oxygen species during stress can result in damage to cells, there is an in-built mechanism present in animals that helps to mitigate and mop up the reactive oxygen species. This is due to the presence of some antioxidant enzymes such as superoxide dismutase (SOD) and catalase, that mops up these reactive oxygen species under natural conditions thereby protecting the cells. Any damage or inhibition of any one of these enzymes will therefore compromise the defensive mechanisms of the cell. When an imbalance between free radicals and antioxidants occurs in favor of free radicals, an oxidative stress will be induced which can lead to chronic permanent damage (Halliwell, 1989). Increased generation of ROS has been implicated in the pathogenesis of several diseases and in the toxic effects of a wide variety of compounds (Okoro and Kadiri, 2019).

The decreased in lipid peroxidation across treated groups is associated with increased activities of the antioxidant enzymes-catalase and SOD which was more pronounced in the pre-treatment of 400mg/kg *F. capensis* extract, than in animals co-administered/post-administered with 200mg/kg or 400mg/kg. *F. capensis* had been shown to possess phytochemicals, most especially flavonoids (Uzoekwe and Mohammed, 2015). This could be linked to the prophylactic actions of bioactive compounds contained in the leaf extract at steady state. Hence, pre-treatment with *F. capensis* extract is more effective compared to other mode of treatments as demonstrated in this study.

Conclusion

The present study has demonstrated that administration of *F. capensis* extract to rat may effectively prevent tissue damage or oxidative stress caused by cyanide in rats. Thus, the antioxidant effect of this flavonoid rich extract from *F. capensis* is reflected in the present study. This effect was more pronounced at 400 mg/kg pre-administration.

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