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Research Article

Effects of Drinking Gammalin-20 Contaminated Water: A Biochemical Study in Rattus norvegicus Rats

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Abstract: Gammalin-20 is a widely used organochlorine pesticide in veterinary and human medicine for the treatment of ectoparasite and pediculosis. This study was aimed at determining the effects of drinking Gammalin-20 contaminated water on some biochemical parameters in male Rattus norvegicus rats. Each of the ten rats in the experimental group drank approximately 2.5 Liters-3.0 Liters of 0.01% Gammalin-20 contaminated water within the period of four weeks which this study lasted while each of the ten rats in the control group drank approximately 10 Liters- 12 Liters of distilled water within the same period. Five milliliters blood specimens were collected from each experimental and control rats into lithium heparin anti-coagulated bottles. The blood specimens were spun and plasma samples used for the quantitative measurement of alanine aminotransferse, aspartate aminotransferase, urea, creatinine and C-reactive protein using a spectrophotometer. The results revealed statistically significant elevations ($p<0.05$) in the mean values of all the biochemical parameters measured in the experimental rats as compared to that of the control. In conclusion, drinking of 0.01% Gammalin-20 contaminated water for a period of four weeks may lead to hepato-renal and inflammatory disorders in Rattus norvegicus rats. It is therefore recommended that the use of Gammalin-20 in veterinary, killing of fish, human medicine etc should be well guided and properly regulated so as to avoid the likelihood of these adverse effects on humans.

Keywords: Gammalin-20 contaminated water, Effects of drinking, Biochemical study, *Rattus norvegicus* rat.

INTRODUCTION

Gammalin-20 is an organochlorine pesticide that is mainly used in veterinary and human medicine for the treatment of ectoparasites and pediculosis, control of a broad spectrum of phytophagous and soil-inhibiting insects, public health pests, control of crops pests and seed treatment (Adedeji *et al.,* 2008).

The active ingredient in Gammalin-20 is lindane which is widely used in the treatment of seeds, lotion cream as well as shampoo to control lice and mites in humans (Lawson *et al.,* 2011). This active ingredient which is highly toxic to fish, bees and aquatic invertebrates is also used in fish industries for killing of fish and on a wide range of crops to control aphididae larvae of coleopteran, dipteral etc (Joshi *et al.,* 2002). Some of its toxicological signs and symptoms among several others include irritability, difficulty in breathing, convulsion, staggering and death (Dede and Dogara, 2004).

The application of environmental toxicology studies on non-mammalian vertebrates has gained rapid expansion and for aquatic system, fish have become an indication to assess the effects of noxious compounds. Pesticides are among one of the major chemicals that are encountered daily by man as a result of being deliberately added to the environment for the purpose of killing, posing injury or at times enhancing the development of some form of life (Omitonyin *et al.,* 2006).

Gammalin-20 is a well-known toxic chemical with numerous side effects. Regardless of these effects, it is still very much in use for killing of fish as well as local treatments of some disease conditions in villages along the riverine areas of the studied community. It is therefore vital to carry out this study using male *Rattus norvegicus* rats as the experimental animal. This however, is with a view to further enlighten the public on the possible adverse effects it has on humans particularly when used indiscriminately.

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MATERIALS AND METHODS

Study area

This study was carried out in the Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

Animals used

The male *Rattus norvegicus* rats used for this study were purchased from the Pharmaceutical Department, University of Port-Harcourt animal house, Port-Harcourt, Rivers State, Nigeria and transported to the animal house of the Department of Medical Laboratory Science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria via a private transport. The rats were allowed to acclimatize in the animal house for two weeks and were observed for physical deformity or any ailments that may render them unfit prior to the commencement of the study. The rats which were placed in ventilated iron standard cages were fed with pre-mix rat feed and water.

Ethical clearance

This study which got the ethical approval from the ethical committee of Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria was carried in conformity to the National Guidelines for Animal usage in research.

Gammalin-20

The Gammalin-20 used for this study was manufactured by Morton Grove Pharmaceutical MC Grove, IL 60053, REV. 06-05 and distributed by Alliant Pharmaceuticals, Inc Alpharetta, GA-30004-FDA REV United States. It was bought in a local store in Amassoma, Bayelsa State, Nigeria and stored in line with the manufacturer's instructions.

Scope of experimental design

The study was carried out on four months old male *Rattus norvegicus* rats with each of them within the weight range of 0.23+_0.05g. The rats were grouped as follows

(i) Control group: Each of the rats in this group drank approximately 10 Liters-12 Liters of distilled water for the period of four weeks within which this study lasted.

(ii) Experimental group: Each of the rats in this group drank approximately 2.5 Liters-3.0 Liters of 0.01% Gammalin-20 contaminated water for the period of four weeks within which this study lasted.

Reagents used

Commercially available alanine aminotransferase, aspartate aminotransferase, urea, creatinine (Randox Diagnostic kit, UK) and C-reactive protein (Spin-react Diagnostic kit, Spain) used for this research were purchased in Timko Medicals, Idumota, Lagos State, Nigeria. The manufacturers' standard operational procedures were strictly adhered to while carrying out these tests.

Equipment used

Medical equipment and scientific Limited Vis-spectrophotometer with model number S23A13192 was used for absorbance measurement of the respective biochemical parameters. Other equipment and materials that were used include: Gulfex medical equipment and scientific Limited macro centrifuge with model number 800D, Incubator with model number DNP-9052A, Refrigerator, and automatic micropipettes.

Pilot study

A pilot study was carried out to ascertain the minimum concentration of Gammalin-20 contaminated water drunk that caused 100% death (LC_{100}) in the experimental rats. A total of 4 (four) rats having approximately the same weight of 0.23±0.05g were used and the LC_{100} was obtained with 0.06% of Gammalin-20 contaminated water.

Also, a pilot study was carried out to ascertain the minimum concentration of Gammalin-20 contaminated water drunk that caused 50% death (LC_{50}) in the experimental rats. A total of 4 (four) rats having approximately the same weight of 0.23±0.05g were used and the LC_{50} was obtained with 0.03% of Gammalin-20 contaminated water.

Sub-chronic toxicity study

In this study the rats were divided into two groups, with ten rats per group categorized as experimental and control respectively. Approximately 2.5 Liters-3.0 Liters of 0.01% Gammalin-20 contaminated water was drunk by each of the experimental rats weighing 0.23±0.05g within the period of four weeks which the experiment lasted, while each of the control rats weighing 0.23±0.05g drank approximately 10 Liters-12 Liters of distilled water within the same period which the experiment lasted.

At the end of this experiment, the control and experimental rats were anaesthetized using chloroform technique after which five milliliters blood specimens were withdrawn from the cardiac of each rat for biochemical investigations

Biochemical parameters analyzed with specified methods

The following biochemical parameters as shown below were analyzed.

I. Alanine aminotransferase in accordance with the colorimetric method as described by Ifenkwe et al. (2018) using reagents manufactured by Randox Laboratories, Limited, 55, Diamond Road, Crumlin, County, Antrim, BT294QY, United Kingdom.

II. Aspartate aminotransferase in accordance with the colorimetric method as described by Ifenkwe et

al. (2018) using reagents manufactured by Randox Laboratories, Limited, 55, Diamond Road, Crumlin, County, Antrim, BT294QY, United Kingdom.

III. Urea in accordance with Urease Berthelot method as described by Egoro et al. (2019) using reagents manufactured by Randox Laboratories, Limited, 55, Diamond Road, Crumlin, County, Antrim, BT294QY, United Kingdom.

IV. Creatinine in accordance with Jaffe reaction method as described by Obodo et al. (2020) using reagents manufactured by Randox Laboratories, Limited, 55, Diamond Road, Crumlin, County, Antrim, BT294QY, United Kingdom.

V. C-reactive protein in accordance with latex turbidimetry method as described by Egoro et al. (2019) using reagent manufactured by Spin-react Diagnostic, Spain.

Statistical analysis

The results obtained from the control and experimental groups were expressed as mean and standard deviation while the differences between the groups compared using the student's'‘t’ test. A p-value of $p \leq 0.05$ was considered statistically significant.

DISCUSSION

In this study comparison was made between the mean values of plasma liver, renal and inflammatory biochemical parameters in male *Rattus norvegicus* rats that drank approximately 2.5 Liters-3.0 Liters of 0.01% Gammalin-20 contaminated water for the period of 4 (four) weeks which this study lasted (experimental group) and that of male *Rattus norvegicus* rats that drank approximately 10 Liters-12 Liters of distilled water within the same period which this study lasted (control group).

Alanine aminotransferase is an aminotransferase enzyme that catalyzes the inter-conversion of amino acid to 2-oxo-acid by transfer of amino group from alanine to the alpha keto group of ketoglutaric acid to generate pyruvic acid (Brissot, 2007). This enzyme which is clinically measured as part of liver function test (Lala *et al.,* 2020) and more hepatocellular specific than aspartate aminotransferase (Kunutsar, 2013) was firstly characterized by Arthur Karmen and colleagues in the mid-1950s (Karmen *et al.,* 1955) The results from this research showed that the mean value of plasma alanine aminotransferase was significantly higher statistically ($p<0.05$) in the male *Rattus norvegicus* rats that drank approximately 2.5 Liters-3.0 Liters of 0.01% Gammalin-20 contaminated water for the period of 4 (four) weeks which the study lasted (experimental group) as compared with that of the control group which drank approximately 10 Liters-12 Liters of distilled water within the same period the experiment lasted as shown in Table-1. It is presumed that this statistically significant elevation may be due to damage imposed on the liver which led to the leakage and subsequent release of this enzyme from the liver to the plasma as established in this study since there are no literatures on any previous work linking the effects of drinking Gammalin-20 contaminated water on plasma in *Rattus norvegicus* rats.

Aspartate aminotransferase is an aminotransferase enzyme that catalyzes the inter-conversion of amino acids to 2-oxo-acids by transfer of amino group from aspartate to the alpha keto group of ketoglutaric acid to generate oxaloacetic acid (Brissot, 2007). It is found in all tissues except bone (Evans, 2009). The results from this study showed that the mean value of plasma aspartate aminotransferase was significantly higher statistically ($p<0.05$) in the male *Rattus norvegicus* rats that drank approximately 2.5 Liters-3.0 Liters of 0.01% Gammalin-20 contaminated water for the period of four weeks within which the study lasted (experimental group) as compared with that of the control group that drank approximately 10 Liters-12 Liters of distilled water for the same period within which the study lasted as shown in Table-1. It is however, presumed that the statistically significant elevation may be due to liver damage as a result of the drunk 0.01% Gammalin 20 contaminated water which led to the leakage and subsequent release of this enzyme from the liver to the plasma as established in this study, as there are no literatures on previous work linking the effect of drinking Gammalin-20 contaminated water on plasma aspartate aminotransferase in *Rattus norvegicus* rats.

Table-1: The liver biochemical parameters of control and experimental groups

Parameters	Control group (n=10)	Experimental group (n=10)	p-value	Remarks
ALT (U/I)	4.40 ± 0.28	17.60 ± 1.32	$p < 0.05$	**S**
AST (U/I)	3.79 ± 0.20	15.60 ± 1.03	$p < 0.05$	**S**

Values are in mean and standard deviation
Keys
ALT = Alanine aminotransferase
AST = Aspartate aminotransferase
S = Statistically significant
n = Number of rats

Urea is the main nitrogenous breakdown product of protein metabolism in mammals which is excreted in urine with more than 90% of its industrial production worldwide used as a nitrogen release fertilizer (Meessen and Petersen, 2010). The results from this study showed that the mean value of plasma urea was significantly higher statistically ($p<0.05$) in the male *Rattus norvegicus* rats that drank approximately 2.5 Liters-3.0 Liters of 0.01% Gammalin-20 contaminated water for a period of four weeks within which the study lasted (experimental group) as compared with the mean value in the control group which drank 10 Liters-12 Liters of distilled water for the same period within which the experiment lasted as shown in Table-2. This statistically significant elevation may be suggestive of renal impairment resulting from the 0.01% Gammalin-20 contaminated water that was drunk as established in this study, as there are no literatures on previous work linking the effect of drinking Gammalin-20 contaminated water on plasma urea in *Rattus norvegicus* rats.

Creatinine is a nitrogenous waste product that is derived from creatine and creatine phosphate. It is not reutilized, but is excreted from the body in the urine via the kidney with its serum level increasing slightly significant 24 hours after exercise (Spada *et al.,* 2018). As a consequence of the way in which it is excreted by the kidney, its measurement is used almost exclusively in the assessment of kidney function. The results from this study showed that the mean value of plasma creatinine was significantly higher statistically ($p<0.05$) in the male *Rattus norvegicus* rats that drank approximately 2.5 Liters-3.0 Liters of 0.01% Gammalin-20 contaminated water for the period of four weeks within which the study lasted (experimental group) as compared with the mean value in the control group which drank approximately 10 Liters-12 Liters of distilled water for the same period within which the study lasted as shown in Table-2. This significant elevation may be suggestive of renal impairment resulting from the 0.01% Gammalin-20 contaminated water that was drunk as established in this study, as there are no literatures on previous work linking the effect of drinking Gammalin-20 contaminated water on plasma creatinine in *Rattus norvegicus* rats.

Table-2: The renal biochemical parameters of control and experimental groups

Parameters	Control group (n=10)	Experimental group (n=10)	p-value	Remarks
Urea (mmol/I)	3.30 ± 1.02	10.50 ± 3.02	$p < 0.05$	S
Creatinine (umol/L)	66.50 ± 3.38	99.92 ± 4.01	$p < 0.05$	S

Values are in mean and standard deviation
Keys
S = statistically significant
n = Number of rats

C-reactive protein is a pentraxin family of protein which derived its name from its ability to react with C-polysaccharides isolated from pneumococcal cell walls. The production rate of C-reactive protein increases in inflammation, infection, cystitis or bronchitis, this rate falls drastically once the inflammation subsides, this however, is as a result of its short half life (Bray and Christopher, 2016). The results from this study showed that the mean value of plasma C-reactive protein was significantly higher statistically ($p<0.05$) in the male *Rattus norvegicus* rats that drank approximately 2.5 Liters-3.0 Liters of 0.01% Gammalin-20 contaminated water for the period of four weeks within which the study lasted (experimental group) as compared with the mean value in the control group which drank approximately 10 Liters-12 Liters of distilled water for the same period within which the study lasted as shown in Table-3. This statistically significant elevation is presumed to be due to systemic inflammation following drinking approximately 2.5 Liters-3.0 Liters of 0.01% Gammalin-20 contaminated water with the resultant release of interleukin-6 and other cytokines which may have triggered the synthesis of C-reactive protein as established in this study.

Alteration in the chemical composition of a natural aquatic environment as a result of contact with hazardous substances such as Gammalin-20 may influence the behaviours, physiology as well as biochemistry of aquatic animals including fish. One of the most important natural resources on earth is water which is so vital to humans, fish, wildlife, agriculture, industries, urban as well as social development (Lawrence and Temiotan, 2010).This study went further to show that the experimental rats drank $1/4^{th}$ of the 0.01% Gammalin-20 contaminated water as compared to the total amount of distilled water drunk by the control rats. This gross reduction in the amount of 0.01% Gammalin-20 contaminated water drunk by the experimental rats as established in this study due to the lidane, an active ingredient in the Gammalin-20 contaminated water may expose the aquatic ecosystem to risk of biodiversity loss as a result of the unregulated release of this organochlorine pesticide into the river (Rahman *et al.,* 2002). Also, this observation may be a pointer to the fact that the human inhabitants of the studied community and beyond whose primary source of bath, washing of plates and clothes etc is solely dependent on this Gammalin-20 contaminated river water may be at the risk of some health challenges.

Table-3: The inflammatory biochemical parameter of control and experimental groups

Parameters	Control group (n=10)	Experimental group (n=10)	p-value	Remarks
CRP (mg/L)	1.80 ± 0.40	12.50 ± 3.13	$p < 0.05$	S

Values are in mean and standard deviation
Keys
CRP = C-reactive protein
S = Statistically significant
n = Number of rats

CONCLUSION

In conclusion this study has established that drinking approximately 2.5 Liters-3.0 Liters of 0.01% Gammalin-20 contaminated water for a period of four weeks is capable of causing hepato-renal and inflammatory disorders in *Rattus norvegicus* rat.

Recommendation

a. The use of Gammalin-20 for medicine, killing of fish, veterinary and other purposes should be well guided and properly regulated in order to avoid adverse health effects and subsequent death in humans
b. The persistence of Gammalin-20 in aquatic environment should be discouraged based on the fact that it is a very toxic organochlorine pesticide.
c. The biochemical profile of individuals living in the studied environment should be monitored regularly

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