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RESEARCH ARTICLE

PROTECTIVE EFFECT OF METHANOL EXTRACT OF *RUSSELIA EQUISETIFORMIS* AGAINST PARACETAMOL-INDUCED HEPATOTOXICITY IN WISTAR RATS

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Abstract

Objectives: Liver diseases are among the health challenges facing many people and health care providers worldwide. In their search for solution to these problems, researchers are increasingly advocating the use of herbal preparations with proven efficacy in protecting against hepatic disorders. They also investigate medicinal plants with the aim of developing new drugs. *Russelia equisetiformis* is a plant which contains phytoconstituents that were reported to have biological activities, such as anti-inflammatory, antidiabetic, and membrane-stabilizing properties. In this study, the effect of methanol extract of *R. equisetiformis* (MEREQ) on paracetamol-induced hepatotoxicity was investigated in rats.
Method: Rats were pretreated orally with graded doses (100–400 mg/kg b.w) of MEREQ for 7 days. On the 8th day, hepatotoxicity was induced in the pretreated rats with a single intraperitoneal injection of paracetamol (2 g/kg b.w). Rats were sacrificed on the 15th day; blood samples were taken for biochemical analysis, and the liver was excised for histopathological study. Biochemical parameters analyzed are alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, and bilirubin.
Results: Administration of paracetamol in the rats resulted in significant increase ($p<0.05$) in the serum levels of AST, ALT, ALP, and bilirubin compared with the control. Treatment with MEREQ significantly reduced ($p<0.05$) the levels of these parameters in a dose-dependent manner, compared with the untreated rats. No significant changes were observed in the serum levels total protein and albumin. Histopathological examination showed that administration of paracetamol caused distortions in the architecture of the liver, but the degree of degeneration of hepatocytes was reduced in the MEREQ-treated rats.
Conclusion: The results of this study showed that methanol extract of *R. equisetiformis* has protective effect on paracetamol-induced hepatic injury.
Keywords: Hepatotoxicity, *Russelia equisetiformis*, paracetamol, liver, rats.

INTRODUCTION

The liver accounts for about 5 percent of the total body mass and is considered the second largest organ and the largest gland in the body[1]. It is uniquely situated within the circulatory system to receive the blood coming from the gastrointestinal tract and abdominal space before it is pumped through the lung and into the general circulation. The liver is a very important organ as it is responsible for many physiologic functions in the body. It is involved in the metabolism and storage of carbohydrate, metabolism of hormones, endogenous wastes and ingested chemicals, formation of urea and bile and metabolism of fats[2]. The liver receives potentially toxic chemicals in high concentration as it is the first organ these chemicals come in contact with following absorption from the gastrointestinal tract. For this reason, it is especially susceptible to chemical attacks which result in tissue injury. Being the primary organ for biotransformation of chemicals, the liver is also exposed to toxic reactive chemicals and intermediates that are formed during the biotrans-formation process[3]. Acute or chronic exposure to chemicals results in different kinds of liver injury. For example, hepatic mitochondria are damaged when the liver is exposed to carbon tetrachloride, cocaine,

hydrazine, and phosphorus. Likewise, plasma membrane of the hepatocytes is disrupted when exposed to acetaminophen[4]. Drug-induced liver diseases are among diseases that are currently posing serious challenge to public health. In spite of the advances already made in the fields of science and medicine, incidences of cholestasis, cirrhosis and hepatitis are on the increase. Therefore there is the need to search for more drugs that are effective and safe for the treatment of chemical-induced liver diseases. In their search for such drugs, scientists have turned their attention to medicinal plants, recognizing the enormous potential of these plants.

Russelia equisetiformis is a plant that belongs to Scrophulariaceae family. It is native to Mexico but it has naturalized in Florida, Hawaii and countries with tropical climate such as Nigeria and Kenya. Its common names are firecracker plant, coral plant and fountain plant. *Russelia equisetiformis* has been reported to have anti-inflammatory, analgesic and membrane stabilizing properties. It has also been reported to promote hair growth[5,6]. Methanol extract of the plant has also been shown to possess central nervous system depressant activities in mice[7]. The plant has also been reported to be useful in treating kidney stones and diabetes mellitus[8,9]. Some reference works reported that *Russelia equisetiformis* contains bioactive compounds such as triterpenes of the lupane type, russectinol and russeliaoside[10]. Total phenolic content of the plant has also been determined and quantified[11]. However, the hepatoprotective effect of *R. equisetiformis* has not been scientifically investigated, and since triterpenes and the other phytoconstituents of the plant are well-known for their biological activities, and some important biological properties of the plant have been reported, especially its membrane-stabilizing effect, this study was designed to investigate the effect of extract of *R. equisetiformis* on paracetamol-induced liver injury in rats.

MATERIALS AND METHODS

Fresh aerial part of *Russelia equisetiformis* was collected from New Bodija Extension Ibadan, Southwest Nigeria. It was authenticated by a taxonomist in the herbarium of the Forestry Research Institute of Nigeria (FRIN) Ibadan, where voucher specimen was deposited (voucher number: 106998). The plant was washed thoroughly with tap water and air-dried at room temperature. The dry sample was then milled into fine powder in a commercial blender. Five hundred grams (500 g) of the pulverized plant was extracted in 70% methanol for 72 hours. Whatman filter paper (No. 1) was subsequently used to filter the extract. Evaporation and concentration of the filtrate were done on a water bath at 40°C, and a solid extract (MEREC) was obtained, with a yield of 6.2% w/w. MEREQ was kept at 4°C until use. Wistar rats of both sexes weighing 180±20 g were used for the study. They were kept under standard environmental conditions of 50±10% relative humidity and 12 h light and 12 h dark cycle throughout the experiment. The animals acclimatized in the laboratory environment for five days prior to the commencement of the experiments. They were provided with standard rat pellets and clean drinking water *ad libitum.*

Ethical Consideration

Experimental procedures and protocols used in this study conform to the "Guide to the care and use of animals in research and teaching" [12].

Experimental Procedure

Rats were randomly divided into five groups of six animals each. The doses of the extract were chosen based on the reported median lethal dose (LD_{50}) of *R. equisetiformis* in rats[13]. In groups 3-5, the extract was administered orally for seven days. To induce liver injury, paracetamol (PCM) was given intraperitoneally on the 8th day to the rats that were pretreated with the methanol extract of *R. equisetiformis* (MEREQ). The animals were treated as follows:

Group 1; 10 ml/kg distilled water (normal control)
Group 2; PCM only (2 g/kg)
Group 3; PCM (2 g/kg) + MEREQ (100 mg/kg)
Group 4; PCM (2 g/kg) + MEREQ (200 mg/kg)
Group 5; PCM (2 g/kg) + MEREQ (400 mg/kg)

On the 15th day, the animals were sacrificed under ether anesthesia and blood samples were collected into heparinized tubes for biochemical analysis. The liver was also harvested for histopathological examination.

Biochemical Analysis

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities were estimated using test kit (BioVision Inc. USA). Plasma bilirubin was estimated as previously described[14]. Total protein and plasma albumin were determined by the method of Tietz[15].

Histopathological Study

Liver tissue was harvested and fixed in 10 % formalin. It was dehydrated in graded concentrations of ethanol, cleared in xylene and embedded in paraffin. Sections were prepared and then stained with hematoxylin and eosin (H & E) for photomicroscopic examination.

Statistical Analysis

Data are expressed as mean±standard error of mean (SEM) and then subjected to one-way analysis of variance (ANOVA) and Student's t- test. Values with $p < 0.05$ were taken to be significant.

RESULTS AND DISCUSSION

Paracetamol is one of the most common causes of poisoning worldwide[16]. It is metabolized in the liver by hepatic cytochrome P450 mixed-function oxidase system to a reactive intermediate, N-acetyl-p-benzo-quinoneimine (NAPQI)[17]. This metabolite is normally conjugated with sulfhydyl groups of hepatic glutathione before it is excreted by the kidney[18].

When paracetamol is ingested in large amount, hepatic glutathione is depleted and this initiates covalent binding of NAPQI with macromolecules in the liver cells[19]. These cellular events ultimately culminate in hepatic necrosis. Intracellular proteins and other macromolecules are usually released into the blood by cells that are undergoing acute degeneration and injury[20].

Table 1: Effects of MEREQ on biochemical parameters of rats following induced liver injury.

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
AST (IU/L)	123.24±24.32	154.68±26.06[a]	148.76±27.45	142.93±22.40	131.51±24.09[b]
ALT (IU/L)	26.74±12.41	41.86±11.62[a]	38.90±10.55	33.25±9.11	30.41±9.32[b]
ALP (IU/L)	116.20±24.06	152.84±28.22[a]	147.71±26.17	130.60±19.73[b]	121.30±25.24[b]
TP (g/L)	84.57±10.15	77.10±13.53	78.44±18.60	80.57±16.20	82.72±18.22
ALB (g/L)	58.41±12.63	49.65±13.45	50.33±19.24	52.91±18.42	55.57±16.19
BIL(mg/dL)	12.10±2.4	19.51±4.63[a]	17.92±3.73	16.65±4.33	13.82±2.15[b]

MEREQ=methanol extract of *R. equisetiformis*. [a]$p<0.05$ compared with the Group 1; [b]$p<0.05$ compared with Group 2. ALT=alanine aminotransferase, AST=aspartate aminotransferase, ALP=Alkaline phosphatase, TP=total protein, ALB=albumin, BIL=bilirubin

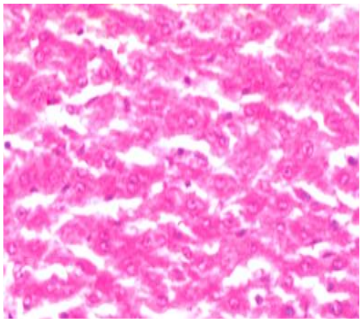

Figure 1: Liver section of rats treated with distilled water(x 200 magnification).

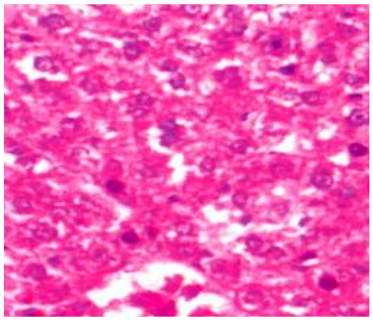

Figure 2: Liver section of rats treated with Paracetamol (2g/kg b.w)(x 200 magnification).

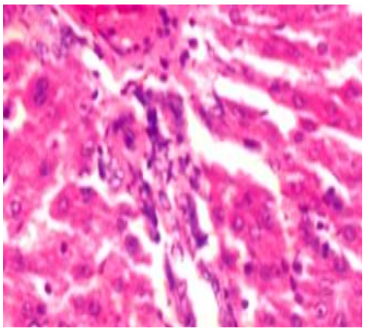

Figure 3: Liver section of rats treated with Paracetamol and MEREQ (100 mg/kg b.w)(x 200 magnification).

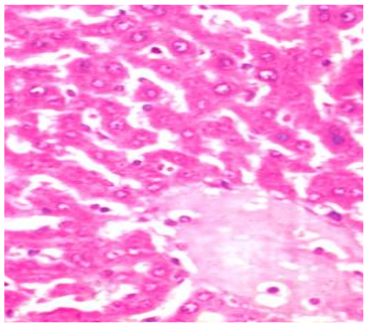

Figure 4: Liver section of rats treated with Paracetamol and MEREQ (200 mg/kg b.w)(x 200 magnification).

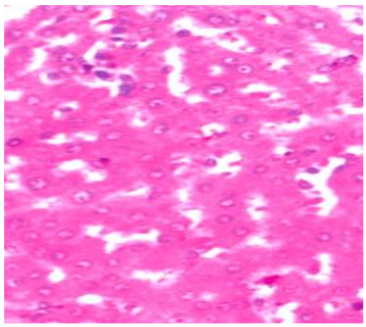

Figure 5: Liver section of rats treated with Paracetamol and MEREQ (400 mg/kg b.w)(x 200 magnification).

When these substances are detected in the blood in measures that are above the normal baseline levels, cell injury is confirmed. Several enzymes are found primarily in the hepatocytes and their presence in blood in elevated levels is the basis for some of the common tests for hepatotoxicity. Severe hepatic injury induced by paracetamol can result in significant increase in the concentration of serum AST and ALT, but only modest increase in ALP[21]. In the present study, administration of paracetamol in rats resulted in significant increase ($p<0.05$) in the serum levels of ALT, AST and ALP compared to normal control. Pretreatment with MEREQ significantly lowered the serum levels of these enzymes in paracetamol-induced hepatic injury (Table 1). As shown in Table 1, there was no significant change in plasma total protein in rats treated with paracetamol when compared to normal control. Although there was reduction in the serum level of albumin in paracetamol-treated rats, the decrease was not significant ($p>0.05$) compared with normal control. Albumin is synthesized in the liver and secreted into blood. The ability of the liver to synthesize albumin is impaired when there is a hepatic injury. The decrease in serum level of albumin following paracetamol-induced hepatotoxicity was not significant probably due to the slow turnover time for albumin and therefore it takes a long time for impaired albumin synthesis to manifest as changes in serum albumin[22]. However,

treatment of rats with MEREQ after induction of liver injury increased the level of albumin to near normal. Bilirubin is the breakdown product of heme from red blood cells. It is conjugated by the liver and the glucuronide conjugate is secreted into the bile. In the hepatocellular injury, conjugation and excretion of bilirubin is impaired leading to jaundice as it accumulates in the blood[23]. Therefore elevation of serum bilirubin level is an indication of acute hepatocellular injury, cholestatic injury or biliary obstruction. Administration of the large dose of paracetamol to rats resulted in a significant increase in the serum level of bilirubin. This effect was significantly reduced when the rats were treated with MEREQ, suggesting that the extract protected the rats against hepatic injury.

The results obtained for biochemical analysis was supported by the observations from the histopathological study. Figure 1 shows the liver section of rats treated with distilled water (normal control). The hepatocytes appear normal with well preserved cytoplasm and prominent nucleus. The hepatotoxic effect of paracetamol is evident in figure 2 which shows the liver section of rats treated with paracetamol only. There is diffuse vacuolar degeneration of the hepatocytes and extensive periportal congestion, edema, fibrosis and infiltration by mononuclear cells. The degree of degeneration of the hepatocytes was reduced in rats treated with graded doses of MEREQ as observed in Figure 3, Figure 4 and Figure 5. *Russelia equisetiformis* was reported to contain flavonoids which are free radical scavengers[24]. This may be responsible for its hepatoprotective effect since one of the mechanisms through which paracetamol causes hepatotoxicity is oxidative damage[25].

CONCLUSIONS

Based on the results of this study, we conclude that methanol extract of *Russelia equisetiformis* protects rats against paracetamol-induced hepatotoxicity. Further studies are needed to determine the mechanism by which the extract exhibits its therapeutic effect and to isolate and characterize the bioactive compounds in the plant.

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AUTHOR'S CONTRIBUTIONS

Wakeel OK: designed the study and wrote the protocol. **Ayankunle AA:** managed the literature searches and performed the statistical analysis. **Kolawole OT:** wrote the first draft of the manuscript. **Oluogun WA:** performed the histopathological examinations. **Adeyeba OA:** analyzed the biochemical parameters. All authors revised the article and approved the final version.

DATA AVAILABILITY

The data supporting the findings of this study are not currently available in a public repository but can be made available upon request to the corresponding author.

CONFLICT OF INTEREST

None to declare.

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