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EFFECT OF *RANA GALAMENSIS*–BASED DIET ON THE ACTIVITIES OF SOME ENZYMES AND HISTOPATHOLOGY OF SELECTED TISSUES OF ALBINO RATS

Basiru Olaitan Ajiboye, Nasir Olarewaju Muhammad, Oluwafemi Adeleke Ojo

ABSTRACT

The effect of *Rana galamensis*-based diet on the activities of some enzymes and histopathology of selected tissues of albino rats was investigated for eight weeks. A total of sixteen albino rats weighing between 29.15 and 26.01g (21 days old) were divided into two groups. The first group contains animals fed on casein–based diet (control); the second group was fed on *Rana galamensis*–based diet. The animals were fed with their appropriate diet on daily basis and on the eight weeks of the experiment the animals were sacrificed using diethyl ether as anesthesia, blood was collected by cardiac puncture and organs of interest were harvested. Thereafter, organ to body weight ratio, some biochemical parameters and histopathology examination were carried out. There was no significant difference (p >0.05) in the organ to body weight ratio of the animals fed on control and *Rana galamensis*–based diets. Also, there was no significant different (p >0.05) in the activities of all the enzymes (ALP [alkaline phosphatase], AST [asparate transaminase], ALT [alanine transaminase], and γGT [gamma glutamyl transferase]) investigated in the selected tissues and serum of rats fed on *Rana galamensis*–based diet when compared with the control. In addition, histological examinations of hepatocyte's rats fed on *Rana galamensis*– based diet show normal architecture structure when compared with the control. The insignificant different in the activities of all the enzymes studies (ALP, AST, ALT and γGT) indicated no organ damage, supported by the normal histology studies. The obtained results may imply that *Rana galamensis* is safe for consumption.

Keywords: *Rana galamensis*, ALP, AST, ALT, γGT, histological examination

INTRODUCTION

Food has been one of the most important sources of life to both human and livestock, that is "no food no life". Foods are substances which are capable of producing energy, promoting growth, and repairing of tissues **(Naik, 2011)**. The chemical components of food which perform these functions are called nutrients **(Murray et al., 2009)**. One of the important classes of food or nutrient is protein. The biological roles of this macronutrient in the system includes cells growth, enzymes, antibodies (also called immunoglobulin) which are specific protein produced by specialized cells of the immune system in response to foreign antigen, transport materials from one place to another in the body (for example transport of iron from the liver to the marrow), hormones or regulatory protein (for example insulin and glucagons) and to provide energy during starvation **(Naik, 2011)**. They are the basis of many animals bodies structure (for examples muscle, skin and hair). Inadequate intakes of protein cause protein-energy malnutrition **(Harvey and Ferrier, 2010)**.

Most people cannot afford the exorbitant prices of egg, milk, meat and fish **(Oloyede and Fowomola, 2003)**. Thus, it is important for the nutritionist to search for alternative source of high protein quality. One of the most readily available and cheap sources of protein is *Rana galamensis* **(Muhammad and Ajiboye, 2010)**. *Rana galamensis*, otherwise known as edible toad, belongs to the family of *Ranidae*, which has widest distribution of any frog family, and the class amphibian. Its common name is galam white-lipped frog. *Rana galamensis* is abundant throughout most of the continents except Antartica. In Africa they are found in savannah region of West Africa, South Africa and East Africa **(Ajiboye and Muhammad, 2015; Ajiboye et al., 2014)**. In Nigeria, they are found especially in Lagos State, Ogun State, Oyo State, Kwara State, Osun State, Ondo State, Ekiti State, ZariaCity and Benin City **(Muhammad and Ajiboye, 2010)**. They are strongly aquatic species in savannah area, where they live in and around permanent lakes, rivers, ponds and swamps **(Ajiboye et al., 2014)**. But information on the effect of *Rana galamensis*–based diet on the activity of some enzymes and histopathology of selected tissues of albino rats is still very scanty in literature. Therefore, the objective of this study was to determine effect of *Rana galamensis*–based diet on the activity of some enzymes and histopathology of selected tissues of albino rats.

MATERIAL AND METHODOLOGY

Rana galamensis

These were purchase from 'Oja Tuntun' market, in Ilorin, North Central, Nigeria. They were then authenticated at the Department of Zoology, University of Ilorin, Kwara State, Nigeria.

Albino rats

The albino rats used for this research were inbred in the Animal Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. This was approved by Animal Ethical Committee of the same University.

Kits and Chemicals

AST, ALT and GGT kits were obtained from Randox Laboratories Ltd, UK while ALP and all other chemicals were obtained from Quinica Aplicada (QCA), S. A Spain.

Formulated Diets

The diets were composed as earlier reported by **Ajiboye and Muhammad (2015)** and **Ajiboye et al. (2014)**.

Animal's Treatment

Sixteen weanling albino rats (weighing between 29.15 and 26.01 g) which were twenty one days old were used in this study, divided into two groups. The first group was placed on casein-based diet which serves as the control while the second group was placed on *Rana galamensi*s-based diet which serves as the tested. Each group comprises of eight rats. The animals were fasted for twelve hours and then acclimatized for a week in the laboratory to normalize them for the experiment before being placed on the different diets.

The animals in each group were housed in metal cage. The diet and water were fed to the animals *ad libitum* throughout the period of 8 weeks and sanitary measures were ensured.

Serum and Tissues Homogenates Preparation

At the end of eight week the animals were sacrificed by anaesthetizing them in a jar containing cotton wool that had been soaked in diethyl ether. The anaesthetized animals were then sacrificed by cutting the jugular vein. The blood was collected into clean, dry glass beaker and allowed to coagulate for 1 hour, using pasture pipette. The serum was removed from the clot and collected into centrifuge tube. Clear serum was then obtained by centrifugation at 1,500 g for 15 minutes. The samples were kept frozen and used for analyses within 24 hours **(Akanji, 1986)**. The animals were quickly dissected after sacrifice and organs such as liver, heart, kidney, stomach and small intestine were removed quickly into ice – cold 0.25 M sucrose solution to maintain the integrity of the organs. Each organ was homogenized separately in ice – cold 0.25 M sucrose solution (×6 dilution) using mortar and pestle by cutting a known weight of the tissues finely with a clean scissors and homogenized. All operations were carried out at 0 °C to 4 °C. The homogenates were stored in the freezer and used for analyses within 24 hours while the organs (liver) for histological studies were immersed in 10% formalin.

Determination of Organ to Body Weight Ratio

This was determined using **Drury and Wallington (1973)** formular:

Percentage of organ to body weight ratio = weight of organ/weight of animal X 100%.

Determination of Enzymes Activities

Alkaline phosphates (EC 3.1.3.1) activities were determined according to the methods described by **Wright et al. (1972)** Aspartate transaminase (EC 2.6.1.1) and Alanine transaminase (EC 2.6.1.2) activities were determined as described by **Reitman and Frankel (1957**) while gamma-glutamyl transferase (EC 2.3.2.2) was estimated by method described by **Persijin (1976)**.

Histological Examination

This was done as described by **Krause (2001)**; the photomicrographs were observed using the Leitz, DIALUX research microscope at X 400 magnification.

Statistical Analysis

Statistical analysis was carried out using the students' t-test **(Adamu and Johnson, 1997)**.

RESULTS AND DISCUSSION

Effect of Rana galamensis-Based Diet on Organ to Body Weight Ratio

The percentage of organ to body weight ratio of the selected rat's organs is presented in Table 1. There was no significant difference (p >0.05) in the organ to body weight ratio of the animals fed on control and *Rana galamensis*–based diet.

This suggests that *Rana galamensis* supports normal growth of the organs, thereby causing no inflammation or constriction of the organs studied **(Moore and Dalley, 1999)**. **Akanji and Ngaha (1989)** reported that biochemical parameters like tissue enzyme can indicate tissue or cellular damage long before structural damage can be picked up by conventional histological techniques.

Effect of Rana galamensis-Based Diet on Alkaline Phosphatase (ALP) Activities

The ALP activities in the tissues of the rats fed *Rana galamensis*-based diet is depicted in Table 2. The results shows that there were no significant difference (p >0.05) in ALP activities in comparing the albino rats fed on control (casein-based diet) and *Rana galamensis*-based diet.

ALP is a 'marker' enzyme for the plasma membrane and endoplasmic reticulum **(Shahjahan et al., 2004)**; it is frequently used to assess the integrity of the plasma membrane **(Akanji et al., 1993)**. In all the five tissues studied ALP activities was highest in small intestine followed by kidney and least in the liver. This is in agreement with the report of **Bonting et al. (1960)** that the main mammalian organ, which have very high ALP activities are those involved in active transport mechanisms.

Moreover, ALP is a membrane bound enzyme which is used frequently to detect damage to the plasma membrane. The non-reduction of ALP activities in the tissues which occurred in this study may indicate that there are no likely damages to the plasma membrane, therefore leakage did not occur. This may give an indication that there may not likely be the presence of diseases such as obstructive jaundice, bone diseases, cancer and heart infections which mostly results from increase in serum ALP activities due to leakage from the tissues **(Akanji et al., 2013)**.

Table 1 Changes in organ to body weight ratio (%) of selected tissues of animals fed with *Rana galamensis*-based diet for 8 weeks.

Tissues	Control (casein)	*Rana galamensis*
Liver	3.13 ±0.18	2.81 ±0.11
Heart	0.43 ±0.03	0.36 ±0.01
Kidney	1.03 ±0.09	0.97 ±0.02
Stomach	0.92 ±0.10	0.75 ±0.04

Each value is a mean of eight determinations ±SEM. The values are not significantly different (p >0.05).

Table 2 ALP activity ($IU.L^{-1}$) in the tissues of rats fed Rana galamensis-based diet for eight weeks.

Tissues	Control (casein)	*Rana galamensis*
Liver	11.96 ±2.95	12.02 ±3.07
Heart	12.67 ±1.73	12.68 ±1.25
Kidney	206.09 ±14.75	188.56 ±12.60
Small Intestine	293.52 ±16.35	288.98 ±10.96
Serum	5.75 ±0.52	5.63 ±0.71

Each value is a mean of eight determinations ±SEM. The values are not significantly different (p >0.05).

Table 3 AST activity ($IU.L^{-1}$) in the tissues of rats fed Rana galamensis-based diet for eight weeks.

Tissues	Control (casein)	*Rana galamensis*
Liver	654.67 ±17.67	659.0 ±15.09
Heart	218.63 ±11.09	207 ±10.61
Serum	30.47 ±8.36	33.43 ±8.51

Each value is a mean of eight determinations ±SEM. The values are not significantly different (p >0.05).

Table 4 ALT activity ($IU.L^{-1}$) in the tissues of rats fed *Rana galamensis*-based diet for eight weeks.

Tissues	Control (casein)	*Rana galamensis*
Liver	508.2 ±6.57	504.75 ±6.70
Heart	156.67 ±6.79	158.5 ±6.70
Serum	156.9 ±6.66	150.46 ±3.27

Each value is a mean of eight determinations ±SEM. The values are not significantly different (p >0.05).

Table 5 γGT activity ($IU.L^{-1}$) in the tissues of rats fed *Rana galamensis*-based diet for eight weeks.

Tissues	Control (casein)	*Rana galamensis*
Liver	190.5 ±5.43	182.4 ±4.97
Serum	14.9 ±0.67	14.9 ±0.67

Each value is a mean of eight determinations ±SEM. The values are not significantly different (p >0.05).

Effect of Rana galamensis-Based Diet on Some Transaminases and Transferase Activities

The activities of AST, ALT and γGT in the tissues of the rats fed *Rana galamensis*-based diet is shown in Table 3, table 4 and Table 5. The results shows that there were no significant difference (p >0.05) in the activities of all these transaminases as compared to the control.

AST is one of the enzymes involved in the transfer of anions group to keto acid without formation of ammonia as intermediate. In general, transaminases form an important link between protein and carbohydrate metabolism and are widely distributed in animal tissues **(Yakubu et al., 2008)**. It has been reported that enzymes from damage tissues or disease tissues may become recognizable in the serum, presumably by leakage through altered cell membrane **(Yakubu et al., 2008)**. But in this study, (Table 3) no leakage of AST from tissue was observed showing that there was no alteration in the cytosolic content of the tissues studied. In addition, AST and ALT are widely used for hepatic disorders **(Yakubu et al., 2003)**. Also, high serum level of ALT is an indicator for some form of hepatic diseases. Therefore, the non–significant difference in the serum level of ALT indicates (Table 4) that there is probably absence of any form of hepatic disease **(Abubakar et al., 2010)**.

γGT, the most common enzymatic indicator of hepatobiliary disease, is a membrane-localized enzyme that functions in glutathione metabolism and reabsorption of amino acids from the glomerular filterate and intestinal lumen **(Yakubu et al., 2001)**. It is a group of enzyme called peptidases, which catalyses the hydrolytic cleavage of peptides to form amino acid or smaller peptides. Elevated serum levels of the enzyme are found in association with hepatobiliary and pancreatic disorders, alcohotics and heavy disorders, in myocardial disorders and in diabetics **(Yakubu et al., 2001)**. The non-significance difference in the serum γGT (Table 5) giving an indication that there may not likely be any form of hepatobillary and pancreatic disorders. Also, γGT is more sensitive than ALP, AST and ALT in detecting jaundice **(Mayne, 1998)**. Moreover, the non-significant differences in the ALP, AST, ALT and γGT may be ascribed to the non-toxic of *Rana galamensis* coupled with the present of essential amino acids and minerals in.

Effect of Rana galamensis-Based Diet on Histological examination

Plates 1 to 2 show the histology section of the control and *Rana galamensis* fed rat livers. The results revealed that no significance difference between the two groups. The liver of both animals have normal architecture structure. The photomicrographs show portal tract at the centre with the normal hepatocytes.

This indicate no alterations in the photomicrograph of the tissues studied (Figure 1 and Figure 2) corroborates the results of the enzymes and blood parameters. This shows that *Rana galamensis* is safe for consumption coupled with its high digestibility as reported by **Ajiboye et al. (2014)**.

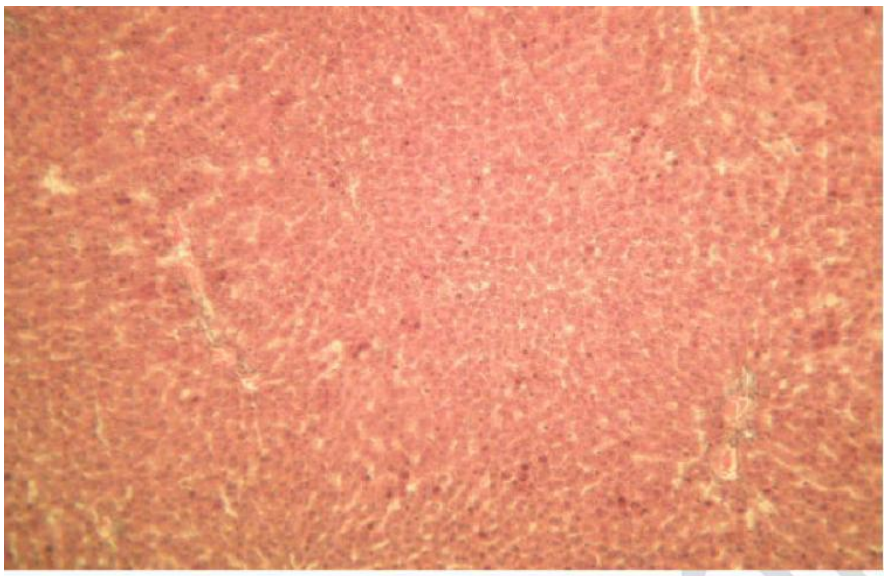

Figure 1 Plate 1: Photomicrograph of the liver of rats fed casein (control) – based diet (Magnification x 400).

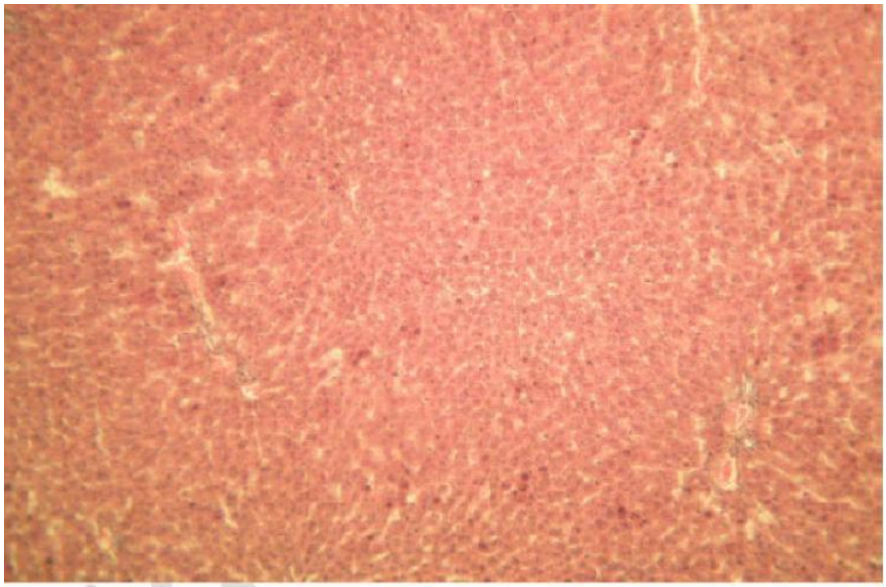

Figure 2 Plate 2: Photomicrograph of the liver of rats fed *Rana galamensis* – based diet (Magnification x 400).

CONCLUSION

The lower activities of all the serum enzymes studies (ALP, GOT, GPT and γGT) indicated no organ damage coupled with the organ to body weight ratio of the animals. This was also supported by the result of liver histological studies. This may probably be attributed to high nutritional status of *Rana galamensis*.

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Contact address:

Basiru Olaitan Ajiboye, Phytomedicine, Biochemical Toxicology and Diabetes Research Group, Department of Biochemistry, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria, E-mail: bash1428@yahoo.co.uk.

Nasir Olarewaju Muhammad, Department of Biochemistry and Molecular Biology, Federal University, Dustin-ma, Kastina State, Nigeria, E-mail: α_mn02@gmail.com.

Oluwafemi Adeleke Ojo, Phytomedicine,Biochemical Toxicology and Diabetes Research Group, Department of Biochemistry, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria, E-mail: oluwafemiadeleke08@gmail.com.