DEXAMETHASONE INDUCES BIOCHEMICAL AND HISTOMORPHOLOGICAL CHANGES IN HEPATO-RENAL SYSTEM OF NORWAY RATS (*RATTUS NORVEGICUS)*

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ABSTRACT

This study was aimed at evaluating dexamethasone induced biochemical and histomorphological changes in hepato-renal system of *Rattus norvegicus.* The rats were divided into 3 groups. Each rat was injected intraperitoneally with 0.02ml/0.166 ± 0.05 kg of dexamethasone on daily basis for two weeks, (experimental group one), 0.02ml/0.166 ± 0.05 kg of dexamethasone on daily basis for four weeks (experimental group two) and those in control group not injected. At day 14 and 28, the rats were sacrificed respectively using chloroform technique and 5ml of blood collected via cardiac puncture into lithium heparin anti-coagulated bottles. The blood specimens were separated using a Gulfex macro-centrifuge model 800 D and used for biochemical assay: alanine aminotransferase, aspartate aminotransferase, urea and creatinine. The organs (liver and kidneys) were collected into 10% BFS, processed and stained using haematoxylin and eosin staining technique. The results showed that the mean values of all the biochemical parameters in the plasma of the experimental group two *Rattus norvegicus* were significantly higher ($p<0.05$) than they were in the control group, which showed no significant changes ($p>0.05$). When compared to the control group, the histomorphological analysis of the liver and kidney tissues indicated central vein congestion and glomerular enlargement, respectively. The mean values of all the measured biochemical parameters in the experimental group one *Rattus norvegicus* did not significantly differ from those in the control group, however ($p>0.05$). The liver and kidneys' histomorphological examinations showed no changes. Prolonged administration of dexamethasone for four weeks may adversely effect changes in the hepato-renal system of *Rattus norvegicus.*

Keywords: Biochemical, Dexamethasone, Histomorphological, *Rattus norvegicus*, Toxicity.
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INTRODUCTION

Dexamethasone is one of the corticosteroid drugs used to treat a variety of illnesses, including rheumatism, severe allergies, asthma, chronic obstructive pulmonary disease, tuberculosis etc. [1]. This medication is included on the World Health Organization's list of important, moderately inexpensive, and widely accessible medications [1]. It may be administered orally, intramuscularly, or intravenously. Long-term usage might cause thrush, cataracts, and muscular weakness; thus, it is strongly urged that nursing women avoid using this medication. However, the United States Food and Drug Administration (FDA) has authorized intravitreal steroid implants for the treatment of ocular diseases including uveitis, central retinal vein occlusion, and diabetic macular edoema [2]. This substance was estimated to be the 359th most prescribed pharmaceutical in the United States in 2019, with fewer than one million prescriptions [2].

According to Roila *et al.*; Holte & Kehlet [3, 4], cancer patients undergoing chemotherapy are typically given dexamethasone to counteract the side effects of their anti-tumor treatments. The exact mechanism for this, which is not well understood, has been theorized to involve inhibition of prostaglandin synthesis, immunosuppressive effects, inflammatory effects, or decreased release of endogenous opinoids, or a combination of the aforementioned.

Philip Shawalter Hench initially synthesized this medication in 1957, and it was approved for medicinal use in 1958 [5, 6]. It is classified as pregnancy category C in the United States (i.e., it should only be taken when the benefits outweigh the risks) [7], however in Australia it is classified as oral use category A (i.e., after regular usage during pregnancy, no detrimental effects on the foetus have been seen) [7].

This medicine, provided as a single dosage in tiny doses before and/or after some dental procedures, can minimize airway swelling to improve breathing and alleviate pain [8]. It may also be taken prior to the administration of antibiotics in cases of bacterial meningitis. It is known to lessen the body's inflammatory reaction to antibiotic-killed microorganisms [9]. This medicine may be administered to cancer patients receiving chemotherapy to alleviate specific negative effects of their anti-tumour therapies [10]. In addition, this medication may be administered for adrenal insufficiency and Addison's disease when the patient responds poorly to prednisolone or methylprednisolone. It can be used to inhibit production

in older adolescents and adults with congenital adrenal hyperplasia [10].

This medication is frequently used on mountain climbing expeditions to prevent altitude sickness consequences. One dosage of dexamethasone has been observed to alleviate some throat sickness [11]. It has been associated with forgetfulness, birth defects, acne, confusion, cataract, euphoria, dyspepsia, hiccups, headache, hypertension, hyperglycemia, sleeplessness, vomiting, and weight gain, among others [12]. Despite all of these incidents, the use of this substance without discrimination is still prevalent, especially among young people. Therefore, it is expedient to conduct this study to evaluate its influence on biochemical and histomorphological alterations in the hepato-renal system of *Rattus norvegicus.*

MATERIALS AND METHODS

Study area

This research was conducted at Niger Delta University, Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Wilberforce Island, Bayelsa State.

Animals

Public transportation was employed to carry male *Rattus norvegicus* from the animal house of the University of Port Harcourt's Pharmaceutical Department to the animal house of the Niger Delta University's Department of Medical Laboratory Science on Wilberforce Island, Bayelsa State. The rats were kept for two weeks in ventilated rat cages and provided growers mash hybrid meals (pre-mixed rat food) and water ad libitum.

Ethical clearance

This study was carried out in conformity with the National Guideline for Animal usage in research as approved globally.

Dexamethasone injectable

Injectable dexamethasone ampoules (4 mg/ml)., USP Product made by Hubei Tianyao Pharmaceutical Company Limited Dufu Block, Jianshe Road, Xiangfan town, Hubei City, China were purchased from a Pharmacy in Yenagoa, Bayelsa State and used for this study in accordance to the manufacturer's instructions.

Experimental design

Pilot study

A pilot research was conducted to determine the minimal dosage of dexamethasone that might induce 50% mortality (LD_{50}) in *Rattus norvegicus*. This was achieved by administering 0.4 mg/0.1 ml of dexamethasone intraperitoneally to 0.265 kg *Rattus norvegicus.*

Sub-chronic toxicity study

In this study, the experimental *Rattus norvegicus* were divided into 3 groups as follows:

(i) **Experimental group one:** This consisted of six *Rattus norvegicus* each intraperitoneally injected with 0.02ml/0.166kg of dexamethasone on daily basis for a period of two weeks.

(ii) **Experimental group two:** Six *Rattus norvegicus* were daily injected intraperitoneally with 0.02ml/0.166kg of dexamethasone for four weeks.

(iii) **Control group:** This comprised of six rats of the *Rattus norvegicus* species that were not treated with dexamethasone or any other medicines and were provided with normal food and water.

Both the control and experimental rats were anaesthetized with chloroform at the conclusion of this experiment. Blood was taken through heart puncture and kidney and liver were extracted, blotted dry, and preserved in 10% formalin.

Laboratory Investigations

(i) Measurement of alanine aminotransferase

This was measured in accordance with the colorimetric method as described by Egoro *et al.* (2018) [13] using reagents manufactured by Randox Laboratories Limited, 55, Diamond Road, Crumlin County, Antrim, BT294QY, United Kingdom.

(ii) Measurement of aspartate aminotransferase

This was measured in accordance with the colorimetric method as described by Egoro *et al.* (2020)[14] using reagents manufactured by Randox Laboratories Limited, 55, Diamond Road, Crumlin County, Antrim, BT294QY, United Kingdom.

(iii) Measurement of creatinine

This was measured in accordance with the Jaffe reaction method as described by Egoro *et al.* (2020) [14] using reagents manufactured by Randox Laboratories Limited, 55, Diamond Road, Crumlin County, Antrim, BT294QY, United Kingdom.

(iv) Measurement of urea

This was measured in accordance with the urease Berthelot's method as described by Obodo *et al.* (2020) [15] using reagents manufactured by Randox Laboratories Limited, 55, Diamond Road, Crumlin County, Antrim, BT294QY, United Kingdom.

(v) Preparation of the histology slides of liver and kidneys of the *Rattus norvegicus*

The excised organs were dehydrated using 50%, 70%, 80%, 90%, 95%, and 100% concentrations of alcohol, and were subsequently immersed in molten paraffin wax, trimmed and sectioned into 4 mm thickness as reported by Tijani *et al.* (2014) [16]. This was followed by clearing of the dehydrated agent using xylene. After staining the tissue slides with Haematoxylin and Eosin, an Olympus binocular light research microscope (XSZ-107BN, No 071771) was used to evaluate them histomorphologically (H&E). The Kodak digital camera was then used to take photomicrographs (Kodak Easyshare C183).

Statistical analysis

Data collected from *Rattus norvegicus* in both the experimental and control groups were reported as mean ± SD, and analysis was carried out using SPSS version 23.0. Student's "t" test was used to express group differences. A 0.05 p-value was regarded as statistically significant. One-way ANOVA was used to compare the research groups' means, with a 95% confidence level being used.

RESULTS

Intraperitoneal injections of 0.02ml/0.166kg dexamethasone for two weeks (experimental group one) and four weeks (experimental group two) as well as those not injected with dexamethasone or any other medicines which served as the control group are presented in Tables 1 and 2 correspondingly.

Table 1: The biochemical parameters of *Rattus norvegicus* injected intraperitoneally with 0.02ml/0.166kg of dexamethasone on daily basis for a period of two weeks (experimental group one) compared to that of the control group

Parameters	Control group (n=6)	Experimental group (n=6)	P-values	Comment
ALT (U/I)	6.72 ± 0.48	6.75 ± 0.50	0.71	NS
AST (U/I)	5.97 ± 0.29	6.01 ± 0.31	0.35	NS
Urea (mmol/l)	6.10 ± 4.15	6.13 ± 4.17	0.57	NS
Creatinine (µmol/l)	61.56 ± 5.35	61.60 ± 6.20	0.82	NS

Values are in mean and standard deviation

Keys:

ALT=Alanine aminotransferase

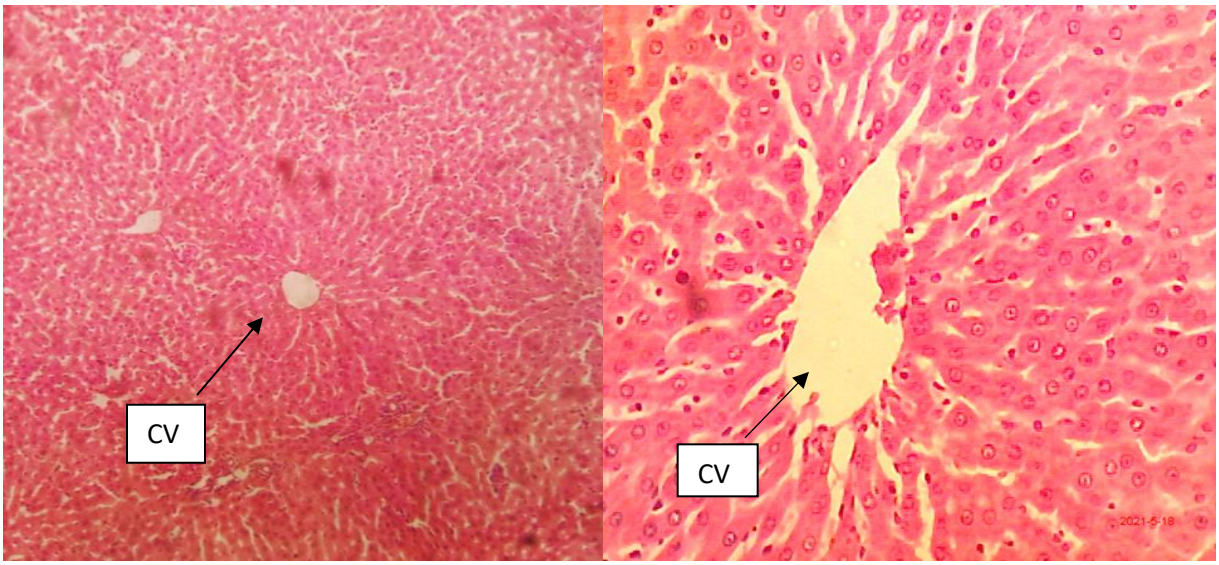
AST=Aspartate aminotransferase

n=Number of rats

NS=Not statistically significant

The findings demonstrated that there was no significant difference between the mean values of all the measured biochemical parameters in the dexamethasone-injected *Rattus norvegicus* group and the control group ($p>0.05$).

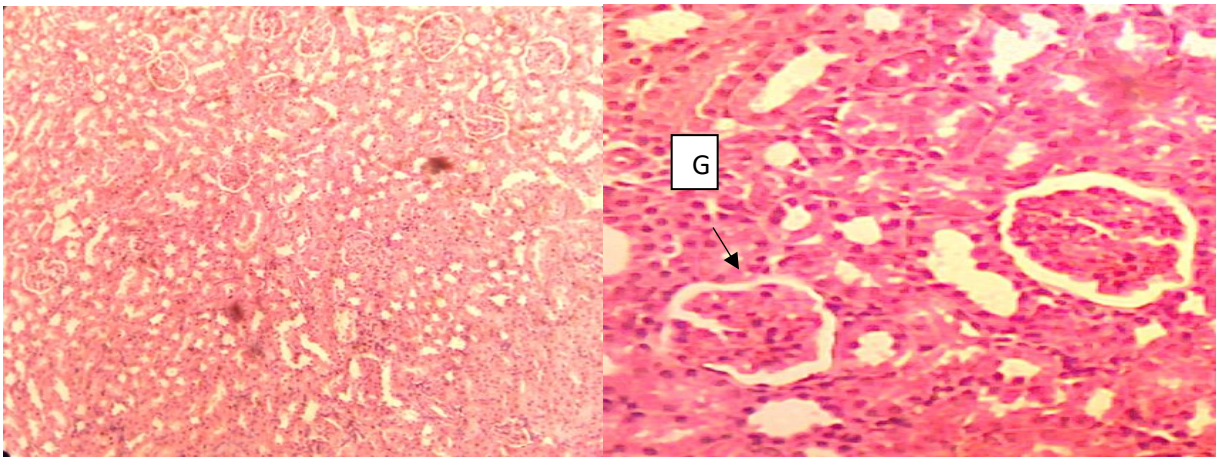
The histomorphological study of the liver and kidneys of the experimental *Rattus norvegicus* in this group, which likewise found no toxicological changes as shown in Plates I and II, respectively, is consistent with these biochemical findings.



Control Group **Experimental Group**

Plate I: Photomicrographs of liver tissue stained with Haematoxylin and Eosin staining technique (H and E ×100 and ×400)

The result showed no toxicity in the central vein (CV) of the experimental group of the *Rattus norvegicus* as indicated by the arrow when compared with that of the control group.



Control Group **Experimental Group**

Plate II: Photomicrographs of kidney tissue stained with Haematoxylin and Eosin staining technique (H and E ×100 and ×400)

The result showed no toxicity in the glomeruli (G) of the experimental group of the *Rattus norvegicus* as indicated by the arrows when compared with that of the control group.

Table 2: The biochemical parameters of *Rattus norvegicus* injected intraperitoneally with 0.02ml/0.166kg of dexamethasone on daily basis for a period of four weeks (experimental group two) compared to that of the control group.

Parameters	Control group (n=6)	Experimental group (n=6)	P-values	Comment
ALT (U/I)	6.72 ± 0.48	12.38 ± 1.02	0.003	S
AST (U/I)	5.97 ± 0.29	9.87 ± 0.88	0.004	S
Urea (mmol/l)	6.10 ± 4.15	10.20 ± 2.10	0.004	S
Creatinine (µmol/l)	61.56 ± 5.35	102.00 ± 4. 94	0.001	S

Values are in mean and standard deviation

Keys:

ALT=Alanine aminotransferase

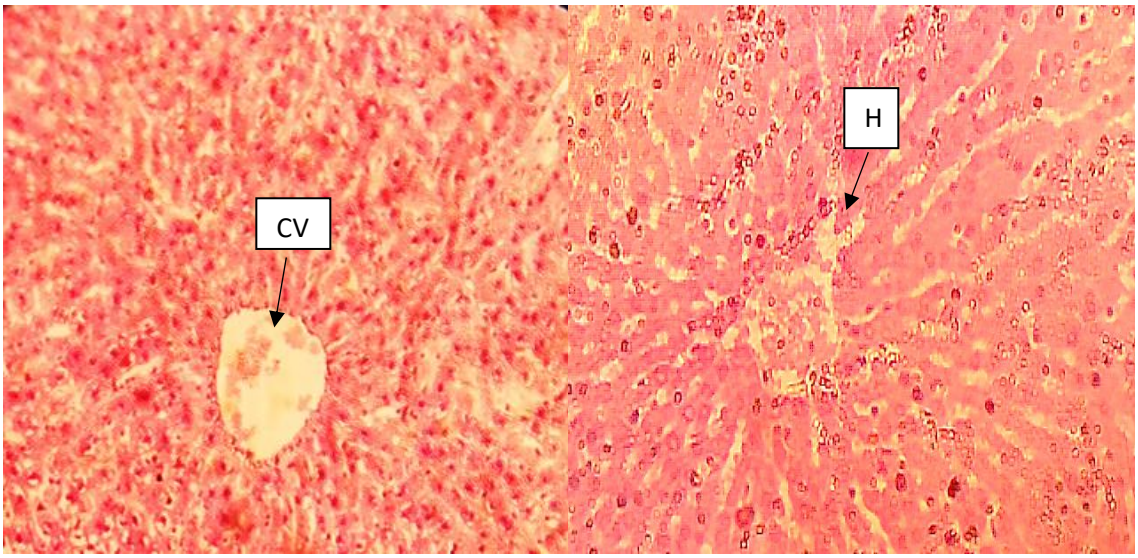
AST=Aspartate aminotransferase

n=Number of rats

S=Statistically significant

The results indicated that the biochemical parameters of *Rattus norvegicus* daily injected with dexamethasone for four weeks were substantially affected ($p<0.05$) compared to those of the control group.

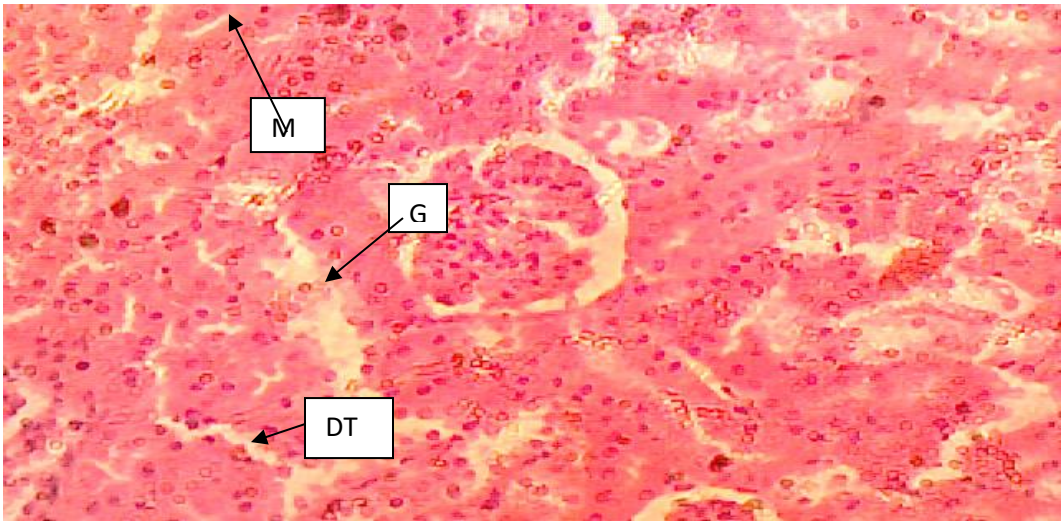
These biochemical findings are in conformity with the histomorphological examination of the liver and kidneys of the experimental *Rattus norvegicus* in this group which revealed toxicological alterations as shown in Plates III and IV respectively.



Experimental Group

Plate III: Photomicrographs of liver tissue stained with Haematoxylin and Eosin staining technique (H and E ×100 and ×400)

The result showed congestion of the central vein (CV) in the experimental group of the *Rattus norvegicus* as indicated by the arrows when compared with that of the control group.



Experimental Group

Plate IV: Photomicrographs of the kidney tissue showed glomeruli (G) swelling, vacuolar tubular degeneration (DT) and marked presence of mesangial cells (MS) in the experimental group of *Rattus norvegicus* (H and E, ×400)

DISCUSSION

In this study, the plasma biochemical parameters of male *Rattus norvegicus* administered with 0.02ml/0.166kg of dexamethasone injectable intraperitoneally daily for two weeks (experimental group one) and four weeks (experimental group two) were compared to those of male *Rattus norvegicus* that were not administered with dexamethasone injectable or any other drug (control group), as shown in Tables 1 and 2, respectively.

Table 1 demonstrates that the mean values of plasma alanine aminotransferase (p=0.71), aspartate aminotransferase (p=0.31), urea (p=0.57), and creatinine (p=0.82) in male *Rattus norvegicus* administered 0.02ml/0.166kg of dexamethasone injectable intraperitoneally on a daily basis for two weeks (experimental group one) were not statistically significant when compared to the control group. However, this biochemical finding established in the present study that daily administration of 0.02ml/0.166kg of dexamethasone intraperitoneally for two weeks has no adverse effect on plasma concentration of the aforementioned biochemical parameters is consistent with the histomorphological examination of the liver and kidneys organs of these *Rattus norvegicus*, which revealed no toxicity when compared to that of the control group.

As indicated in Table 2, the mean levels of plasma alanine aminotransferase (p=0.03) and aspartate aminotransferase (p=0.04) were substantially higher in male *Rattus norvegicus* injected with 0.02ml/0.166kg of dexamethasone intraperitoneally on daily basis for four weeks than in the control group. This significant biochemical elevation as established in this study, which is consistent with the histomorphological examination of the liver organ of these *Rattus norvegicus* which revealed congestion of the central vein when compared with that of the control *Rattus norvegicus* as shown in Plate III, may be suggestive of damage imposed on the liver organ which may be caused by the reactive metabolite of dexamethasone triggered by its prolonged administration for a period of time. This study's confirmation of a large rise of these liver enzymes in rats is consistent with prior research [17] showing a considerable elevation of these enzymes in rats.

As indicated in Table 2, the mean values of plasma urea (p=0.04) and creatinine (p=0.03) were considerably higher in male *Rattus norvegicus* injected with 0.02ml/0.166kg of dexamethasone intraperitoneally on daily basis for four weeks than in the control group. Significant biochemical elevations were found in this study, which is consistent with previous research [18] and in agreement with the histomorphological examination of the kidney organ of

these *Rattus norvegicus,* which revealed glomeruli swelling as shown in Plate IV, when compared with that of the control group. These biochemical elevations are presumed to be the result of increased bioaccumulation of dexamethasone, which may have contributed to the elevated concentration of acidity in the *Rattus norvegicus*.

CONCLUSION

According to the findings of this study, daily intraperitoneal injection of 0.02ml/0.166kg injectable dexamethasone for a period of four weeks may have negative effects on biochemical parameters such as urea and creatinine (kidney biomarkers), alanine aminotransferase and aspartate aminotransferase (liver enzyme biomarkers), and histomorphological changes in the status of hepato-renal organs in male *Rattus norvegicus*.

RECOMMENDATION

The competent authorities should inform the public of the risks linked with extended dexamethasone intake especially without a physician's prescription.

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