

## **The effects of *Rauwolfia vomitoria* extract on the liver enzymes of mercury induced hepatotoxicity in adult wistar rats**

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### **Abstract**

*Rauwolfia vomitoria* is a natural medicinal plant which has been used over the years for the treatment of various ailments. This work focuses on the effects of extract of *Rauwolfia vomitoria* on liver enzymes of mercury induced hepatotoxicity were observed in twenty adult wistar rats weighing between 120g and 210g. They were divided into four groups A,B, C and D of five rats each. Group A served as the control and received 0.5ml of distilled water. The experimental groups B, C and D received different doses of drugs as follows: group B received 0.6ml of *Rauwolfia vomitoria* extract, group C received 0.5ml of mercury and group D received 0.5ml of mercury and 0.6ml of *Rauwolfia vomitoria* extract. The drugs were administered once in a day using intubation method for a period of thirty four days. Twenty four hours after the last administration, the animals were anaesthetized under chloroform vapour and dissected. liver tissues were removed and weighed. Blood samples were collected by cardiac puncture and Serum samples were separated from clot by centrifugation using bench top centrifuge. Activities of serum aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were determined using randox kit method. The relative liver weight for mercury treated group were significantly higher ( $p<0.001$ ) than the control and groups B and D. serum aspartate aminotransferase, alanine aminotransferase and alkaline phosphate level of group C were statistically higher ( $p<0.001$ ) than the control. The extract exhibited a liver protective effect against mercury induced hepatotoxicity.

**Key words:** Liver enzymes, mercury, Liver weight, *Rauwolfia vomitoria*, Wistar rats

### **1. Introduction**

Liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy production and reproduction. Because of its unique metabolism and relationship to the gastrointestinal tract, the liver is an important target for toxicity produced by drugs, xenobiotics and oxidative stress.<sup>6</sup> More than 900 drugs, toxins and herbs have been reported to cause liver injury and drugs account for 20–40% of all instances of fulminant liver failure.<sup>1</sup>

One of the plants of medicinal value from the humid tropics is *Rauwolfia Vomitoria*. It is traditionally used in treatment of variety of ailment such as snakebites, fever and nervous disorders. In the absence of reliable liver protection drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders and quite often claimed to offer significant relief. Attempts are being made globally to get scientific evidences for these traditionally reported herbal drugs. This scenario provides a severe necessity to carry out research in the area of hepatotoxicity<sup>1</sup>.

From 1931, Indian doctors researched on possible utilization of *Rauwolfia vomitoria* in neuro-psychiatry. The

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extract from this plant was first extracted by Swiss chemists in 1952 and becomes the first natural neuroleptic. Today, this plant is still the source of a lot of drugs used in psychiatry<sup>5</sup>. In traditional medicine, the roots and leaves of *Rauwolfia vomitoria* are brewed as tea and used in humans for treatment of hypertension, insanity, snakebite and cholera<sup>9</sup>.

Mercury is a toxic heavy metal which is widely dispersed in nature. Most human exposure results from fish consumption or dental amalgam<sup>11</sup> known to induce liver damage. Hence, this study aims at painstakingly investigating the effects of *rauwolfia vomitoria* extract on liver enzymes of mercury induced hepatotoxicity in adult Wistar rats.

## 2. Materials And Methods

**2.1 Breeding of Animals:** Twenty four Wistar rats were obtained from the animals house of the Pharmacy Department, Nnamdi Azikiwe University Agulu, Anambra state, Nigeria and bred in the Animal house of Nnamdi Azikiwe University, Nnewi campus, Anambra State, Nigeria. They were allowed for a period of ten days for acclimatization under normal temperature (27°C -30°C) before their weights were taken. They were fed with water and guinea feed pellets from Agro feed mill Nigeria Ltd.

**2.2 Drug Preparation:** *Rauwolfia vomitoria* leaves were collected from Eket in Akwa Ibom State and was dried in an oven at a temperature of 50°C and crushed using laboratory blender. Extraction was done using ethanol. Ethanol was poured into the grinded leaf of *Rauwolfia vomitoria* and was allowed to stay for twenty four hours. It was filtered into a stainless basin with a white cloth and placed in a water bath so as to dry up the ethanol. 300mg of this extract /kg body weight was dissolved in 10mls of distilled water and administered to the animals.

Mercury was obtained from the Department of Biochemistry, Nnamdi Azikiwe University, Nnewi campus, Anambra State, Nigeria.

**2.3 Experimental Protocols:** The twenty animals were weighed and allocated into five groups of four animals each. The groups were designated as groups A, B, C, and D. Group A animals served as the control and received 0.5ml of distilled water. The experimental groups B, C and D received different doses of drugs as follows: Group B received 0.6ml of extract of *Rauwolfia vomitoria*, Group C received 0.5ml of mercury, Group D received 0.6ml of extract + 0.5ml of mercury. The drugs were administered once in a day between the hours of 12-3.30pm for a period of thirty four days. The drugs were administered orally using intubations method. After the twenty first day, the animals were weighed and their weight recorded.

Twenty four hours after the last administration, the animals were anesthetized under chloroform vapour and were dissected. Blood samples were collected by cardiac puncture using sterile syringes with needles. Blood for serum preparation was collected into sterile plain tubes without an anti-coagulant. Serum samples were separated from the clot by centrifugation at 3,000rpm for 5minutes using bench top centrifuge (MSE, Minor, England). Serum samples were separated into sterile plain tubes and were stored in the refrigerator for analysis. Liver tissues were removed from the animals and weighed. The activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined using randox kit method.

## 3. Results

**3.1 Morphometric Analysis of Body Weights:** The result obtained from calculation of initial, final and weight change of the various groups are presented in table 1.0 below

**Table 1.0: Comparison of mean initial and final body weight and weight change in all the groups (A,B,C &D)**

	Group A	Group B	Group C	Group D	F-Ratio	PROB.OF SIG.
Initial Body Weight	110.60±4.64	117.50±6.62	136.62±6.64	124.43±6.64	67.220	<0.0001
Final Body Weight	116.74±5.40	129.64±5.70	123.72±5.53	129.75±8.01	30.510	<0.0001
Weight Change	6.14±6.67	12.14±6.18	13.90±5.01	8.25±5.67	17.150	<0.0001

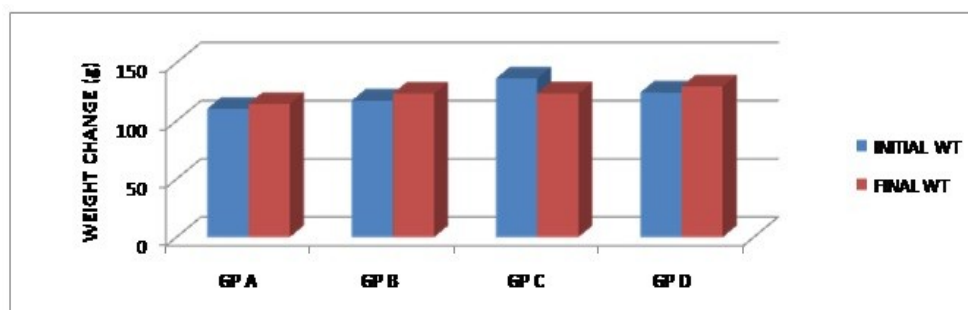
(Mean ± SEM given for each measurement)

The final body weight for group A (Control), groups B, C, and D showed a statistically significant decrease (P<0.001)

The final body weight for group C treated with mercury was significantly higher ( $P<0.001$ ) than the control and other experimental groups (B and D) animals. The weight change for group C showed a statistically increase compared with the control and other experimental groups ( $P<0.001$ ).

Figure 1.0 are the bar chat representation of the mean initial and final body weight. After the administration, the weight of animals in group A (control) and group B, and C increased statistically.

Figure 1.0 is the bar chat representation of the mean initial and final body weight.



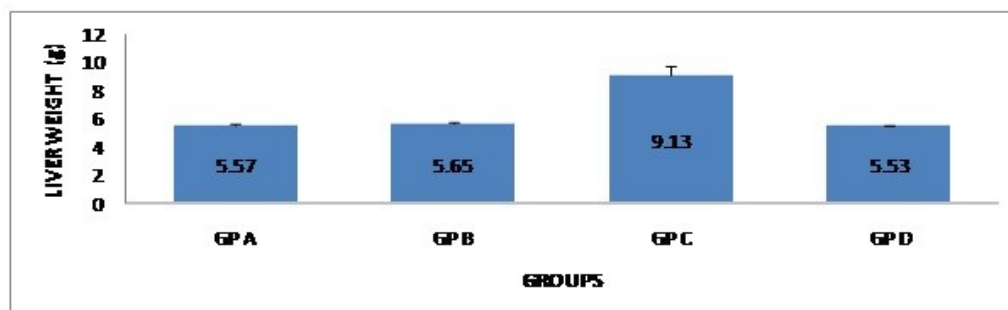
**3.2 Morphometric Analysis of Liver Weights:** The results obtained from calculations of relative liver weight of the various groups are presented in table 2.0. The relative liver weight for group C (mercury administered) were significantly higher ( $P<0.001$ ) than that of the group A (control) and other experimental groups (B, and D). The values for groups B and D were similar to the group A (control)

Table 2.0: comparison of mean relative liver weight for group A (control) and experimental groups (B,C and D)

	Group A	Group B	Group C	Group D	F-Ratio	PROB. OF SIG.
Liver Weight	5.57±0.045	5.65±0.161	9.13±0.625	5.53±0.070	53.64	<0.0001

(Mean ± SEM given for each measurement)

Figure 2.0. The bar chart representation of the relative liver weight of the various groups.



### 3.3 Activities of serum levels of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP)

Table 3.0 Effects of *Rauwolfia vomitoria* on biochemical Parameters

Biochemical Parameters	Group A	Group B	Group C	Group D	F-Ratio	PROB. OF SIG.
Aspartate Aminotranse- Ferase (AST)	83.80±28.55	79.75±13.51	129.15±3.20	92.66±5.77	60.04	<0.0001
Alanine Aminotranse Ferase (ALT)	45.01±15.74	34.56±11.12	75.43±7.01	41.33±8.08	12.30	<0.0001
Alkaline Phosphotase (ALP)	381.39±31.22	299.93±37.83	473±46.75	337.21±161.29	7.58	< 0.0012

(Mean -+SEM given for each measurement)

From the results obtained from calculations of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), there was a significant decrease ( $P < 0.001$ ) in the AST activity levels at all doses of the drugs relative to the control (A) except in group C treated with mercury. The group C activity level statistically was significantly higher ( $P < 0.001$ ) than the control (A) and other groups.

The alanine aminotransferase (ALT) activity levels showed a significant decrease ( $P < 0.001$ ) in groups B and D relative to the control (A) except in group C treated with mercury. The alkaline phosphatase (ALP) level in group C was significantly higher than the control group (A) and other groups.

The alkaline phosphatase (ALP) activity levels in groups B and D were significantly lower ( $P < 0.001$ ) than the control (A). The alkaline phosphatase activity levels in group C were significantly higher ( $P < 0.001$ ) than the control (A) and other groups.

### 3. Discussion

The results of this study agree with previous researchers that mercury has a toxicological effect on the liver enzymes of wistar rats. There was no significant difference ( $P < 0.001$ ) in the serum and tissue levels of AST, ALT and ALP in groups B and D compared with the control as shown in table 2.0. There was a significant difference ( $P < 0.001$ ) in the serum and tissue levels of AST, ALT and ALP in group C compared with the control and groups B and D. These results indicated that the extract from *Rauwolfia vomitoria* did not bring about cellular damage in the liver during the experimental period. Enzyme activities in the serum and tissues are often used as “marker” to ascertain toxic effects of administered foreign compounds to experimental animals [3]. ALP is a membrane bound enzyme [10] while ALT and AST are cytosolic enzymes [2]. These enzymes are highly concentrated in the liver and kidney and are only found in serum in significant quantities when the cell membrane becomes leaky and even completely ruptured [4,8]. A rise in serum level or decrease in tissue level of these intracellular enzymes is an index of damage to the liver cells [7].

Observation of the body weight difference in groups reveals a gradual increase in weight of animals for the control group A. This could have been physiological as the only substance they were exposed to was water and food. Comparing the results of weight difference reveals severe loss of weight by the mercury exposed group. This is probably as a result of loss of appetite by the animals in the group. The groups that were treated with extract of *Rauwolfia vomitoria* only, extract of *Rauwolfia vomitoria* and mercury, showed an increase in weight which is similar to the control group. Extract of *Rauwolfia vomitoria* in this instance functions primarily as a dietary supplement enhancing growth. Previous researches cited in literatures of *Rauwolfia vomitoria* did not state pre and post experimental weight, hence weight changes were not determined in their works.

The relative weights of the organ also showed significant differences in groups. There was a relative increase in liver weight for the mercury exposed animals compared to the control. This organ weight increase was irrespective of the fact that there was total body weight loss. This could have been pathological and one may deduce that the increase in liver weight was not growth but inflammation. Antioxidant properties of *Rauwolfia vomitoria* could have been responsible for the control or prevention of inflammation in the groups treated with them.

The animals in group D give a particularly interesting observation about the dynamics of reactions to the presence of various substances in our systems. On administration of extract of *Rauwolfia vomitoria* respectively to the groups, the animals showed an increase in overall body weight similar to that of the control. Administration of extract of *Rauwolfia vomitoria* alone did not cause weight loss to the animals compared with the animals in control group B. By these observations one may deduce that administration of extract of *Rauwolfia vomitoria* may boost the tolerance capacity for mercury induced toxicity.

### 4. Conclusion

The extract of *Rauwolfia vomitoria* did not induce adverse alterations in biochemical parameters of serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) and no histopathological lesions were observed in the liver tissues of the rats. This study has demonstrated the potential ability of *Rauwolfia vomitoria* to protect against mercury induced toxicity in the liver enzymes of rats. The findings of this study suggest that *Rauwolfia vomitoria* administered to individuals exposed to mercury poisoning could provide some protection against its effects on the liver enzymes.

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