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Hepatoprotective and Antioxidant Activities of Methanol Root Extract of *Sphenocentrum jollyanum* on Rifampicin Induced Liver Damage in Wistar Rats

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ABSTRACT

The methanol root extract of *Sphenocentrum jollyanum* was investigated for hepatoprotective and antioxidant activities against rifampicin-induced hepatic damage in Wistar rats. Thirty male Wistar rats were randomized into six groups of five rats each. Hepatotoxicity was induced by administering rifampicin (50mg/kg) orally and methanol root extracts (50, 100, and 200mg/kg) were administered orally to the rats one hour before rifampicin induction. The treatment lasted for 28 days after which the animals were sacrificed and blood and liver were collected for biochemical and histological studies. Administration of methanol root extract at 50, 100, and 200mg/kg showed a significant ($P<0.05$) reduction in ALT, AST, ALP, and total bilirubin levels in a dose-dependent manner with 50mg/kg having the least therapeutic effect while 200mg/kg showed a highest therapeutic effect. It was observed that the administration of the root extract showed a significant ($P<0.05$) increase in total protein and albumin. There was also a significant ($P<0.05$) increase in the activity of superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx). However, there was a significant ($P<0.05$) decrease in malondialdehyde (MDA) level of the extract-treated group when compared to the rifampicin induced untreated group The changes in the biochemical parameters were supported by histological profile. These results indicated that the methanol root extract of *Sphenocentrum jollyanum* possessed hepatoprotective activity against rifampicin-induced liver damage and this effect may be due to its strong antioxidant property.

INTRODUCTION

The liver is the chief metabolizing organ in the body and as such any damage done to this organ will affect metabolism[1]. Liver diseases such as hepatitis and necrosis are caused by drugs, toxic chemicals, excess consumption of alcohol, infections, and autoimmune disorders[2]. Drug-induced hepatic injury is the most common reason for the withdrawal of many approved drugs[3]. Most hepatotoxic substances including drugs damage liver cells by inducing lipid peroxidation and other oxidative stress in the liver[2]. Free radicals such as superoxide, hydroxide radicals, nitric oxide, and other reactive species (hydrogen peroxide, hypochlorous acid, and peroxynitrite) produced during aerobic metabolism in the body can cause oxidative damage to amino acids, lipids, proteins, and DNA[4]. Free radicals can cause oxidative damage to amino acids, lipids, proteins, and DNA[4,5]. The most effective way to eliminate free radical which causes oxidative stress is with the help of antioxidants[3].

Antioxidants may protect the body against ROS toxicity either by preventing the formation of ROS, by the interruption of ROS attack, or by scavenging the reactive metabolites or converting them to less reactive molecules[6]. When oxygen traps a singlet electron, it becomes unstable and thus very reactive since it generates harmful chain reactions against many biological molecules.

Herbs and spices are recognized as sources of natural antioxidants that can protect man from oxidative stress and thus play an important role in the chemo-protection of diseases that have their etiology and pathophysiology in reactive oxygen species[7]. Various plants have been used effectively as hepatoprotective agents. *Sphenocentrum jollyanum* is a plant that has wide therapeutic value in traditional medicine.

Traditionally the plant is used as a remedy for feverish conditions, cough, and wound dressing and as an aphrodisiac[8,9]. Studies have shown the leaf possesses significant antipyretic and analgesic activities[10]. The roots and leaf have been reported to be active[11]. The roots are bright yellow with a sour taste while the ovoid-ellipsoid bright yellow or orange fruits occur in clusters and are edible when ripe[12]. In Nigeria, the roots of *Sphenocentrum jollyanum* are used as chewing sticks, as relief for constipation, and as a cure for stomach problems.

In this present study, we assessed the hepatoprotective and antioxidant activities of *Sphenocentrum jollyanum* root against rifampicin-induced hepatotoxicity in rats.

METHODOLOGY

Plant sample collection and identification

The roots of *Sphenocentrum jollyanum* Pierre Menispermaceae were collected from Agbo-ihegboma forest in Ekwusigo LGA of Anambra State, Nigeria, and identified in the herbarium unit in the Department of Biological Sciences Ahmadu Bello University, Zaria Nigeria with voucher number 3290.

Experimental animals

Male Wistar rats (7 – 8 weeks old) weighing between 150-200g were used for the experiment. The rats were kept and maintained in well-ventilated cages under standard laboratory conditions, that is the temperature and relative humidity was maintained at 25^0C and 50%, respectively. Light and dark cycles were maintained at 12h each. They were maintained on grower's mash (Vital Feeds Nigeria Ltd) and provided with water *ad libitum*. They were allowed to acclimatize to the laboratory conditions for two weeks before the experiment.

Plant preparation and extraction

The roots and leaf of *Sphenocentrum jollyanum* were washed with tap water; shade dried for about two weeks and pulverized using mortar and pestle. Two hundred and fifty grams of the root was also placed in another glass jar and soaked in 500ml of methanol. The jar was kept in a room with the lid tightly closed and the mixture was stirred 3-4 times daily. This type of cold maceration was carried out for 15 days[13]. The resulted in dark brown methanol extract was filtered using Whatman filter paper No 1 and dried using a rotary evaporator under reduced pressure at 45^o C. The yield of 39g of the root, was stored in a refrigerator at 4^o C until it was needed[13]. An aliquot portion of the crude extract was dissolved in distilled water for use on each day of the experiment.

Animal grouping and treatment

Thirty male Wistar albino rats were dived into six groups of five rats each.

Group 1: Normal control (NC) rats given feed and water only

Group 2: Normal rats that received 50mg/kg body weight rifampicin and were not treated (RF).

Group 3: Normal rats treated with 50mg/kg body weight root extract of *Sphenocentrum jollyanum* and after 1 hour received 50mg/kg body weight rifampicin (R_{50} + RF).

Group 4: Normal rats treated with 100mg/kg body weight root extract of *Sphenocentrum jollyanum* and after 1 hour received 50mg/kg body weight rifampicin (R_{100} + RF).

Group 5: Normal rats treated with200mg/kg body weight root extract of *Sphenocentrum jollyanum* and after 1 hour received 50mg/kg body weight rifampicin (R_{200} + RF).

Group 6: Normal rats were treated with vitamin E (100mg/kg) and after 1 hour received 50mg/kg body weight rifampicin (Vit E + RF).

Induction of experimental hepatotoxicity

Rifampicin was prepared in sterile distilled water. Rifampicin-induced liver damage was achieved by administering the rats with a 50mg/kg dose of rifampicin orally through an insulin syringe for 28 days using the method as described byJehangir[14]. For hepatoprotective studies *Sphenocentrum jollyanum* extracts were administered orally to the rats 1hour before rifampicin doses were according to the method of Pal[15].

Statistical Analysis

Data obtained were expressed as mean ± SD. The data were statistically analyzed using analysis of variance (ANOVA). The difference between the various extracts and animal groups was compared using the Duncan Multiple Range Test. The values of $p<0.05$ were considered significant[16].

RESULTS AND DISCUSSION

The result of phytochemical screening of methanol root extract of *Sphenocentrum jollyanum*

Revealed the presence of carbohydrates, cardiac glycoside, flavonoids, and alkaloids in the methanol root extract (Table 1).

LD50 result

Results of the lethal dose (LD50) for methanol leaf extract of *Sphenocentrum jollyanum* showed that no death was recorded up to 5000 mg per kg body weight. Therefore, the LD50 is greater than 5000 mg/kg body weight.

Table No. 1: Phytochemical screening result

Test	Observation
Carbohydrate	+
Anthraquinone	-
Cardiac glycosides	+
Saponins	-
Flavonoids	+
Tannins	-
Alkaloids	+
Triterpenes	-

+ = Present, - = Not detected

Table No. 2: Effect of Methanol Root Extract of *Sphenocentrum jollyanum* on some Liver Marker enzymes

GROUPS (n-5)	Serum AST (U/L)	Serum ALT (U/L)	Serum ALP (U/L)
NC	18.80□□ 2.28^{a}	37.60□□ 3.85^{a}	56.20□□ 7.46^{a}
RF	71.20□□ 9.63^{c}	97.20□□ 7.56^{c}	101.40□□ 10.78^{c}
R_{50} + RF	25.80□□ 3.70^{b}	42.60□□ 4.21^{a}	72.40□□ 7.77^{b}
R_{100} + RF	24.20□□ 2.80^{b}	41.00□□ 3.40^{a}	71.60□□ 14.77^{b}
R_{200} + RF	22.60□□ 3.40^{ab}	32.80□□ 5.40^{a}	63.40□□ 6.88^{ab}
Vit E + RF	22.40□□ 3.05^{ab}	42.60□□ 3.51^{ab}	61.60□□ 8.38^{ab}

Values are means of five determinations + SD. Values with different superscripts down the column are significantly different ($p<0.05$).NC: Normal rats control, RF: Normal rats + Rifampicin (50mg/kg), RF + Vit E: Normal rats + Rifampicin (50mg/kg) + Vitamin E (100mg/kg), RF + R50: Normal rats + Rifampicin (50mg/kg) + root extract (50mg/kg), RF + R100: Normal rats + Rifampicin (50mg/kg) + root extract (100mg/kg): RF + R200: Normal rats + Rifampicin (50mg/kg) + root extract (200mg/kg). AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase.

Table No. 3: Effects of Methanol Root Extract of *Sphenocentrum jollyanum* on Total Protein and Albumin

GROUPS (n=5)	Total protein(g/l)	Albumin (g/l)
NC	76.00□□ 6.20^{c}	40.80□□ 4.10^{b}
RF	48.40□□ 4.30^{a}	28.60□□ 3.70^{a}
R_{50} + RF	65.80□□7.50^{b}	32.60□□ 3.80^{ab}
R_{100} + RF	66.20□□ 4.20^{b}	35.40□ 4.20^{bc}
R_{200} + RF	67.20□□ 4.50^{b}	36.40□□ 4.10^{bc}
Vit E + RF	73.60□□ 8.70^{bc}	39.00□□ 5.70^{c}

Values are means of five determinations + SD. Values with different superscripts down the column are significantly different ($p<0.05$).NC: Normal rats control, RF: Normal rats + Rifampicin (50mg/kg), RF + Vit E: Normal rats + Rifampicin (50mg/kg) + Vitamin E (100mg/kg), RF + R50: Normal rats + Rifampicin (50mg/kg) + root extract (50mg/kg), RF + R100: Normal rats + Rifampicin (50mg/kg) + root extract (100mg/kg): RF + R200: Normal rats + Rifampicin (50mg/kg) + root extract (200mg/kg). AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase.

Table No. 4: Effect of Methanol Root Extracts of *Sphenocentrum jollyanum* on some Serum Antioxidants

GROUPS (n-5)	MDA (nmol/mg protein)	SOD (U/ml)	Catalase (Umol/min/ mg protein)
NC	1.46 □0.54^{a}	1.58 □ 0.56^{b}	49.20□ 8.32^{b}
RF	2.62 □0.41^{b}	0.80 □ 0.25^{a}	38.80 □□8.38^{a}
R_{50} + RF	1.70 □ 0.40^{a}	1.30 □□0.47^{b}	38.00 □ 7.87^{ab}
R_{100} + RF	1.62 □□ 0.38^{a}	1.38 □ 0.43^{b}	39.00 □ 8.00^{ab}
R_{200} + RF	1.52 □ 0.36^{a}	1.42 □□0.36^{b}	44.00 □ 7.52^{ab}
Vit E + RF	1.48 □ 0.62^{a}	1.50 □ 0.38^{b}	48.40 □ 7.13^{b}

Values are means of five determinations + SD. Values with different superscripts down the column are significantly different ($p<0.05$).NC: Normal rats control, RF: Normal rats + Rifampicin (50mg/kg), RF + Vit E: Normal rats + Rifampicin (50mg/kg) + Vitamin E (100mg/kg), RF + R50: Normal rats + Rifampicin (50mg/kg) + root extract (50mg/kg), RF + R100: Normal rats + Rifampicin (50mg/kg) + root extract (100mg/kg): RF + R200: Normal rats + Rifampicin (50mg/kg) + root extract (200mg/kg). AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase.

Table No. 5: Effect of Methanol Root extracts of *Sphenocentrum jollyanum* on Liver Homogenate Antioxidants

GROUPS (n=3)	MDA (nmol/mg protein)	Catalase (Umol/min /mgprotein)	SOD (U/ml)	GPX (Umol/min/mgprotein)
NC	2.13 □ 0.51^{a}	49.67 □ 7.23^{b}	2.63 □ 0.55^{b}	52.00 □ 8.71^{b}
RF	3.20 □ 0.62^{b}	25.67 □7.37^{a}	1.17 □ 0.32^{a}	34.00 □ 6.00^{a}
R_{50} + RF	2.57 □ 0.51^{ab}	41.00 □ 9.64^{b}	2.00 □ 0.44^{ab}	47.67 □ 8.74^{b}
R_{100} + RF	2.43 □ 0.51^{ab}	43.00 □ 6.24^{b}	2.33 □ 0.42^{b}	48.00 □ 7.55^{b}
R_{200} + RF	2.20 0.44^{ab}	44.00 □ 7.21^{b}	2.53 □ 0.50^{b}	48.67 □ 8.50^{b}
Vit E + RF	2.20 □ 0.61^{ab}	47.00 □ 9.00^{b}	2.60 □ 0.72^{b}	51.33 □ 6.66^{b}

Values are means of five determinations + SD. Values with different superscripts down the column are significantly different ($p<0.05$).NC: Normal rats control, RF: Normal rats + Rifampicin (50mg/kg), RF + Vit E: Normal rats + Rifampicin (50mg/kg) + Vitamin E (100mg/kg), RF + R50: Normal rats + Rifampicin (50mg/kg) + root extract (50mg/kg), RF + R100: Normal rats + Rifampicin (50mg/kg) + root extract (100mg/kg): RF + R200: Normal rats + Rifampicin (50mg/kg) + root extract (200mg/kg). AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase.

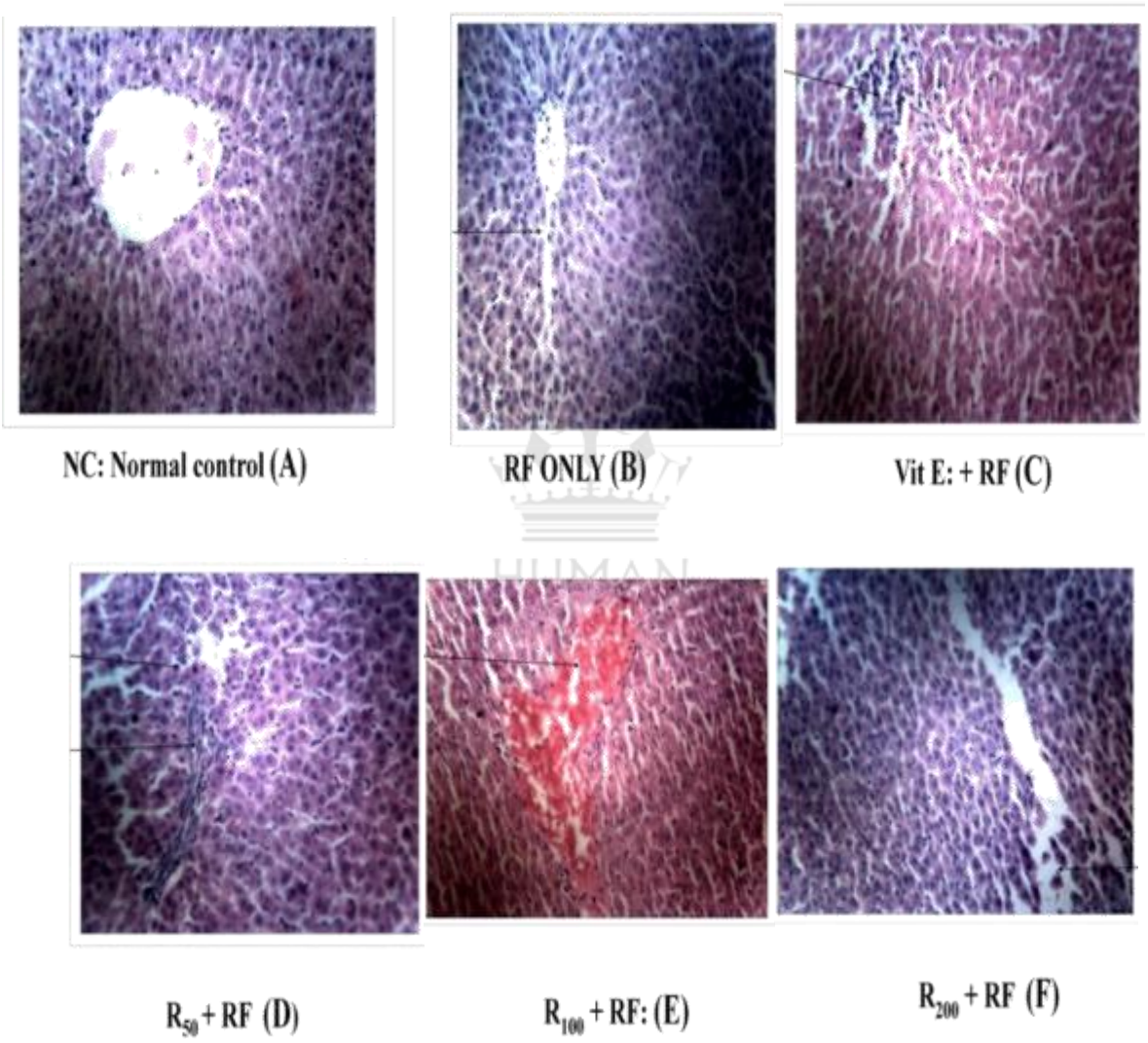

Plate 1: Histology of the liver

The result of phytochemical screening of methanol root extract of *Sphnocentrum jollyanum* showed the presence of carbohydrates, cardiac glycoside, flavonoids, and alkaloids (Table 1).

There was a significant ($p<0.05$) increase in the level of liver maker enzymes found in the rifampicin group. However, administration of methanol root extract of *Sphenocentrum jollyanum* prevented significant ($p<0.05$) increases in the level of these enzymes- AST, ALT, and ALP in the methanol root extract treated groups (Table 2).

The present study revealed a significant ($p<0.05$) decrease in levels of total protein and albumin in the rifampicin-induced untreated group (Table 3). The decreased levels of total protein and albumin are due to a reduction in the function of the liver to synthesize these total proteins and albumin and maybe result in hepatocellular damage[17]. The methanol root extract was able to significantly ($p<0.05$) increase the activity of SOD in the methanol root extract treated groups compared to the rifampicin-induced untreated group (Table 4). However, there was no significant ($p<0.05$) increase in the activity of catalase enzyme of the methanol extract treated groups compared to rifampicin-induced untreated groups except in the group that received 200mg/kg bodyweight methanol leaf extract of *S. jollyanum*.

A similar trend of the result was observed in the result of liver organ homogenate antioxidants when methanol root extract of *Sphenocentrum jollyanum* was administered to rifampicin-induced hepatotoxic rats (Table 5). Interactions between the aforementioned antioxidant enzymes (SOD, catalase, and GPX) adequately protected the integrity of the liver cells. For instance, superoxide dismutase (SOD) is a sensitive index in hepatocellular damage[18]. SOD scavenges superoxide anion to form hydrogen peroxide[18] and thus diminishing the toxic effect caused by this radical.

Catalase on the other hand decomposes hydrogen peroxide and protects the tissues from highly reactive hydrogen radicals[19] while glutathione peroxidase in a redox cycle protects the cell against hydrogen peroxide radicals and maintains membrane protein thiols[15]. The observed reduction in MDA level and increase in the activity of antioxidant enzyme seen in the various extract-treated groups was due to the antioxidants present in the plant extract as can be seen from the result of the phytochemistry and it indicates the protection of structural integrity of hepatic cell membrane or regeneration of damaged liver cells by the antioxidants present in the plant extract. Hence it is likely that the mechanism of hepatoprotection of *S. jollyanum* root extract is due to its antioxidant effect.

Histopathological examination of the liver of the rifampicin-induced untreated group revealed moderate perivascular necrosis and inflammation of the liver in the centrilobular region (Plate

1). The histopathological findings were in conformity with Pal[15] and Mohammed[20] which showed that rats administered with rifampicin 50mg/kg body weight and 100mg/kg body weight respectively showed hyperplasia, necrosis, portal triaditis, congestion, and degeneration of the liver cells. Treatment with methanol root extract was able to reduce the effect of rifampicin on the liver to slight lymphocyte hyperplasia and moderate necrosis.

CONCLUSION

This study indicated that methanol root extract of *Sphenocentrum jollyanum* has significantly lowered liver marker enzymes AST, ALT, and ALP and increased the activity of endogenous antioxidant enzymes catalase, SOD, and GPX. These suggest that methanol root extract of the plant has hepatoprotective and antioxidant activity against rifampicin-induced hepatotoxicity in Wistar albino rats.

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