

# Investigation into the Haematologic and Hepatotoxic Effects of Rinbacin in Rats

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An evaluation of the effects of rinbacin on the liver and blood of albino rats was carried out. Low (26.25 g/l) and high (52.50 g/l) dose levels of rinbacin were administered in the drinking water of albino rats for 13 weeks. Food and fluid intake were measured daily, and animal body weight taken weekly. Biochemical analysis of the liver function was carried out as well as some haematological parameters and histology of the liver. Results showed a significant ( $p \leq 0.05$ ) increase in all the liver function parameters tested at both dose levels. There was also an increase in packed cell volume (PCV) in the high dose group. Histological examination indicates that rinbacin at both dose sizes induced severe pathologic changes in the forms of degeneration of hepatocytes, necrosis, oedema, cellular infiltration, nuclear fragmentation and chromatinolysis. Administration of rinbacin, though could raise the PCV, may lead to hepatic damage, which might result in increased bilirubin and liver enzymes in rats. Rinbacin is toxic to the rat liver.

**Key words** — rinbacin, hepatotoxicity, bilirubin, alanine transaminase, alkaline phosphatase, packed cell volume

## INTRODUCTION

Medicinal herbs are being used by a large number of patients, who seek or do not seek advice of their physicians regarding their use.<sup>1)</sup> It has been estimated roughly, that more than half of the total population of the earth, presently use herbal drugs.<sup>2)</sup> Because humans have used herbal drugs for a long time, they are considered to be very safe and non-toxic, and so the toxicological actions of these agents have been largely ignored, even while the effectiveness is either already known or under study.<sup>1)</sup>

Rinbacin is a trademark herbal preparation registered in Nigeria (registration number 016283) and used in homeopathic medicine. It is a greenish powdered herbal mixture, said to comprise of 48% roots, 18% seeds, 22% leaves and 12% flowers (personal communication). A large number of people in the Eastern Nigeria use this herbal remedy. The herbalists claim that it has a curative effect on a wide range of bacterial infections, sexually transmitted diseases

and diabetes. However, information is scarce on the toxicology of the preparation. So far, only a recent work by Orisakwe *et al*, which reported the toxic effects of rinbacin on rat testis, is known to the authors.<sup>3)</sup> Variations in the constituents of rinbacin are unlikely, since it is standardized and manufactured by only one company (Mabro Homeopathic Products Ltd., Aba, Nigeria).

The liver occupies a central role in major functions of the organism, because of its interposition between the digestive tract and the rest of the body. It receives large amount of nutrients and noxious compounds entering the body through the digestive tract and portal vein. It is also the principal organ involved in the biotransformation of exogenous substances, with the capacity to covert hydrophobic substances into water-soluble products that can be secreted readily from the body. More than one thousand xenobiotic substances however, are potentially hepatotoxic.<sup>4)</sup> The ability of a chemical to produce liver damage *in vivo* often results from the interaction of a series of complex cellular processes that are involved in the uptake, biotransformation and elimination of these potentially toxic compounds.

In the developing countries, several cases of unreported adverse reactions of drug products and

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**Table 1.** Mean Daily Food and Fluid Intake, Body Weights, and Liver Weights of Drug-exposed Rats

Treatment	Food intake (g/rat)	Fluid intake (ml/rat)	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Absolute weight (g)	Relative weight (%)
Control	17.65 ± 1.06	31.47 ± 3.57	40.16 ± 1.10	180.44 ± 6.92	140.29 ± 7.25	5.80 ± 0.33	3.24 ± 0.21
Low dose	16.72 ± 0.99	26.69 ± 2.99	36.51 ± 3.51	179.23 ± 3.93	142.71 ± 5.59	5.69 ± 0.38	3.16 ± 0.14
High dose	16.73 ± 1.07	25.16 ± 2.74	41.89 ± 1.81	179.26 ± 8.11	137.37 ± 9.02	6.14 ± 0.21	3.51 ± 0.22

Values are expressed as mean ± S.E.M. for  $n = 7$ .

especially, herbal preparations abound. These are obvious results of poor record keeping, lack of or inadequate drug monitoring and ignorance on the part of many drug users. Since rinbacin has been shown to induce toxic effects on the rat testis, this study is set to investigate the effects of rinbacin on the blood parameters, liver function and histology, in rats, with a view to elucidating its toxic effects in prepubertal rats.

## MATERIALS AND METHODS

**Preliminary Studies** — Rinbacin was extracted in distilled water, at the homeopaths' recommended doses of 26.25 g/l (low dose) and 52.50 g/l (high dose). The supernatant was filtered off after 24 hr standing at room temperature, and stored in the refrigerator until use.<sup>3</sup> Phytochemical screening of the extract, for the presence of alkaloids, flavonoids, essential oils, saponin, sugars, proteins and lipids, were carried out by standard methods.<sup>5,6</sup> The method of Litchfield and Wilcoxon<sup>7</sup> was employed in the determination of the acute oral LD<sub>50</sub>.

**Experimental Design** — Three to five week old male albino Wistar rats of initial body weights of 36–42 g were divided into three weight-matched groups of seven rats each. To the different groups were administered, in the drinking fluid, 25.25 g/l (low dose), 52.50 g/l (high dose), or distilled water only, respectively. The animals had free access to the drinking solution for 13 weeks.

Food and fluid (water + drug) intake were measured daily, while the animals' body weights were taken weekly. At the expiration of the 13-week drug exposure, the animals were weighed and blood collected from the orbital sinus and by cardiac puncture. The blood was allowed to clot and the serum separated and used for the liver function tests. Bilirubin (total and conjugated) was analysed by the method of Malloy and Evelyn, alkaline phosphatase (ALP) by the method of King and Armstrong, and aspartate and alanine transaminases (AST and ALT)

by the methods of Reitman and Frankel.<sup>8</sup> The haematological tests were carried out in an ethylene diamine tetra-acetic acid (EDTA)-anticoagulated blood. Haemoglobin (Hb) concentration was analysed by the cyanmethaemoglobin method, packed cell volume (PCV) by micro-method, and white blood cell (WBC) (total and differential) and platelet counts by visual methods.<sup>9</sup> The mean cell haemoglobin concentration (MCHC) was calculated by dividing Hb by PCV.

After the blood collection, the animals were sacrificed by cervical dislocation under chloroform anaesthesia. The liver was harvested, weighed, and fixed in 10% buffered formalin for 48 hr. The tissue was processed using an automatic tissue processor, embedded in paraffin wax, and sections (5  $\mu$  thick) cut using a rotary microtome. The sections were stained by haematoxylin and eosin (H & E) method, and examined and photographed using a light microscope. Two histopathologists examined the sections, independently.

**Statistical Analyses** — Data were analysed using the analysis of variance (ANOVA), and comparison between groups carried out using the Scheffe multiple comparison method.<sup>10</sup> Significance was determined at  $p \leq 0.05$ .

## RESULTS

According to a previous report,<sup>3</sup> the drug extract gave a positive reaction for alkaloids, flavonoids and essential oils in the ratio of 3 : 2 : 1, while the LD<sub>50</sub> was found to be 3.18 ± 0.15 g/kg (i.p.). There was no significant difference in the mean fluid intake between the two dose levels of the drug (Table 1).

The initial and final body weights, and body weight gain of the different experimental groups were statistically equal, while all the animals demonstrated a progressive increase in body weight during the exposure period (Table 1). There were no significant differences in the absolute and relative

**Table 2.** Mean Liver Function Test Values of Drug-exposed Rats

Treatment	Total Bilirubin ( $\mu\text{mol/l}$ )	Conjugated Bilirubin ( $\mu\text{mol/l}$ )	ALT (IU/l)	AST (IU/l)	ALP (IU/l)
Control	14.43 $\pm$ 1.28	3.94 $\pm$ 1.01	39.42 $\pm$ 8.78	57.0 $\pm$ 13.38	176.43 $\pm$ 9.15
Low dose *	40.97 $\pm$ 5.80	13.70 $\pm$ 2.75	121.00 $\pm$ 21.35	127.43 $\pm$ 5.67	289.43 $\pm$ 19.54
High dose *	30.09 $\pm$ 4.37	8.60 $\pm$ 0.94	147.00 $\pm$ 32.80	120.29 $\pm$ 20.63	256.43 $\pm$ 16.82

Values are expressed as mean  $\pm$  S.E.M. for  $n = 7$ . \* Value significantly increased compared to corresponding control at  $p \leq 0.05$ . ALT = alanine transaminase, AST = aspartate transaminase, ALP = alkaline phosphatase.

**Table 3.** Haematological Values of Drug-exposed Rats

Parameter	Control	Low dose	High dose
Hb (g/dl)	13.77 $\pm$ 0.30	13.13 $\pm$ 0.31	13.57 $\pm$ 0.24
PCV	0.43 $\pm$ 0.01	0.44 $\pm$ 0.02	0.49 $\pm$ 0.02 *
MCHC (g/dl)	31.78 $\pm$ 0.65	30.29 $\pm$ 1.27	27.77 $\pm$ 0.85 **
WBC ( $\times 10^9$ )	4.36 $\pm$ 0.19	4.10 $\pm$ 0.41	5.13 $\pm$ 0.57
Platelet ( $\times 10^9$ )	247.86 $\pm$ 15.19	231.00 $\pm$ 11.70	232.00 $\pm$ 8.25
Neutrophil (%)	43.43 $\pm$ 4.02	42.29 $\pm$ 1.71	43.57 $\pm$ 1.25
Lymphocyte (%)	53.00 $\pm$ 4.24	54.14 $\pm$ 1.55	54.14 $\pm$ 5.23
Eosinophil (%)	1.86 $\pm$ 0.26	2.43 $\pm$ 0.43	2.14 $\pm$ 0.51
Monocyte (%)	1.71 $\pm$ 0.29	1.14 $\pm$ 0.34	0.86 $\pm$ 0.34 **
Basophilic (%)	0	0	0

Values are expressed as mean  $\pm$  S.E.M. for  $n = 7$ . \* Significantly increased compared to control at  $p \leq 0.05$ . \*\* Significantly decreased compared to control at  $p \leq 0.05$ .

liver weights of the animals in the three experimental groups.

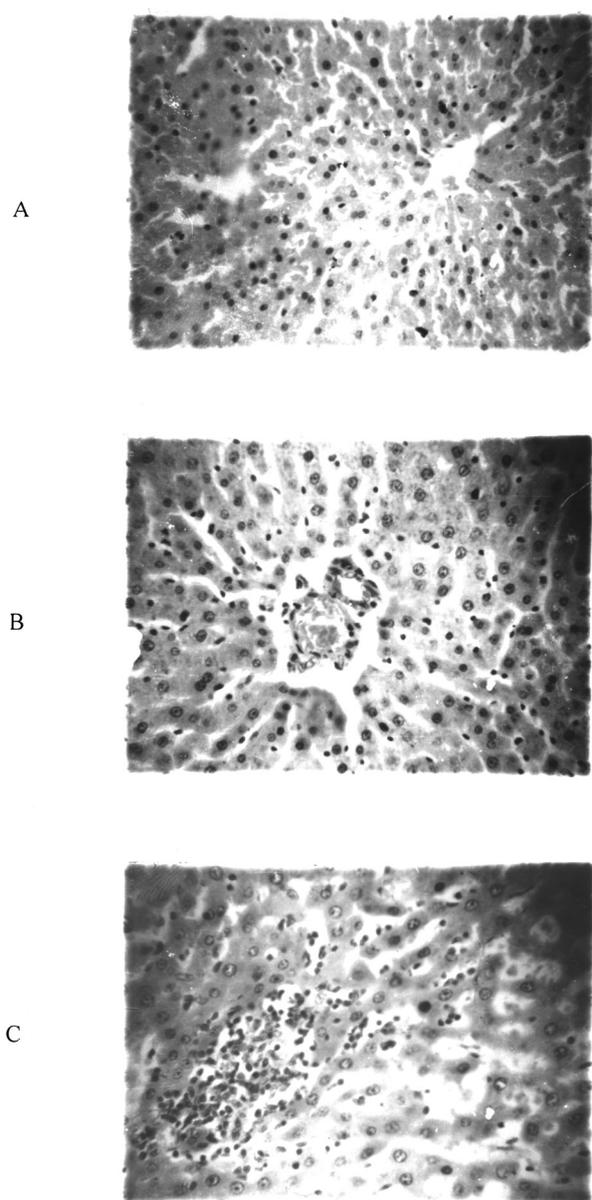
Table 2 shows the mean liver function test values of the rats. From the table, both the low and high dose levels of the drug significantly ( $p \leq 0.05$ ) increased all the biochemical parameters compared to the control. The low dose however, caused a non-significant increase in all the parameters more than the high dose, except the ALT, which was increased more in the high dose group (Table 2).

From Table 3, there were no statistical differences in the haematological parameters, between the different treatment groups, except for the high dose level of the extract, which significantly ( $p \leq 0.05$ ) decreased the MCHC and percent monocyte count, and significantly ( $p \leq 0.05$ ) increased the PCV.

The histological changes of liver in the different treatment groups are shown in Fig. 1. From the figure, the control group showed normal histological structure (Fig. 1A). The low dose treatment induced severe degeneration and necrosis of hepatocytes, moderate oedema, and minimal cellular infiltration and chromatinolysis (Fig. 1B). The high dose level exhibited severe degeneration and necrosis of hepatocytes, oedema, and cellular infiltration. There were also moderate nuclear fragmentation and chromatinolysis in the high dose group (Fig. 1C).

## DISCUSSION

The acute oral toxicity testing of the drug extract (3.18 g/kg) gives an indication that the drug is safe. At present the following chemical labeling and classification of acute systemic toxicity based on oral LD<sub>50</sub> values are recommended by the Organisation for Economic Co-operation and Development (OECD, Paris, France): very toxic,  $\leq 5$  mg/kg; toxic,  $> 5 \leq 50$  mg/kg; harmful,  $> 50 \leq 500$  mg/kg; and no label,  $> 500 \leq 2000$  mg/kg.<sup>11</sup> It has, however, been reported, that an LD<sub>50</sub> cannot be described in terms of accuracy, only of precision, adding that the precision is only relevant for the experiment for which the LD<sub>50</sub> was derived and does not increase the probability that in subsequent experiments the LD<sub>50</sub> will be identical or even similar. The median lethal dose is not an absolute value but is an inherently variable biologic parameter that cannot be compared to constants such as molecular weight or melting point.<sup>12</sup> It does not necessarily guarantee the safety of the tested agent notwithstanding its value. Even with such a high LD<sub>50</sub> value, rinbacin has been reported to induce toxic effects to the rat testis.<sup>3</sup> Phytochemical screening of the drug shows that the drug contains alkaloids, flavonoids and essential oils, chemical substances known to possess a wide range of



**Fig. 1.** Photomicrographs of Rinbacin — Exposed Rat Liver Stained with H & E

(A) Control showing normal liver parenchyma, (B) Low-dose exhibiting degeneration of hepatocytes and necrosis, (C) High-dose presenting with severe necrosis, degeneration of hepatocytes, and cellular infiltration. *Magnification X200.*

pharmacological activities.<sup>13)</sup>

Rinbacin did not exert significant effects on the mean daily food and fluid intake, final body weight and body weight gain, and absolute and relative liver weights of the rats (Table 1). All the serum biochemical parameters measured in this study were significantly raised at both dose levels, with the low dose showing a greater but non-significant increase than the high dose, except for the ALT (Table 2). The liver is the principal organ involved in biotransformation

of exogenous substances, and therefore is involved in a lot of enzyme synthesis that enable it to carry out its functions. Liver enzymes are usually raised in acute hepatotoxicity but tend to decrease with prolonged toxication due to damage to the liver cells.<sup>14)</sup> The greater increase in liver enzymes observed in the low dose group could be attributed to adaptive resistance and/or damage to hepatocytes at the high dose level, and hence inability to synthesize further enzymes. This effect is similar to that observed by Bruckner and co-workers.<sup>15)</sup>

Increased organ weight (either absolute or relative) has been observed as a sensitive indicator of organ toxicity by known toxicants.<sup>16)</sup> The present work however, did not agree with this observation, as there was no increase in either the absolute or relative weights of the organ even with the increase in the biochemical parameters, which are indications of hepatotoxicity. Other works have, however, shown induction of liver damage through elevation of liver enzymes. In a study on Beagle dogs, a novel lipid was shown to induce hepatocellular hypertrophy, with ALT and AST being elevated, while there was a 2–3-fold increase in cytochrome P<sub>450</sub> content of hepatic microsomes.<sup>17)</sup> Oral administration of human immunodeficiency virus (HIV) protease inhibitor (L-689502) was shown to cause cholestasis and hepatocytic injuries in rats and dogs, with elevation in serum transaminases observed as early as 6 hr after dosing in dogs.<sup>18)</sup> Dalbey and Feuston,<sup>19)</sup> on the other hand, observed an increase in liver weights of rats exposed to diisopropyl ether, without any increase in biochemical parameters.

Presence of cellular abnormalities like basophilic stripping, Heinz inclusion bodies and ferritin/haemosiderin deposition on red cells are suggestive of haemotoxicity. Decrease or increase in cell counts, and depletion of plasma constituents or their elevation beyond reference range could equally demonstrate haemotoxicity. Rinbacin produced only a significant increase in the haematocrit level (PCV); with a decrease in the MCHC and percent monocyte count at the high dose level (Table 3). The high PCV level invariably resulted in the decrease in MCHC since the Hb level did not differ significantly. Blood is produced principally in the bone marrow, and, at the early stages of life, also in the liver and spleen, and destroyed mainly in the liver to yield bilirubin. Kirk *et al.*,<sup>20)</sup> using 1,2-dichloropropane in a developmental toxicologic study in rats and rabbits, demonstrated a decrease in red cell count, PCV, and Hb concentrations, with an increase in WBC, platelet and reticu-

locyte counts. It is not deducible from this study that rinbacin is haemotoxic.

Exposure to rinbacin caused pathologic changes in prepubertal rats, which included degeneration and necrosis of hepatocytes, oedema, cellular (mononuclear cell) infiltration of the portal tracts, nuclear fragmentation and chromatinolysis. These changes were more severe in the high than low dose level. Other works using halofantrine (20 mg/kg) administered subchronically resulted in focal granuloma, necrosis, haemorrhage and dilation of sinusoids, while fluconazole was shown to cause hepatocellular damage, cholecystic injury and cirrhosis.<sup>21)</sup>

Mechanism of action of hepatocellular toxicity by rinbacin may be mediated by lipid peroxidation. Arthemeter (universal antimalaria of Chinese origin) has been shown to cause hepatocellular injury through free-radicals-mediated high-level lipid peroxidation.<sup>22)</sup> Whatever the agent responsible for injury, the reaction of liver involves a common sequence of events that can be analysed at the tissular, cellular, and molecular levels. It is however, difficult *in vivo* to distinguish the primary effects of a compound from those induced secondarily, because liver functions are under the influence of various endogenous and exogenous factors that result in complex interactions with other organs.<sup>4)</sup> Most of the understanding of liver injury induced by chemicals remain confined to animal models, and data obtained in animals cannot be extrapolated with certainty to the human situation. Because of the drawbacks of *in vivo* studies of drug-and chemical-induced hepatotoxicity, *in vitro* liver systems may be approached to investigate mechanisms by which rinbacin induces liver damage.

Rinbacin increases the PCV, bilirubin and liver enzyme levels of rats. It also induces histopathologic changes in rats. Based on the above findings, caution should be employed in the administration of rinbacin; while epidemiological studies involving individuals taking the drug is called for. Rinbacin is toxic to the prepubertal rat liver.

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