Effects of Rinbacin Extract on Rat Kidney

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The aqueous leaf extract of rinbacin was tested for toxic effects on prepubertal rat kidneys following chronic administration. Two doses of rinbacin extract (26.25 g/l and 52.50 g/l) were administered in the rats’ drinking water for 13 weeks, and various toxicologic indices tested. Histological study of the kidneys was also carried out at the expiration of the test period. Rinbacin at both dose sizes significantly (p<0.05) increased the absolute and relative kidney weights. Also the serum HCO₃⁻ level was significantly (p<0.05) increased, while the serum K⁺ level was decreased significantly at both dose levels. Only the high dose significantly (p<0.05) increased the serum urea level of the rats. Histological study showed that rinbacin at both dose sizes caused renal pathologic changes, which included necrosis and cellular infiltration of glomeruli and epithelia of the tubules. The effects were less marked in the low dose than the high dose group. Chronic administration of rinbacin extract induces nephrotoxicity in young rats.

Key words rinbacin; renal function; nephrotoxicity; medicinal herb; prepubertal rat

There is little question that many medicinal herbs have preventive and/or therapeutic effects. Thus, like any therapeutic agent, when overdosed or incorrectly used they also have the potential to induce adverse effects. The historic role of medicinal herbs in the treatment and prevention of disease, and their role as catalysts in the development of pharmacology do not, however, assure their safety for uncontrolled use by an uninformed public. There has been minimal research to address possible adverse reproductive, immunologic, or neurologic effects or even systemic toxicity and/or carcinogenicity that might be associated with high doses or prolonged use of these products. This concern was frequently expressed at the International Workshop to Evaluate Research Needs on the Use and Safety of Medicinal Herbs, where it was strongly emphasized that medicinal herbs could not be assumed safe because they are “natural.”

Rinbacin is a greenish powder used as an herbal remedy and homeopathic medicine, and is registered in Nigeria (Reg. No. 016283). It is quoted (in the leaflet contained inside the pack) as consisting of 48% roots, 18% seeds, 22% leaves, and 12% flowers. It is manufactured (by Mabro Homeopathic Products Ltd.) in Aba, Nigeria. There are claims by homeopaths that rinbacin is used for treating various disease conditions, notable among which are bacterial infections and diabetics.

It has long been known that the developing fetus, the young, and the elderly may be more sensitive to the effects of certain drugs than the general population. In most instances, these sensitive subpopulations show an elevated or adverse response as a result of compromised capacity to metabolize the respective drugs. The fact must be recognized that not all compounds are equally toxic to all parts of a living system because the toxic actions of many compounds are manifested in specific organs. Many chemicals, however, affect the kidney, but because of its large functional reserve, which masks signs of dysfunction, early diagnosis of renal disease is often difficult.

There are fears that the wide use of rinbacin may not be unconnected with some of the unreported adverse effects noticeable among users, since the extract is administered without any toxicologic study or monitoring. Recently, Orisakwe and co-workers have shown that the extract can induce toxic actions on rat testis. There is therefore, a need to study the effects of this extract on the kidney, considering the significant role of this organ in the excretion of most pharmacologic agents. The present study therefore, was undertaken to study the effects of chronic administration of rinbacin extract on the renal function and histology of young rats.

MATERIALS AND METHODS

Preparation of Extract The extract was dissolved in water at the doses of 26.25 g/l (low dose) and 52.50 g/l (high dose), as recommended by the homeopaths.

Acute Toxicity Study LD₅₀ was determined intraperitoneally by the method of Litchfield and Wilcoxon.

Phytochemical Analysis Presence of alkaloids, flavonoids, saponin, essential oils, sugar, protein and lipids were tested for using the methods of Trease and Evans, and Odebiyi and Sofowora.

Animals Male albino rats aged 3 to 5 weeks and weighing between 36 and 42 g were obtained from the Toxicology Unit of the Department of Pharmacology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Nigeria. The animals were housed singly in a cross-ventilated room at a temperature 22±3°C and 12 h light/12 dark cycle. They were fed with standard rat pellets (Pfizer Pharmaceuticals PLC, Ikeja, Nigeria), and water ad libitum.

Experimental Design The animals were divided into three groups of seven rats each. These were administered 26.25 g/l (low dose), 52.50 g/l (high dose) and distilled water only (control), respectively. The extracts were given in the drinking water of the animals for a period of 13 weeks, and the animals had free access to the drinking solution.
out the experimental period. The volume of fluid ingested, and weight of food consumed were measured daily, while the body weight of the animals was taken weekly.

**Renal Function** At the expiration of the study period, the animals were weighed, and blood taken from the orbital sinus for the following renal function tests: serum electrolytes, blood urea nitrogen (BUN), and creatinine. Na\(^+\) and K\(^+\) were estimated by the flame photometric method, Cl\(^-\) and HCO\(_3\)\(^-\) by the titrimetric method, urea by the diacetyl monoxime method, and creatinine by the alkaline picrate method.\(^9\)

**Histology** After the blood collection, the animals were killed and the kidneys harvested, weighed and fixed in 10% buffered formalin for 48 h. These were processed using an automatic tissue processor, embedded in paraffin wax, and 5 \(\mu\) thick sections cut with a rotary microtome. The sections were stained by the haematoxylin and eosin (H & E) method, and two histopathologists independently assessed the pathologic changes. Photomicrographs of the sections were taken using a Leitz photographing microscope.

**Statistics** Data were analysed using one-way analysis of variance (ANOVA), and post-hoc tests done with the Scheffe method.\(^10\)

**RESULTS**

According to Orisakwe et al., the drug extract was shown to contain 50% alkaloids, 33.3% flavonoids and 16.7% essential oils. There were no saponin, sugars, proteins or lipids to contain 50% alkaloids, 33.3% flavonoids and 16.7% essential oils.\(^3,11\)

From Table 1, the aqueous extract did not elicit any significant effect on the food and fluid intake, or the body weight gain of the animals. The absolute and relative weights of the animals’ kidneys showed a significant \((p<0.05)\), dose-dependent increase compared to the control.

Figure 1 shows the effect of the drug extract on serum urea and creatinine levels of rats. It is evident that the high dose significantly \((p<0.05)\) increased the BUN level compared to the control. The low dose was statistically equal to the control. There were no significant changes on the creatinine levels at either dose. Also, the Na\(^+\) and Cl\(^-\) levels of the animals were statistically equal to the control. However, K\(^+\) levels were significantly \((p<0.05)\) decreased, while the HCO\(_3\)\(^-\) levels were significantly \((p<0.05)\) increased with both dose sizes, compared to the control (Table 2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Food intake (g/rat/d)</th>
<th>Fluid intake (ml/rat/d)</th>
<th>Body weight gain (g)</th>
<th>Absolute weight (g)</th>
<th>Relative weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17.65±1.06</td>
<td>31.47±3.57</td>
<td>140.29±7.25</td>
<td>0.56±0.03</td>
<td>0.32±0.02</td>
</tr>
<tr>
<td>26.25 g/l</td>
<td>16.72±0.09</td>
<td>26.69±2.99</td>
<td>142.71±5.59</td>
<td>0.74±0.04*</td>
<td>0.42±0.03*</td>
</tr>
<tr>
<td>52.50 g/l</td>
<td>16.73±1.07</td>
<td>25.16±2.74</td>
<td>137.37±9.02</td>
<td>0.92±0.02*</td>
<td>0.52±0.02*</td>
</tr>
</tbody>
</table>

Values are expressed as mean±S.E.M. \((n=7)\). \(* p<0.05\).  \(\dagger\) Significant decrease relative to control \((p<0.05)\).

Fig. 1. Effect of Rinbacin Extract on Serum BUN and Creatinine Levels

Table 2. Effect of Rinbacin Extract on Electrolyte Levels (in nmol/l) of Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Na(^+)</th>
<th>K(^+)</th>
<th>Cl(^-)</th>
<th>HCO(_3)(^-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>123.86±2.50</td>
<td>4.49±0.17</td>
<td>88.00±4.92</td>
<td>6.29±0.64</td>
</tr>
<tr>
<td>26.25 g/l</td>
<td>123.29±1.27</td>
<td>4.03±0.16*</td>
<td>91.71±1.27</td>
<td>19.14±2.80*</td>
</tr>
<tr>
<td>52.50 g/l</td>
<td>127.71±2.67</td>
<td>4.09±0.11*</td>
<td>96.00±1.54</td>
<td>16.00±2.47*</td>
</tr>
</tbody>
</table>

Values are expressed as mean±S.E.M. \((n=7)\). \(\ast\) Significant increase relative to control \((p<0.05)\). \(\dagger\) Significant decrease relative to control \((p<0.05)\).

**DISCUSSION**

This study investigated the effects of rinbacin, an herbal medicine used for a wide range of disease conditions in Nigeria, on the renal function tests and histology of the rat kidney. The results showed that rinbacin at the doses of 26.25 g/l, and 52.50 g/l \((p.o.)\), did not have any effect on the food and fluid intake or the body weight of rats. But the kidney weight and its relativity to the body weight were increased significantly \((p<0.05)\) from the control group. There were significant increases in the bicarbonate ion and BUN levels, while potassium ion was reduced. Histological studies indicated that rinbacin induced some toxic actions on the rat kidney.

Some major concerns regarding the marketing and use of medicinal herbs are those of standardization and stability.\(^3,11\)

Unlike pharmaceuticals, botanical products are complex mixtures in which the active ingredients may not be known or may constitute only a small percent of the total product.
Some products are, in fact, believed to achieve their beneficial effects through the combined actions of several chemical ingredients, each of which accounts for a very small portion of the total product. Rinbacin extract was found to contain alkaloids, flavonoids and essential oils. These biological substances are known to have a wide range of pharmacological activities. The manner in which medicinal herbs are produced complicates quality control. They are gathered in the wild, or grown in relatively small plots. Thus, the active ingredients may vary significantly with the part of the plant used, the season in which it is harvested, and/or the growing conditions (e.g., weather, soil etc.). Rinbacin is a mixture of roots, leaves, flowers and seeds, and these make for potential misidentification or cross contamination of the active ingredients.

Toxic agents may affect the kidney and impair its physiological functions. These effects are detectable and/or quantifiable by cross checking the normally expected functions of the kidney in excreting the non-threshold nitrogenous waste products of metabolism like urea and creatinine. Also, by determining the ability of the kidney to filter and reabsorb the body-needed threshold substances like electrolytes, toxicity to the kidney may be detected. Some authors have demonstrated that estimation of urea and creatinine levels is not sensitive enough in detecting a low level of renal toxicity or damage. Others have observed the absolute kidney weight to be a relatively sensitive indicator of nephrotoxicity for known nephrotoxicants. Nephrotoxicity has, therefore, been defined as increased kidney weight (either absolute or relative) coupled with a significant alteration in at least one serum parameter. In the present study, rinbacin extract altered significantly (p<0.05) the absolute and relative kidney weights, and also the serum urea, K and HCO\textsubscript{3} of the rats. Rinbacin, by this indicator is therefore, nephrotoxic in prepubertal rats.

In a therapeutic study (unpublished article), rinbacin was found to lower the serum glucose level of rats. Some antidiabetic agents are known to deplete potassium. Potassium was significantly (p<0.05) reduced by rinbacin at the two doses used in this study. Regeneration of H\textsuperscript{+} to aid reabsorption of HCO\textsubscript{3} is a renal tubular function, and potassium depletion equally forces renal tubular regeneration of HCO\textsubscript{3}, hence the increase in HCO\textsubscript{3} level observed in this study by rinbacin. It may thus be said that the raised plasma HCO\textsubscript{3} level was due to acidosis accompanying rinbacin-induced potassium depletions.

Necrosis and cellular infiltration were noticed in some of the glomeruli and renal tubules of the rats in the high dose group. The glomeruli, because they receive one-quarter of the cardiac output and are perfused at the highest pressure of any capillary bed in the body, are vulnerable to injury by a number of drugs and other toxic agents. These agents may lead to damage by one of two basic mechanisms: 1) direct, dose-related toxic injury; 2) indirect, immunologically mediated injury, largely dependent on dose. The renal damage observed histologically well correlated with the biochemical indices of toxicity described above, and agreed with Fowler et al. Kidneys from the control animals showed normal renal morphology. Because medicinal herbs are usually self-prescribed by the consumers, recommendations for the use of prescribed drugs like dose, manner, and frequency of administration, which are reviewed and controlled by the prescribing physician, are lacking. These factors increase the risk of toxicity of rinbacin, as consumers use the extract for as long as their conditions last, and even at increased doses.

The chemicals in medicinal herbs may be formed naturally in the plant, but they are not natural to the human body. Any compound with a therapeutic effect has the potential to be incorrectly prescribed or overdosed. This study has indicated the toxic effects of rinbacin to the kidney of young rats. In most instances, sensitive sub-populations like the developing fetus, the young, and the elderly show an elevated or adverse response to certain drugs, as a result of compromised capacity to metabolize such drugs or to an allergic response. This necessitated the choice of prepubertal rats, a sensitive sub-population, in the study.

Isolation and characterization of the active compound(s) responsible for the nephrotoxic action of rinbacin in rats need to be elucidated. This is quite necessary, as medicinal herbs have been known to contain a variety of chemicals that are toxic when consumed at high doses and for which little is known about their chronic toxicity. Reports of adverse or chronic toxic effects of rinbacin are scarce, as medicinal herbs are not prescribed by physicians or dispensed by pharmacists. This makes documentation of adverse effects related
to their use largely limited to those reports associated with overdose and allergic reactions.

There is the need for studies in humans taking rinbacin, so as to establish the effects of this extract in humans. This is necessary, as some agents with known adverse effects in animals have been shown not to exert similar effects in man, whereas some other agents without serious adverse effects in animals may show severe reactions in man. Rinbacin, at the doses of 26.25 g/l and 52.50 g/l is toxic to prepubertal rat kidneys. This nephrotoxic action, however, calls for further studies.

REFERENCES