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POTENCY OF AQUEOUS LEAF EXTRACT OF TELFAIRIA OCCIDENTALIS ON NEVIRAPINE INDUCED LIVER DAMAGE IN RATS

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Abstract

The potency of aqueous leaf extract of *Telfairia occidentalis* on Nevirapine (an anti-retroviral drug) induced liver damage was carried out in Wister albino rats. Forty (40) rats were randomly distributed into 8 groups of 5 rats each. Group 1 served as the normal control and was administered with distilled water only, while group 2 served as test control. Groups 2 to 8 were administered with Nevirapine 6mg/kg for six weeks to induce liver damage after which groups 3 to 8 were administered with 100, 200mg/kg of the aqueous T.occidentalis extract respectively for seven days. Groups 5 to 8 were co-administered with Nevirapine and the same doses of the aqueous vegetable extract for six weeks. Administration of the drug and the aqueous vegetable extracts was done orally. At the end of the treatments, serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase as well as malondialdehyde, total and direct bilirubin concentrations were determined. The result showed that the group administered Nevirapine alone (test control) has significant increases ($P < 0.05$) in the above biochemical parameters compared with normal

KEYWORDS: *Liver damage, leaf extract, Potency, Telfairia occidentalis, Nevirapine*

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control. This shows that Nevirapine administration for 6weeks has induced hepatotoxicity. Administration of aqueous T.occidentalis extracts to groups 3 to 8 resulted in significant decreases in the liver enzyme activities and bilirubin concentrations compared with the test control group. The result of co-administration also showed significant decreases ($P < 0.05$) in the above biochemical parameters. Results f this study indicates that the aqueous vegetable extracts can provide both curative and protective effects against Nevirapine hepatotoxicity.

Introduction

Plants have played a very important role in healing. For the early people, they came easily to hand and were intricately connected to diet and healing. Through observation and experimentation, they learned which plants promoted health and well-being. It is estimated that there are between 200,000 and 700,000 species of tropical flowering plants that have medicinal Properties (Atta and Ajewale, 2003). Their actions include: antibacterial, antifungal, antiviral, antihelmintic, anti allergic, anti carcinogenic, antitoxic and larvicidal. These medicinal value lie in some chemical substances they contain (Oladunmoye, 2007).

Hepatotoxicity is a significant increase in serum Alanine aminotransferase (ALT) and / or Aspartate aminotrasferase (AST) levels. It can also be defined as a chemical-driven liver damage (Friedman,2003).The liver plays a vital role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Certain medicinal plants, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Other chemical agents and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver toxicity are called hepatotoxins (Friedman, 2003).

Nevirapine is an antiretroviral drug which belongs to a class of Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) that is used for the management as well as for the prevention of mother-to-child transmission of the human immunodeficiency virus (HIV). Unfortunately, its adverse effects, mainly hypersensitivity, skin reactions and hepatotoxicity, have

hampered the use of Nevirapine. Since Nevirapine-induced hepatotoxicity commonly occurs between 2-12 weeks of treatment, and Nevirapine is a known inducer of human Cytochrome P450 (CYP3A and CYP2B6) isozymes, it was envisaged that the hepatotoxicity was due to activation of Nevirapine to toxic metabolites by the induced enzymes (Monforte *et al*.,2001).
Human immunodeficiency virus (HIV)-infected patients frequently present with elevated levels of serum transaminases ALT and/or AST. This has often been attributed to the hepatic effects of antiretroviral (ARV) drugs, including non nucleoside reverse-transcriptase inhibitors (NNRTIs). A review of cohort studies investigating the incidence of hepatotoxicity among patients receiving ARV therapy suggests that the overall rate of ALT and/or AST elevations is similar among all ARVs (Dieterich *et al.*, 2004).
Telfairia occidentalis is one of the commonest leaf vegetables consumed in Nigeria. It belongs to a larger family known as Cucurbitaceae. The Cucurbitacea is reported to have been associated with man since 12,000 BC(Sha *et.al*.,2000) and its species are cultivated across lowland humid Tropics of West Africa, mainly because of their nutritional value (Puoti *et al.,* 2001). The fruits of *T.occidentalis* are inedible but the leaves and seeds (without hulls) contain 21.2% and 35.7% protein respectively and are edible to both man and animals (Leitze *et al.,*1998, Pollard *et al.,* 1998) reported that the oil extracted from pumpkin contain a high degree of unsaturation compared to palm oil and is a better alternative for cooking and margarine production. The pharmacologic importance of this family of plants are ample. Momocharin and luffaculin are two abortif proteins isolated from this family with ribosome- inhibition properties which have been used to induce second trimester abortion(Leitze *et al*.,1998).A third proteins trichosanthin (from Tiichosanthe species), has demonstrated the capacity to inhibit the multiplication of human immunodeficiency Virus (HIV) within lymphocytic and phagocytic cells (Leitze *et al.*,1998). Similarly, the aqueous extract of *T. occidentalis* has been shown to be hepatoprotective against garlic-induced oxidative stress while its ethanolic extract has demonstrated hypoglycaemic properties (Haehl, 2001).

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MATERIALS AND METHODS

MATERIALS

GLASSWARES AND REAGENTS

Glasswares used are of standard quality and all the reagents are of analytical grade.

EXPERIMENTAL ANIMALS

Fifty four (54) wistar albino Rats of both sexes were obtained from biological Sciences Department, Bayero University Kano. The animals were kept in cages in the animal house of the same department, and had free access to normal diet (Growers marsh, Vital feeds Grand Cereals and Oil Mills Ltd) and water.

DRUG

Nevirapine (NVP) tablets (200mg each) manufactured by Aurobindo pharmaceutical Limited India were obtained from Sadiq Wali Department of Pharmacy, Aminu Kano Teaching Hospital, Kano- Nigeria.

METHODS

EXTRACTS PREPARATION

The plant materials were obtained from Tarauni Market in Nassarawa Local Government Area of Kano State. They were authenticated at the Botany unit of Bayero University Kano. The leaves were dried and ground to powder using pestle and mortar. The Vegetable powder (40g) was dissolved in 500cm^3 of distilled water and filtered after 24 hours. The residues were dried. The difference between the original weight and final weight was found to be the concentration of the extract. The concentration for *T. occidentalis* extract was found to be 0.07g/cm^3.

The volume of the extract (filtrate) to be administered was based on the weights of the rats. The amount to be given to the rats will be obtained using the following formula;

$$\text{Volume (ml)} = \frac{\text{weight of rats (kg) x dose/kg}}{\text{conc.of extract mg/ml}}$$

The Nevirapine solution administered was prepared by dissolving one tablet (200mg) in 20ml distilled water. The volume of NVP solution to be

administered will be determined based on the weight of the rats using the formula;

$$\text{Volume (ml)} = \frac{\text{weight of rats (kg) x 6mg/kg}}{\text{conc.of Nevirapine mg/ml}}$$

STATISTICAL ANALYSIS

Data is presented as mean± standard deviation. The significant difference between the means groups was assessed by one-way ANOVA. The level of significance is set at $p<0.05$.

RESULTS AND DISCUSSION

RESULTS

Results of Biochemical Analysis.

The results of the potency of T *.occidentalis* aqueous vegetable extract against Nevirapine induced hepatotoxicity in rats (Table 1) showed significant decreases ($p<0.05$) in the activities of AST, ALT, ALP, and the concentrations of MDA, total and direct bilirubin in the serum compared with test control after the administration of 100 and 200mg per kilogram body weight of the vegetable extract for seven days following the induction of liver damage with Nevirapine.

Table 1: Effect of oral administration of *T.occidentalis* aqueous vegetable extracts on Nevirapine induced liver damage in rats.

DOSE	AST (U/l)	ALT (U/l)	ALP (IU/L)	T.BIL (umol/l)	D.BIL (umol/l)	MDA (nmol/l)
0 (Normal control)	18.33 [a] ± 0.57	12.00[a] ± 1.00	37.06[a] ± 2.69	16.62[a] ± 0.41	7.25[a] ± 0.49	3.74 [a] ± 0.56
(Test Control) 6mg/kg NVP	121.67 ± 1.53	130.67 ± 5.13	149.50 ± 1.35	86.71 ± 2.78	21.36 ± 0.90	10.24[+] ± 0.35
100mg/Kg T. Occidentalis	23.67[a] ± 2.52	22.67[a] ± 2.52	40.93[a] ± 0.80	28.34[a] ± 0.79	10.33[a] ± 0.97	9.70[+] ± 0.10

200mg/Kg T. Occidentalis	33.67[a] ± 3.06	21.67[a] ± 1.53	45.75[a] ± 1.80	49.29[a] ± 0.93	25.29[a] ± 4.59	9.27[+] ± 0.21

Values are represented as mean ± SD (n=5)

[a] significantly different from the test control

+ significantly different from the normal control

In Table 2, the vegetable extract were administered concurrently with the Nevirapine; significant decreases ($p<0.05$) in the activities of AST, ALT, ALP, and the concentrations of MDA, total and direct bilirubin in the serum compared with test control after the administration of 100 and 200mg per kilogram body weight of the vegetable extract with Nevirapine drug concurrently for six weeks were obtained.

Table 2: Effect of oral co-administration of Nevirapine with aqueous vegetable extracts of *T. occidentalis* in rats.

GROUP/ TREATMENT	AST (U/L)	ALT (U/L)	ALP (IU/L)	D.BIL (umol/l)	T.BIL (umol/l)	MDA (nMol/ml)
0 (Normal control)	18.33[b] ± 0.58	12.00[b] ± 1.00	37.06[b] ± 2.69	7.25[b] ± 0.49	16.62[b] ± 0.41	3.74[b] ± 0.56
(Test Control) 6mg/kg NVP	121.67 ± 1.53	130.67 ± 5.13	149.5 ± 1.35	21.36 ± 0.90	86.71 ± 2.78	10.24 ± 0.3525
6mg/kg NVP + 100mg/kg T.occidentalis	17.00[b] ± 1.00	12.33[b] ± 1.16	36.19[b] ± 0.89	8.13[b] ± 0.90	16.81[b] ± 1.36	5.00[b] ± 0.10
6mg/kg NVP+ 200mg/kg T.occidentalis	19.67[b] ± 2.52	13.67[b] ± 1.16	37.17[b] ± 2.52	6.11[b] ± 1.01	16.99[b] ± 0.79	5.50[b] ± 0.26

Values are represented as mean ±(SD= 5)

[b] significantly different from the test control

+ significantly different from the normal control

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HISTOPATHOLOGICAL RESULT

Plate 1: Shows normal liver cells (hepatocytes) after the administration of water only .

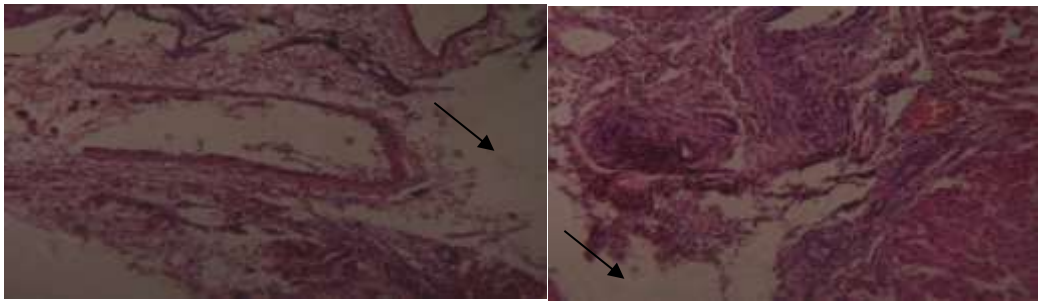

Plate 2: shows severe fibrosis and liver damage after the administration of 6mg/kg Nevirapine only for 6 weeks to induce a liver damage.

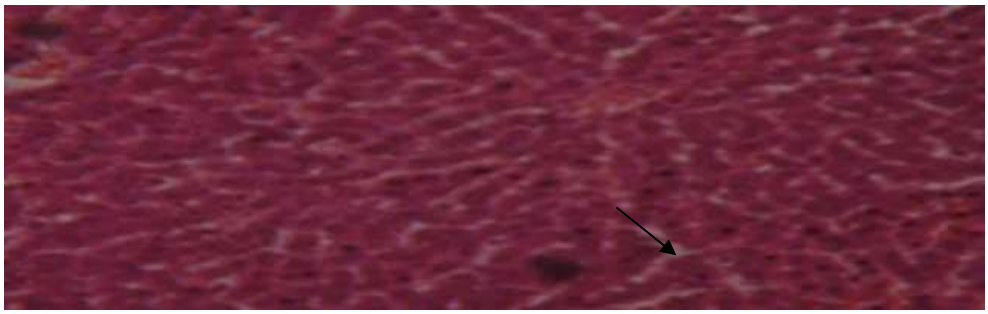

Plate 3: shows moderate fat cells after the administration of 6mg /kg Nevirapine for six weeks and then 100mg/kg *Telfairia occidentalis* for 7 days.

DISCUSSION

The results in Table 1 showed increased activities of ALT, AST, ALP and bilirubin concentrations in serum of the Nevirapine induced rats which might be due to the leakage of the biochemical marker enzymes and bilirubin in the serum. Generally, measurement of ALT, AST and ALP are commonly used as marker enzymes of hepatotoxicity (Yanpallewar *et al.,* 2002; Yen *et al.,* 2007). The significant decrease in the levels of biochemical marker enzymes like ALT, AST, ALP and bilirubin in vegetable extract

administered rats might be due to decreased leakage of the enzymes in liver cells. This suggests that the vegetable extracts could cure the hepatic injury and/or restore the cellular permeability, thus reducing the toxic effect of Nevirapine induced liver toxicity and preventing enzymes leakage into the blood circulation. Other investigators have reported similar observations on the effects of vegetable extracts on liver damage (Molina *et al.,* 2003; Ozaras *et al.,* 2003; Uzun *et al.* ,2003).

In Table 2 there was significant difference (P<0.0001) between the test control and the normal control for serum alanine aminotransferase. Hepatotoxicity is ascertained if there is an increase in ALT three times its level at the start of study (Hosein, 2001) as seen in the control group. This indicates that daily dose of Nevirapine 6mg/kg administered for six (6) weeks has induced hepatotoxic effect on the rats since higher ALT and AST activities are indicators of liver damage or disorder (Kaplan *et al.,* 1995). But as a result of oral co-administration of *Telfairia occidentalis* there was decrease in level of ALT activity. It has been found that there is no difference (P<0.05) between groups administered with 100mg/kg and 200mg/kg of the vegetable extracts.

For serum aspartate aminotransferase, it was found that there was significant difference (P<0.0001) between the test control and the normal control with other groups having no statistically different values at P<0.05 when compared with the normal control. Damage to the structural integrity of the liver is reflected by an increase in the level of serum transaminases (Schmidt and Schmidt, 1963) as observed in test control group because these are cytoplasmic in location and are released into the circulation after cellular damage (Sallie *et al.,*1991). From this table, it has been found that there was no significant difference between the normal control and the extract co-administered groups.

It has been found that there was significant difference (P<0.001) between the test control and the normal control for alkaline phosphatase. But there was significant lower value at P<0.001 between the test control and the extract co-administered groups, since liver ALP is a non-plasma specific enzyme that is secreted from the sinusoidal surface of the liver cell and thus

is present in the serum at low levels in the absence of liver damage (Price and Stevens,1989).

There was significant difference for total bilirubin (P<0.001) between the normal control and the test control. But there was no significant difference between the normal control and the extract co-administered groups at P<0.05.However there was significant difference (P<0.001) between the test control and the extract co-administered groups.

There was significant difference (P<0.001) between the normal control and the test control for direct bilirubin. But there was no significant difference between the normal control and the extract co-administered groups at P<0.05. And there was significant difference (P<0.001) between the test control and extract co-administered groups.

For malondialdehyde,the result showed a significant difference (P<0.001) between the test control and the normal control. There was also significant decrease in the MDA concentration (P<0.001) between the co-administered groups and the test control. Concurrent administration of the vegetable extracts to Groups 11-18 preserved the histological structure of the liver (plates 11-18) by significantly reducing the scores of degeneration, necrosis and fibrosis with evidence of significant regeneration. This was similar to the findings of Mateenuddin *et al.* (2000) on the effect of *Ocimum sanctum* leaf extract on hepatotoxicity induced by anti-tubercular drugs in rats.

This indicates that administration of Nevirapine 6mg/kg to the rats results in the development of oxidative stress which has been reduced to near normal by the co-administration of the vegetable extracts, which are high in phytochemicals and minerals. This is similar to the findings of Fraga *et al.*(1987) where the high significant elevation of MDA level in liver homogenate of rats treated with CCl_4 indicated excessive formation of free radicals and activation of lipid peroxidation of the hydrophobic core and cell damage. Also the administration of different antioxidants together with CCl_4 stimulated the antioxidant protective mechanisms against CCl_4 free radicals by reducing MDA level and elevating the level of reduced glutathione as well as the activities of both SOD and catalase enzymes in liver homogenate.

In general, results from this study showed that rats administered with 6mg per kilogram body weight of Nevirapine i.e the test control have significant increases (P<0.001) in serum ALT,AST,ALP activities and total bilirubin concentrations in comparison with the normal control. Increased activity of alkaline phosphatase, a marker enzyme for plasma membrane and endoplasmic reticulum (Wright and Plummer, 1974) observed after the Nevirapine administration can be employed to assess the integrity of plasma membrane (Akanji *et al*.,1993),since it is localised predominantly in the microvilli of the bile canaliculi located in plasma membrane. Increases in the activities of the other marker enzymes, aspartate and alanine aminotransferases after the Nevirapine administration can be used to assess and monitor liver damage (Wada and Snell,1962).Their presence in the serum may give information on organ dysfunction (Wells *et al*.,1986).These increases in the enzyme activities and total bilirubin concentrations might be due to the fact that the daily dose of 6mg/kg NVP administered for six (6) weeks has induced hepatotoxic effect since higher ALT and AST activities are indicators of liver damage (Kaplan *et al*.,1995).A similar result indicates the restorative ability of *T. occidentalis* in treatment of *Ocimum sanctum* induced liver damage in rats. The results showed no significant difference (P<0.05) between the treatment group and the control but the authors reported that *Telfairia occidentalis* extract may be beneficial in the management of cholesterolemia, liver problems and impaired immune systems (Eseyin *et al*., 2005).The antioxidant capabilities of *Telfairia occidentalis* were earlier demonstrated by Ajebesin *et al*., (2002), who reported that the vegetable helps to restore damage to liver cells, protect the heart and enhance youthfulness. It was reported to have protective role against liver damage caused by agents like drugs and other toxins. The results of this research work further confirms the findings of Ajebesin *et al*.(2002), on *T.occidentalis.*

CONCLUSION

It has been found out that aqueous extracts of *T.occidentalis* can provide curative and protective effects against Nevirapine hepatotoxicity.

Therefore HIV Patients on Nevirapine therapy are adviced to take the drug along with T.occidentalis regularly as part of diet.

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