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 phytocannabinoids that were reported to have biological activities, such as anti-inflammatory, anti-diabetic, 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 were 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. 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. 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. 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. 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. Methanol extract of the plant has also been shown to possess central nervous system depressant activities in mice. The plant has also been reported to be useful in treating kidney stones and diabetes mellitus. Some reference works reported that Russelia equisetiformis contains bioactive compounds such as triterpenes of the lupane type, russetinol and russeliaoside. Total phenolic content of the plant has also been determined and quantified. 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 (MEREQ) 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”.

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 (LD50) of R. equisetiformis in rats. 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 | Treatment |
|-------|-----------|
| 1     | 10 ml/kg distilled water (normal control) |
| 2     | PCM only (2 g/kg) |
| 3     | PCM (2 g/kg) + MEREQ (100 mg/kg) |
| 4     | PCM (2 g/kg) + MEREQ (200 mg/kg) |
| 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. Total protein and plasma albumin were determined by the method of Tietz.

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 | 148.76±27.45 | 142.93±22.40 | 131.51±24.09 |
| ALT (IU/L) | 26.74±12.41 | 41.86±11.62 | 38.90±10.55 | 33.25±9.11 | 30.41±9.32 |
| ALP (IU/L) | 116.20±24.06 | 152.84±28.22 | 147.71±26.17 | 130.60±19.73 | 121.30±25.24 |
| TP (g/L)  | 84.57±10.15 | 77.10±13.53 | 73.48±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 |

MEREQ=methanol extract of R. equisetiformis. *p<0.05 compared with the Group 1; †p<0.05 compared with Group 2. ALT=alanine aminotransferase, AST=aspartate aminotransferase, ALP=Alkaline phosphatase, TP=total protein, ALB=albumin, BIL=bilirubin.
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\(^1\). It is metabolized in the liver by hepatic cytochrome P450 mixed-function oxidase system to a reactive intermediate, N-acetyl-p-benzoquinoneimine (NAPQI)\(^2\). This metabolite is normally conjugated with sulfhydryl groups of hepatic glutathione before it is excreted by the kidney\(^3\). When paracetamol is ingested in large amount, hepatic glutathione is depleted and this initiates covalent binding of NAPQI with macromolecules in the liver cells\(^4\). 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\(^5\). 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\(^6\). 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
CONFLICT OF INTEREST

No conflict of interest associated with this work.

REFERENCES

1. Ozuogwu JC. Physiology of the liver. Int J Res Pharm Biosci 2017; 4(8): 13-24.
2. Boyer JL. Bile formation and secretion. Compr Physiol 2013 3(3):1035-1078. https://doi.org/10.1002/cphy.c120027
3. Asharp F, Zheng G, Wan Y, Li T, Hu W, Li W, Zhang H, Zhang Z, Hu J. Discovery of a widespread metabolic pathway within and among phenolic xenobiotics. PNAS 2017; 114(23): 6062-6067. https://doi.org/10.1073/pnas.1700558114
4. Gamal W, Trespes K, Samuel K, et al. Low-dose acetaminophen induces early disruption of cell-cell tight junctions in human hepatic cells and mouse liver. Sci Rep 2017; 7:37541. https://doi.org/10.1038/srep37541
5. Awe EO, Makinde JM. The hair growth promoting effect of Russelia equisetiformis (Schlect & Cham). J Nat Prod Med 2009; 2: 70-73.
6. Ahmed EM, Yehia S, Fouad M, Kamel M. A pharmacognostical study of Russella equisetiformis Sch Cham. Int J Pharmacogn Phytocoem Res 2016; 8: 174-192.
7. Kolawole OT, Makinde JM, Olajide OA. Central nervous system depressant activity of Russelia equisetiformis. Niger J Physiol Sci 2007; 22(1-2): 59-63. PMID: 18379620
8. Romero-Cerecero O, Reyes-Morales H, Aguilar-Santamaria L, Huerta M, Tortoriello-Garcia J. Use of medicinal plants among patients with diabetes mellitus type 2 in Morelos, Mexico. Bol Latinoam Caribe Plantas Med Aromat 2009; 8(5): 380-388.
9. Gómez-Estrada H, Díaz-Castillo F, Franco-Ospina L, et al. Folk medicine in the northern coast of Colombia: an overview. J Ethnobiol Ethnomed 2011; 7: 27 https://doi.org/10.1186/1746-4269-7-27.
10. Awe EO, Adeoye A, Idowu T, Olajide OA, Makinde J. Antinociceptive effect of Russella equisetiformis leave extracts: identification of its active constituents. Phytomed 2008; 15(4): 301-5. https://doi.org/10.1016/j.phymed.2007.03.012
11. Johnson CE, Oladeinde FO, Kinyua AM, et al. Comparative assessment of total phenolic content in selected medicinal plants. Niger J Nat Prod Med 2008; 12: 40-42. https://doi.org/10.4314/njnpm.v12i1.45664
12. National Institute of Health. Guide for the use of laboratory animals. DHHIS, PHS, NIH Publication 1985; No. 85-23
13. Kolawole OT, Kolawole SO. Effects of Russelaequiisetiformis methanol and aqueous extracts on hepatic function indices. Biol Med 2010; 2(3): 38-41.
14. Gordon DM, Neifer KL, Hamoud, et al. Bilirubin remodels murine white adipose tissue by reshaping mitochondrial activity and the coregulator profile of peroxisome proliferator-activated receptor α. J Biol Chem 2020; 295: 9804-9822
15. Tietz NW. Fundamental of Clinical Chemistry. W.B. Saunders Company, London 2000; 1020-1038.
16. Tittarel R, Pellegrini M, Scarpellini MG, et al. Hepatotoxicity of paracetamol and related fatalities. Eur Rev Med Pharmacol Sci 2017; 21(1 Suppl): 95-101. PMID: 28379590
17. Eberhardt MJ, Schillers F, Eberhardt EM, Risser L, de la Roche J, Herzog C, Echtermeyer F, Leffler A. Reactive metabolites of acetaminophen activate and sensitize the capsaicin receptor TRPV1. Sci Rep 2017; 7: 12775 https://doi.org/10.1038/s41598-017-13054-3
18. Mazaleuskaya LL, Sangkuhl K, Thorn CF, FitzGerald GA, Altman RB, Klein TE. Pharm GKB summary: pathways of 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 albumin22. 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 blood22. 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. Russella equisetiformis was reported to contain flavonoids which are free radical scavengers22. This may be responsible for its hepatoprotective effect since one of the mechanisms through which paracetamol causes hepatotoxicity is oxidative damage22.

CONCLUSION

Based on the results of this study, we conclude that methanol extract of Russella 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.

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. Olouogun WA performed the histopathological examinations. Adeyeba OA analyzed the biochemical parameters.
acetaminophen metabolism at the therapeutic versus toxic doses. Pharmacogenet Genomics 2015; 25(8): 416-426
https://doi.org/10.1097/PGC.0000000000000150
19. Athersuch TJ, Antoine DJ, Boobis AR, et al. Paracetamol metabolism, hepatotoxicity, biomarkers and therapeutic interventions: a perspective. Toxicol Res 2018; 7(3): 347-357. https://doi.org/10.1039/c7tx00340d
20. Miller MA, Zachary JF. Mechanisms and morphology of cellular injury, adaptation, and death. Path Basis Vet Dis 2017; 2:43.e19. https://doi.org/10.1016/B978-0-323-35775-3.00001-1
21. Castañares-Zapatero D, Dinant V, Ruggiano I, Willem H, Laterre P, Hantson P. Pattern of paracetamol poisoning: influence on outcome and complications. Toxics 2018; 6: 58
22. Levitt DG, Levitt MD. Human serum albumin homeostasis: a new look at the roles of synthesis, catabolism, renal and gastrointestinal excretion, and the clinical value of serum albumin measurements. Int J Gen Med 2016; 9: 229-255. https://doi.org/10.2147/IJGM.S102819
23. Méndez-Sánchez N, Vítek L, Aguilar-Olivos NE, Uribe M. Bilirubin as a biomarker in liver disease. In: Patel V., Preedy V. (eds) biomarkers in disease: methods, discoveries and applications 2017; Springer, Dordrecht. https://doi.org/10.1007/978-94-007-7675-3_25
24. Kumar, S, Pandey AK. Chemistry and Biological Activities of Flavonoids: An Overview. Sci World J 2013; 162750. https://doi.org/10.1155/2013/162750
25. Mingzhu Yan, Yazhen Huo, Shutao Yin, Hongbo Hu. Mechanisms of acetaminophen-induced liver injury and its implications for therapeutic interventions. Redox Biol 2018; 17: 274-283. https://doi.org/10.1016/j.redox.2018.04.019