

Effects of *Russelia equisetiformis* methanol and aqueous extracts on hepatic function indices

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Abstract

Russelia equisetiformis is a medicinal plant used by traditional healers to treat malarial, cancer and inflammatory diseases. It is also claimed to promote hair growth. Methanol and aqueous extracts of *Russelia equisetiformis* were administered orally to experimental rats at various doses of 100mg/kg, 200mg/kg and 400mg/kg for 28 days. At the end of the 28-day treatment, the animals were sacrificed under light ether anesthesia. The blood samples were collected separately by carotid bleeding into sterilized dry centrifuge tubes. The clear serum was separated at 2500 rpm for 10min. The effect of the extracts on hepatic function was evaluated by the assay of biochemical parameters (serum protein, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). In both aqueous and methanol extract – treated animals, there was significant dose – dependent increase in total bilirubin, ALT, AST, and alkaline phosphatase levels but a significant reduction in serum protein. However, these effects were more pronounced with the methanol extract. The study showed that chronic use of both aqueous and methanol extracts of *Russelia equisetiformis* could impair normal liver function.

Keywords: *Russelia equisetiformis*; extracts; liver; assay.

Introduction

Russelia equisetiformis is a medicinal plant used by some traditional healers in Nigeria. They claim it cures malaria cancer and inflammation. In an earlier study on the plant it was reported that the methanol extract of the plant has an oral LD50 of 3.6g/kg and a dose-dependent hemolytic effect with chronic use (Kolawole and Wakeel, 2006). Since herbal medicine is a major component of health care delivery system in many places around the world (Phillipson, 1994), the safety of medicinal plants is of great importance to all concerned. The influence of these plants on the liver needs to be established considering its important physiological functions. For example, the liver plays a major role in determining the disposition of drugs. It is often one of the tissues negatively affected by drug toxicity because of its daily exposure to a wide variety of xenobiotics. Many of these xenobiotics can provoke biologic responses, which often depend on conversion of the absorbed substance into an active metabolite (Murray, 1992). The toxic influence of such active metabolites on the liver may result in drug – induced hepatitis, cirrhosis, cholestasis or liver dysfunction (Bossone, 1977). Impairment of liver

function in turn prolongs the half-life of many drugs increasing their duration of action, peak effect and potential for toxicity (Mar, 1982). In the present study, we investigated the effect of aqueous and methanol extracts of *Russelia equiseformis* on some indices of liver function.

Materials and Methods

Animals

Male Wistar rats (180-200g) were used. The animals were bred and housed under normal laboratory conditions of humidity, temperature and light. They were allowed free access to drinking water and animal pellets. The principle of laboratory Animal Care (NIH publication No. 85-23) guidelines and procedures were used in this study (NIH, 1985).

Plant material

Russelia equiseformis (whole plant) was collected from New Bodija extension, Ibadan, South West Nigeria. The plant sample was identified in the herbarium of Forestry and Research Institute of Nigeria (FRIN), Ibadan. A

voucher specimen of the collected sample was deposited in the FRIN herbarium.

Preparation of hot water extract

R. equisetiformis was air-dried for 3-5 days in the shade and cut into small pieces. Six hundred grams were boiled with 3.0 liters of distilled water for 2 hours. The hot water extract was concentrated under vacuum (yield=28.4% w/w drug weight basis) and stored at 4°C until use.

Preparation of methanol extract

The plant sample was air-dried at room temperature, powdered and extracted in a mixture of methanol and water (1:1) for 24 hrs. The extract was filtered and concentrated under vacuum at 24°C. The extract obtained was stored at 4°C prior to pharmacological studies.

Evaluation of liver function

Seven groups of animals (5 rats per group) were used for the study. The animals in group I served as the control and received saline at a dose of 100ml/kg. Groups II, III and IV received 100mg/kg, 200mg/kg and 400mg/kg of aqueous extract (WE) respectively every day for 28 days. Groups V, VI and VII were treated with 100mg/kg, 200mg/kg and 400mg/kg of methanol extract (ME) respectively, also for 28 days. All drugs were administered orally. All the animals were sacrificed on day 28 under light ether anaesthesia. The blood samples were collected separately by carotid bleeding into sterilized dry centrifuge tubes. The clear serum was separated at 2500 rpm for 10min and biochemical investigations were carried out to assess hepatic function. Serum aminotransaminases (ALT and AST) were estimated by the procedure of Bergmeyer et al. (1986). Briefly, 1.0 ml of working solution was incubated in a test tube for 2-3 min at 37°C. 100ul of each sample was added, mixed and absorbance was immediately read at 340nm using a 1cm light path cuvette. Control and standard samples were treated similarly. The mean values of absorbance change per minute for each sample and standard were determined and the respective enzyme activity of the samples was calculated. Alkaline phosphatase (ALP) activities were done by spectrophotometry using an assay kit supplied by Quinica Clinical (Aplicada, Spain) (Babson et al., 1966). Serum bilirubin was estimated colorimetrically using assay kit obtained from BioAssay Systems, USA (Garber, 1981) while total protein was estimated by Biuret method (Kingsley and Frankel, 1939).

Statistical analysis

All data were expressed as mean \pm SD. The results were computed statistically (SPSS software package, version 7.5) using one-way analysis of variance. Dunnett's T3 *post hoc* testing was performed for inter-group comparison. $p < 0.05$ was statistically significant.

Results

Both aqueous and methanol extracts of *R. equisetiformis* caused a marked dose-dependent increase in serum aminotransaminases (ALT and AST), bilirubin and alkaline phosphatase. There was also a significant dose-dependent decrease in serum total protein. These effects were more pronounced with methanol extract (ME) than with water extract (WE). The results are presented in Table 1 and Table 2 below.

Discussion

The study showed that both aqueous and methanol extracts of *Russelia equisetiformis* have the potential to impair normal liver function in rats. This is indicated by the marked dose-dependent increase in serum levels of transaminases (ALT and AST), bilirubin and alkaline phosphatase (ALP) and a significant reduction in total protein level. These results were clear indication of cellular leakage and loss of functional integrity of the cell membrane (Sarawat et al., 1993). Transaminases are among a group of ubiquitous enzymes whose serum activity is elevated in many different disease states, including myocardial infarction, viral and toxic hepatitis, muscular dystrophy and cancer (Nevin and Vijayammal, 2005). Free radicals and peroxidants have been implicated in the pathogenesis of toxic liver injury (Jalalpure et al., 2003). Many of the drugs that precipitate liver disease produce free radicals during metabolism (Khalid et al., 2002). *R. equisetiformis* has been reported to contain triterpenes and sterols, which could be sources of free radicals after metabolism (Burn et al., 2000). In addition, it has been reported that *R. equisetiformis* does not contain flavonoids, which have free radical scavenging and antioxidant activities (Hesham and Shgeru, 2002). Therefore, it is suspected that the free radical generated by the metabolism of *R. equisetiformis* accumulates and adversely affect liver function as indicated by an increase in the serum levels of liver enzymes. It is therefore necessary to carry out further investigations on the toxicity of extracts of this plant and determine how long they can safely be administered. The

study showed that chronic use of *Russelia equisetiformis* has the potential to impair normal

liver function and therefore should be used with care.

Table 1:- Effect of *R. equisetiformis* methanol extract (ME) on indices of liver function.

TREATMENT	BILIRUBIN (mg/dl)	TOTAL PROTEIN (mg/dl)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control	0.61 ± 0.07	9.61 ± 0.26	53.40 ± 0.84	126.72 ± 0.42	198.20 ± 2.13
ME (100mg/kg)	0.88 ± 0.01	8.32 ± 0.32	63.61 ± 0.75*	182.54 ± 0.25*	214.41 ± 1.72*
ME (200mg/kg)	1.06 ± 0.03*	7.54 ± 0.21*	75.20 ± 0.59*	206.47 ± 0.92*	288.60 ± 2.16*
ME (400mg/kg)	1.42 ± 0.12*	6.25 ± 0.46*	86.8 ± 0.96*	232.83 ± 1.02*	253.0 ± 2.31*

n=5; Values are expressed as mean ± SD
*p<0.05 compared to control

Table 2:- Effect of *R. equisetiformis* water extract (WE) on indices of liver function.

TREATMENT	BILIRUBIN (mg/dl)	TOTAL PROTEIN (mg/dl)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control	0.61 ± 0.07	9.61 ± 0.26	53.4 ± 0.84	126.72 ± 0.42	198.20 ± 2.13
WE (100mg/kg)	0.72 ± 0.04	8.84 ± 0.12	58.68 ± 0.27	164.98 ± 0.22*	206.51 ± 1.57*
WE (200mg/kg)	0.86 ± 0.06	7.61 ± 0.25*	62.55 ± 0.45*	188.60 ± 0.68*	218.23 ± 0.98*
WE (400mg/kg)	1.02 ± 0.09	6.83 ± 0.61*	72.58 ± 0.33*	216.26 ± 1.24*	227.32 ± 1.05*

n=5; Values are expressed as mean ± SD
*p<0.05 compared to control

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