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Hepatoprotective Potential of *Russelia Equisetiformis* Plant Extract on Drug-Induced Hepatotoxicity in Experimental Models

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Abstract
Background: *A large number of herbal medicines are widely in-use to offer relief from hepatic injury, although attempts are still ongoing globally to get scientific proofs for these orthodox remedies, there is still paucity of data on Russelia equisetiformes. There are solitary reports of anti-inflammatory, analgesic, and antinociceptive properties. Meanwhile, inflammation is a hallmark of hepatic injury. Further study may pave way for next generation hepatoprotective medication.*
Methods: *The experimental animals were grouped into five (5), with six animals each. Groups 3, 4 and 5 were orally pretreated with the extracts for 7 days at doses of 100, 200 and 400 mg/kg, while groups 1 and 2 served as normal (negative) and paracetamol-induced (PCM-induced;positive) controls. Liver damage was induced on the 8th day using 2000 mg/kg of paracetamol. Liver enzymes such as alanine amino transaminase (ALT) aspartate amino transaminase (AST) and alkaline phosphatase (ALP), along with other liver function tests [plasma total protein (TP), albumin (ALB) and bilirubin (BIL)] were subsequently estimated. Liver samples were examined for histopathological changes.*
Results: *Comparison of case groups with positive controls shows that levels of biomarkers such as AST, ALT, ALP were significantly reduced ($p<0.05$) at a dose of 400mg/kg of the extract. Serum proteins were also significantly elevated ($p<0.05$). Histologically, there were significant changes in the hepatic cellular architecture of the PCM-induced group compared with extract-treated groups.*
Conclusion: *The results obtained from this study show that Russelia equisetiformes may offer hepatoprotective influence against any liver damage as a result of drug toxicity.*
Keywords: *Hepatoprotective Influence; Histopathological Changes; Liver Function Tests; Paracetamol; Plant-Extract; Russelia equisetiformes.*

1 Introduction

World Health Organization (WHO) estimates that up to 80 percent of the world populations now rely on medicinal plants as their main source of health care. Currently, more pharmaceutical drugs on the market contain extracts from medicinal plants [1]. On the other hand, some of these pharmaceutical drugs are hepatotoxic. Drug-induced liver injury (DILI) is increasingly recognized as a strong etiological factor in the development of acute (rapid hepatic) and chronic liver (long-term hepatic) dysfunctions [2]. Liver diseases are unequivocally large contributory factors of registered fatal diseases in the world of today [3]. They pose a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are again employed for the treatment of many liver diseases thus becoming widely appreciated [4]. Meanwhile, Chaterrjee [3] reported that only few drugs are available for the treatment of liver disorders.

From history, a wide range of herbal preparations from various plants exploited for treatment of different liver diseases are on trials such Phyllanthus, Silybum marianum (milk thistle), glycyrrhizin (licorice root extract), and Liv 52 (consisting of mixture of herbs) [4,5].Today, there are emerging explorative studies on*Russelia equisetiformes* on its medicinal importance. *Russelia equisetiforme* is a plant native to Mexico, although it has naturalized in Florida, Hawaii and the Caribbean, as well as other countries with hot climates such as in the tropical African countries like Nigeria. *Russelia equisetiformis* (Schlect & Cham) belongs to the family Scrophulariaceae while the English names are firecracker, fountain plant, coral plant and coral bush [7,12].

Medicinally, the plant is currentlyin wide use for the treatment of diabetes and leukemia in Southwestern Nigeria[8]. It is also reported as a useful medicine for malaria and cancer[9]. *Russelia equisetiformis* is considered to have anti-inflammatory, analgesic and membrane stabilizing properties [10]. Reports also state that the methanol extract of Russelia equisetiformis possesses central nervous system (CNS) depressant activities [11] and further report has subsequently shown that the extract of the whole plant has anti-nociceptive effects [12]. Phytochemically, the plant has been reported to contain triterpenes of lupane type [13]. Two phenylethanoid glycosides, russectinol and russeliaoside, were repeatedly reported and identified as active constituents of the plant [14]. Likewise, total phenolic content has also been determined and quantified byJohnson and coworkers [15]. Again, the active constituent 'lupeol' isolated from extract of *Russelia equisetiformis* is reported with an anti-inflammatory activity in acute and certain aspects of chronic inflammation [12]. Furthermore, many studies have reported probable effect of Russelia equisetiformis in maintaining membrane stability [10]. Surprisingly, there is still dearth of dataon the preventive roles of *Russelia equisetiformis* plant extracts in reversing or preventing hepatic injury which most of lifetime is occasioned by drug toxicity. In addition, the existing literature has not succinctly proved difference in dosage overload and the associated level of impairment reversed with *Russelis equisetiformis* herbal remedy. Consequently, further scientific trialis urgently required and this may probably augment formulation of next generation hepatoprotective medication considering the physiological roles of the liver as a vital organ in disposition of drugs that could be grossly affected owing to wide exposure to various drug overloads or other hepatotoxic substances in our lifetimes.

2.0 Materials and methods

2.1Ethical consideration

Experimental procedures and protocols used in this study were approved by the Ethics committee of the Ladoke Akintola University of Technology, Nigeria and conform to the "Guide to the care and use of animals in research and teaching" (NIH publications number 85-93 revised in 1985).

2.2 Plant materials

Fresh plant leaves of *Russelia equisetiformis* were plucked at Osogbo in the southwestern part of Nigeria. It was authenticated by Dr. S.O Oni, a taxonomist in the herbarium of the Forest Research Institute, Ibadan, Nigeria (FRIN), where voucher specimen was deposited with number 106998.

2.2.1Preparation of plant extract

R. equisetiformes plants were washed thoroughly in tap water, shade-dried and powdered in the laboratory. 500g of powdered plant was weighed and dissolved in 4 litres of methanol for 72 hours. The suspension was filtered using a filter paper. The filtrate was collected and evaporated to dryness with a rotary evaporator at 41 degree centigrade. The crude extract was then used for analysis. A yield of 31g (6.2%.) was obtained. Without any further purification, aliquot portions of the plant crude extract residue were weighed and dissolved in distilled water (at room temperature) for use on each day of our experiments.

2.3 Animals

Healthy, male and female healthy Wistar rats (*Rattusnorvegicus*) weighing 235-250g were used. The animals were kept and maintained under laboratory conditions of temperature and humidity with a 12 h light/dark cycles. They were allowed free access to food (standard pellet diet) and drinking tap water ad libitum.

2.4 Procedure

2.4.1Grouping and dosing

Thirty Wistar rats of either sex weighing 235-250g, divided into five groups with six animals each, were used for the study. The extract was given to the animals for seven days. On the eighth day, paracetamol (graded doses) was given orally for liver damage induction. The doses chosen were based on the medianlethal dose (LD50) obtained (2,250 mg/kg) for the plant's extract.

Group 1: non-pretreated controls; no paracetamol induction (negative controls)

Group 2: non-pretreated control; paracetamol load only (positive controls)

Group 3: pretreated with100mg/kg of extract + followed with paracetamol load

Group 4: pretreated with 200mg/kg of extract + followed with paracetamol load

Group 5: pretreated with 400mg/kg of extract + followed with paracetamol load.

2.4.2 Blood collection

Blood samples (3 mls) was collected each from rats of all groups after seven days of treatment, into heparinized tubes for spectrophotometrical analyses of AST and ALT as described by Bergmeyer *et al.* [16], ALP as described by Babson *et al.* [6], plasma bilirubin using Jendrassik and Groof method as described by Garber [17], plasma albumin using bromocresol green binding method as described by Doumas *et al.* [18] and plasma total protein as described by Kingsley and Frankel [19] respectively.

2.4.3 Histopathological studies

The liver tissue was grossly dissected out and fixed in10% formalin, dehydrated in graded ethanol (50-100%) concentrations, cleared in xylene, and embedded inparaffin. Sections were prepared and then stained with hematoxylin and eosin (H and E) dyes for photomicroscopic observation.

2.5 Statistical Analysis

Values were expressed as mean ± standard deviation. Statistical significance was determined using one-way ANOVA test. Values with $P<0.05$ were considered significant.

3.0 Results and discussion

3.1 Result

The pretreated extract offered at varying doses show significant changes ($P<0.05$) in AST, ALT, ALP, TP and ALB when compared with paracetamol control group (positive group) as shown in the table 1.

Table 1: Effect of methanol extract of *R.equisetiformes* on liver biochemical parameters

Parameters/Group	Group 1	Group 2	Group 3	Group 4	Group 5
ALT (IU/L)	50.30 ± 11.64	109.20 ± 25.53	91.90 ± 27.90	60.76 ± 27.27	*66.72± 14.13
AST (IU/L)	51.78 ± 15.57	93.26 ± 14.42	82.68 ± 13.27	73.10 ± 11.78	*67.10 ± 13.31
ALP (IU/l)	62.38 ± 13.11	146.68 ± 41.48	112.74 ± 21.28	124.04± 35.72	*92.74± 21.28
TOT. BIL (mg/dl)	0.74 ± 0.26	1.76 ± 0.63	1.14 ± 0.23	1.32 ± 0.83	*0.78 ± 0.29
CON. BIL (mg/dl)	0.24 ± 0.13	0.66 ± 0.36	0.68 ± 0.49	0.45 ± 0.22	0.31 ± 0.19
ALB (g/dl)	41.24 ± 4.26	24.30 ± 4.26	34.56 ± 9.34	34.00 ± 4.33	*40.00 ± 13.49
T. PROT (g/dl)	87.60 ± 13.48	55.26 ± 5.34	59.28 ± 3.98	59.36 ± 2.36	61.56 ± 3.16

KEY: Group1- Negative controls (no extract pretreatment; no PCM-load); Group2-Positive controls (no pretreament; but PCM-load); Group3-Pretreatment with100mg/kg of extract before PCM-load; Group 4- Pretreatment with 200mg/kg of extract before PCM-load; Group 5-Pretreatment with 400mg/kg of extract before PCM-load; *= statistically significant when compared with positive group ($p \leq 0.05$); AST-Aspartate transaminases; ALT- Alanine transaminases; ALP- Alkaline phosphatase; TOT.BIL- Total bilirubin; CON.BIL- Conjugated bilirubin.; ALB- Albumin; T.PROT-Total protein.

KEY

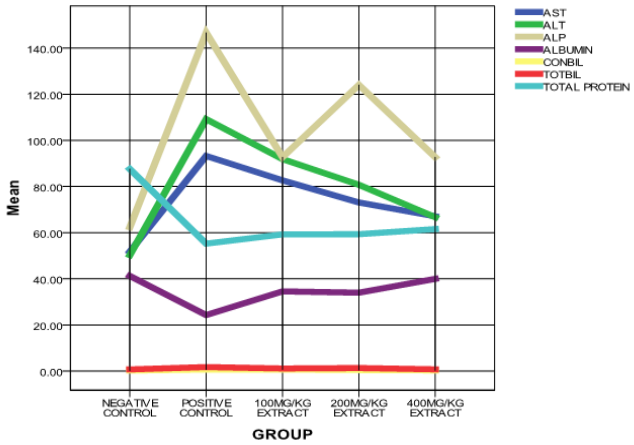

Figure 1: Line graph showing mean distribution of the liver biochemical parameters across the groups

Figure 1 shows impact of extract of *R.equisetiformes* on liver function tests or parameters regarding its counteractive effects against the paracetamol (PCM) toxic load. The upsurge secretion of the liver enzymes (ALT, AST and ALP) and reduced syntheses of albumin and total protein is a cardinal point of changes observed in the positive control group that was treated solely with PCM load without extract pretreatment in comparison with negative group without substance exposure at all. On the other hand, reduced enzyme secretions and increased syntheses of albumin along with total protein were observable effects of extract pretreatment prior to PCM load when compared with positive control group, as represented by the chart.

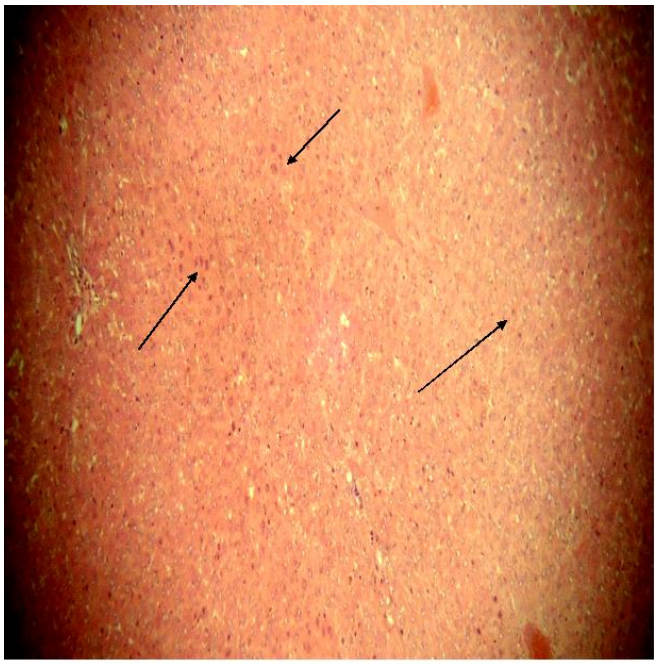

Fig.2: Histopathological examination of H&E stained slide from a representative normal control tissue

Micrograph in figure 2 shows normal hepatic cells with well-preserved cytoplasm; well brought out centralvein; prominent nucleus and nucleolus. This is typical of normal liver tissue on histological assessment.

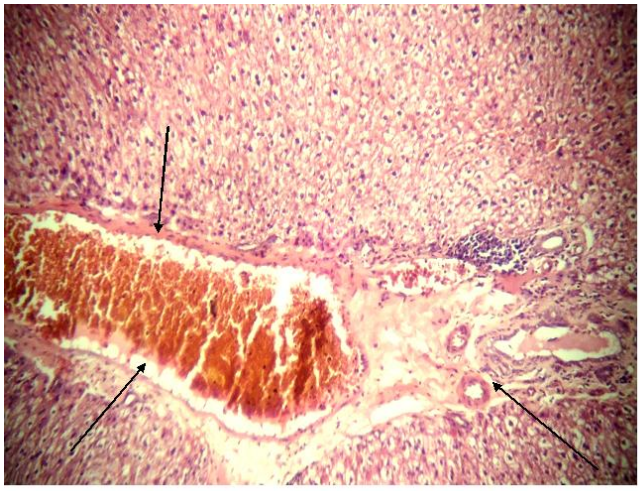

Fig. 3: Histopathological examination of H&E stained slides from a representative tissue of the positive control group

Micrograph in figure 3showsthere is severe diffuse vacuolar degeneration of the hepatocytes. There is extensive periportal congestion and oedema, fibrosis and cellular infiltration by mononuclear cells. This is typical of liver injury that may eventually lead to liver damage when tissue repair fails or in continuous toxic load.

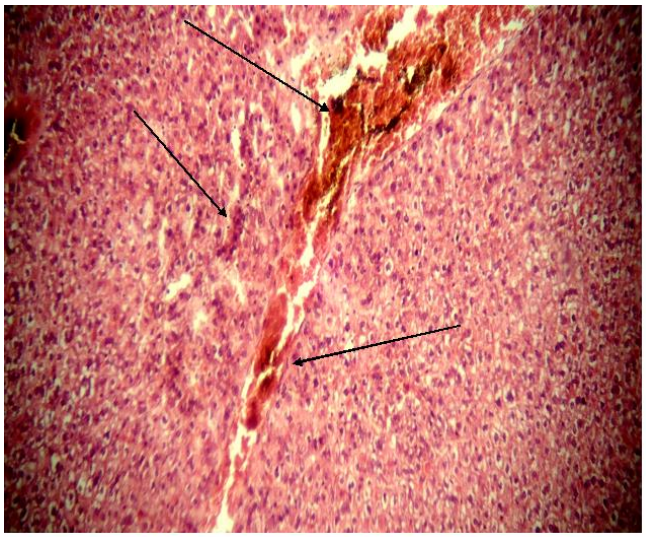

Fig.4: Histopathological examination of H&E stained slide from a representative group with paracetamol induction +100mg/kg extract pretreatment group

Micrograph in figure 4 shows there is severe diffuse vacuolar degeneration of the hepatocytes. Comparison of this level of damage or injury with positive group (without extract pretreatment) shows minimal alteration.

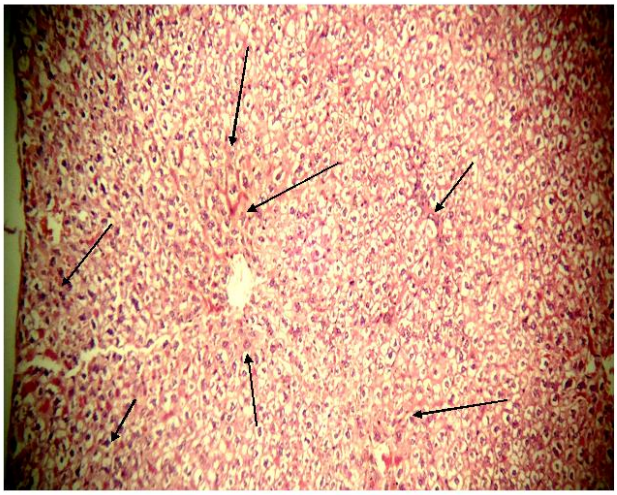

Fig. 5: Histopathological examination of H&E stained slide from a representative group with paracetamol induction +200mg/kg extract pretreatment group

Micrograph in figure 5 shows there is mild to moderate, diffuse vacuolar degeneration of the hepatocytes. There is also a moderate portal congestion. When compared with groups 2 (without extract pretreatment) and 3, the severity of damage process was reduced.

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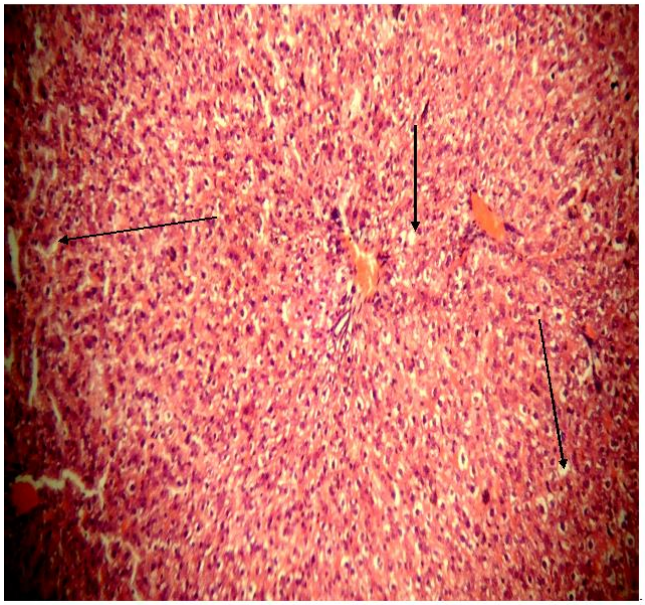

Fig. 6: Histopathological examination of H&E stained slide from a representative tissue of the group with paracetamol induction +400mg/kg extract treatment

Micrograph in figure 6 shows that there is mild to moderate, diffuse vacuolar degeneration of the hepatocytes. There is also a very mild portal congestion. These signs show minimal or greatly reduced effect of PCM damage when compared with groups 2 (without extract pretreatment), 3 and 4 respectively.

3.2 Discussion

The hepatic cytochromeP 450 enzyme system metabolizes paracetamol, forming a minor yet significant alkylating metabolite known as NAPQI. NAPQI is then irreversibly conjugated with the sulfhydryl groups of glutathione. NAPQI depletes glutathione and initiates covalent binding to cellular proteins. These events lead to the disruption of calcium homeostasis, mitochondrial dysfunction, oxidative stress and may eventually culminate in cellular damage and death [20].

Fortifying the above stated mechanisms, biochemical parameters (liver enzymes) demonstrated significant increases in different groups that received toxic doseof paracetamol in the present study. Paracetamol-induced elevation in serum AST, ALT levels has been attributed to the damaged structural integrity of the liver, because these enzymes are normally located in the cytoplasm of hepatocytes and are released into circulation after cellular damage [21]. Reports shows that *R.equisetiformes* possesses membrane stabilizing activities [10]. The decreased concentrations of liver enzymes (AST and ALT) as a result of plant extract administered at 100, 200 and 400m/kg might have been due to prophylactic support of hepatocytes' membrane stabilizing activities of *R.equisetiformes*.

Again,plasma ALP and total bilirubin levels are also related to the status and function of hepatic cells. Increase in serum ALP indicates increased biliary obstruction [22]. Present study indicates that *R.equisetiformes* plant extract at the three doses (in the testing groups) slightly decreases plasma ALP and bilirubin levels when compared to paracetamol-induced group(without extract pretreatment).

More so,hypoalbuminaemia is most frequent in the presence of advanced chronic liver disease. Hence, decline in total protein content can be a useful index for assessment of severity of cellular dysfunction in chronic liver diseases [22]. The lowered level of total protein recorded in plasma of rats that received 2000mg/kg of paracetamol reveals the severity of hepatopathy. There were considerable increases in total protein levels of groups with pretreatment of 100, 200 and 400mg/kg of *R.equisetiformes* leaf extract respectively, compared with paracetamol-inducedcontrol group (without extract pretreatment).

Most hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages [23]. Flavonoids are a large number of phytochemicals that are effective free radical scavengers [24].Reports showed that *R.equisetiformes* possesses flavonoid [10] and also a free radical scavenger [25].When hepatic damages occur, free radicals are released in excess of physiological rate from hepatocytes and bring about associated destabilization of hepatocyte membranes. However, hepatoprotective activities of *Russelia equisetiformes* plant could be due to its membrane stabilizing and free radical scavenging activities.

4.0 Conclusion

Data obtained from this present study show that *R.equisetiformes* plant extract may possess significant hepatotoprotective activities on toxicant-induced liver damage in the experimental models and thereby offer useful prophylactic measure.

5.0 Recommendation

Further studies require assay of biomarkers of inflammation, cellular oxidative membrane damage along with determination of active ingredients to expand the scope and knowledge of mechanisms behind anti-inflammatoryrolesin *R. equisetiformes* extract.

Conflict of Interest: None

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