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Indomethacin Induced Toxicity: A Biochemical Study in Male Wistar Albino Rats

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Original Research Article

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Abstract: Indomethacin is a nonsteroidal anti-inflammatory drug (NSAID) which acts as an analgesic and antipyretic drug. This research work was aimed at demonstrating the effects of oral administration of indomethacin on some biochemical parameters in plasma of male wistar albino rats. Each of the 15 male wistar albino rats in experimental group one was given 2mg/kg of indomethacin capsule daily for two weeks while the other 15 male wistar albino rats in the experimental group two were given 2mg/kg each of indomethacin daily for four weeks. However, the 15 male wistar albino rats in the control group were not administered with indomethacin. Five ml blood specimen was collected from each of the groups into lithium heparinized anticoagulated bottles respectively. The specimen was spun and the plasma obtained was used for the quantitative measurement of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, urea and creatinine. The results revealed significant elevations ($P<0.05$) in the mean values of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, urea and creatinine in the male wistar albino rats administered orally with indomethacin for four weeks as compared to that of the control group. It was further revealed from the result that the mean values of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, urea and creatinine were not significantly elevated ($P>0.05$) in the rats administered with indomethacin for two weeks as compared to that of the control group. The elevated levels in the mean values of the rats orally administered with indomethacin for four weeks may suggest damage on their liver and kidney. It is therefore recommended that further histopathological examinations of these organs be carried out in order to ascertain the extent of damage.

Keywords: Indomethacin, toxicity, biochemical study, male wistar albino rats

INTRODUCTION

Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) which acts as an analgesic and antipyretic drug. It is commonly used as a prescription medication to reduce fever, pain, stiffness, and swelling from inflammation. It works by inhibiting the production of prostaglandins which are hormone-like molecules normally found in the body, where they have a wide variety of effects, some of which lead to pain, fever, and inflammation [1, 2].

Indomethacin has three additional modes of actions and these are inhibiting motility of polymorphonuclear leukocytes, similar to colchicine, uncoupling oxidative phosphorylation in cartilaginous and hepatic mitochondria, like salicylates and decreasing cerebral blood flow. The role of indomethacin in treating certain headaches is unique compared to other non-steroidal anti-inflammatory drugs. In addition to the class effect of cyclooxygenase inhibition, there is evidence that indomethacin has the ability to alter cerebral blood flow not only through modulation of nitric oxide pathways, but also via intracranial pre-capillary vasoconstriction [3]. This may be responsible for the remarkable efficacy in a group of headaches that is referred to as "indomethacin-responsive headaches", such as idiopathic stabbing headache, chronic paroxysmal hemicranial, and exertional headaches [4].

Indomethacin is also an effective tocolytic agent, able to delay premature labor by reducing uterine contractions through inhibition of prostaglandin synthesis in the uterus and possibly through calcium channel blockade [3]. It readily crosses the placenta and can reduce fetal urine production to treat polyhydramnios. It does this by reducing renal blood flow and increasing renal vascular resistance, possibly by enhancing the effects of vasopressin on the fetal kidneys [4]. It has a high acute toxicity both for animals and for humans, with some fatal human cases seen, particularly in children and adolescents [1]. Generally,

overdose in humans causes drowsiness, dizziness, severe headache, mental confusion, paresthesia, numbness of limbs, nausea, and vomiting, others include severe gastrointestinal bleeding, cerebral edema, and cardiac arrest with fatal outcome seen in children [2].

Indomethacin as a relatively less expensive non-steroidal anti-inflammatory drug is widely used by many individuals indiscriminately without the prescription of a physician for the treatment of moderate to severe osteoarthritis, rheumatoid arthritis, gouty arthritis because it reduces pain and fever. It is also used in so many homes in Nigeria for the killing of house rats. Considering the fact that researches as regards the effects of this drug on biochemical parameters in the plasma of humans are very rare it is thus expedite to carry out this study which is aimed at demonstrating the effects of oral administration of indomethacin for a period of two and four weeks respectively on some biochemical parameters in the plasma of male wistar albino rats. This however, is in an attempt to ascertain whether or not this drug has effect on human health when taken indiscriminately particularly without the prescription of a physician.

MATERIALS AND METHODS

Animals

Male albino rats 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, Amassoma, 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 ailment that may render them unfit prior to the commencement of the study. The rats which were placed in well ventilated iron standard cages were fed with pre-mix rat feed and water, *ad libitum.*

Ethical clearance

Informed consent was collected from the Niger Delta University Research Ethical Committee, Amassoma, Bayelsa State of Nigeria.

Experimental study

A packet of indomethacin capsule was bought in a Pharmacy Shop located in Yenagoa, Bayelsa State, Nigeria and stored following strictly the storage instruction as recommended by the manufacturer. The capsules were used for this research.

The study was carried out on male wistar albino rats that were 6 weeks old with an average weight of 138g. These rats were grouped as follow:

- Control group (these rats were not administered with indomethacin)
- Experimental group one (these rats were orally administered with indomethacin for two weeks)
- Experimental group two (these rats were orally administered with indomethacin for four weeks)

Biochemical parameters analyzed with specified methods

The various biochemical parameters analyzed with the specified methods used are as shown. Medifield Equipment and Scientific Limited Vis-Spectrophotometer with model number S23A was used for the absorbance measurement of the respective biochemical parameters.

- Aspartate aminotransferase: colorimetric method as described in the manual of 5th January, 2007 revised edition of Randox Laboratories, Limited, 55, Diamond Road, Crumlin, County, Antrim, BT294QY, United Kingdom [5, 6].
- Alanine aminotransferase: colorimetric method as described in the manual of 11th February, 2009 revised edition of Randox Laboratories, Limited, 55, Diamond Road, Crumlin, County, Antrim, BT294QY, United Kingdom [5, 6].
- Alkaline phosphatase: colorimetric endpoint method as described in the manual of September, 2001, A506 edition of Teco Diagnostics, 1268N, Lakeview Avenue. Anaheim CA92807, 1-800-222-9880 [7].
- Urea: urease method as described in the manual of 7th January, 2011 revised edition of Randox Laboratories, Limited, 55, Diamond Road, Crumlin, County, Antrim, BT294QY, United Kingdom [8-11].
- Creatinine: Jaffe reaction method previously described by Jaffe in 1886 and revised on 15th September, 2010, by Randox Laboratories, Limited, 55, Diamond Road, Crumlin, County, Antrim, BT294QY, United Kingdom [12].

Pilot study

A pilot study was carried out in order to ascertain the minimum dose of indomethacin that can cause 100% death (LD_{IOO}) in the experimental rats. The LD_{IOO} was obtained with 32mg/kg of indomethacin. An acute study was also carried out in order to obtain 50% of death. The LD_{50} was obtained with 17mg/kg of indomethacin.

After the above treatments, the rats were now monitored for a period of 24 hours for any signs and symptoms of indomethacin toxicity as well as death. The following signs and symptoms: dizziness, squeaking, scratching of nose, weakness and loss of appetite were seen to occur at dose levels of 17mg/kg (LD_{50}) and 32mg/kg (LD_{100}). Five out of the ten rats administered with 17mg/kg of the drug died 24 hours

after administration (LD_{50}). While all the ten rats administered with 32mg/kg of the drug died 13 hours after administration (LD_{100}).

Chronic toxicity study

In this study 15 experimental rats weighing 138±2g were administered orally with 2mg/kg of indomethacin daily for two weeks (experimental group one) and four weeks (experimental group two) respectively while another 15 rats which served as the control group were not administered with indomethacin.

At the end of this experiment, the control and experimental rats were anaesthesized using inhaled chloroform technique after which five ml blood specimen was withdrawn from the cardiac aorta of each of the rats into different lithium heparin anti-coagulated bottles for biochemical investigations.

Statistical analysis

The results of this study were expressed as mean and standard deviation, while the differences between the subjects (control and experimental groups) were assessed using the student 't' test. The results were considered statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

The results of the biochemical parameters measured in the control and experimental group one (two weeks) as well as experimental group two (four weeks) are as shown in Tables 1 and 2. The results revealed that the mean values of plasma aspartate aminotransferase (AST), plasma alanine aminotransferase (ALT), plasma alkaline phosphatase (ALP), plasma urea, and plasma creatinine in the male wistar albino rats administered with indomethacin orally for a period of two weeks showed no significant increase ($P>0.05$) when compared with the mean values of the control group while the results of the mean values of plasma aspartate aminotransferase (AST), plasma alanine aminotransferase (ALT), plasma alkaline phosphatase (ALP), plasma urea, and plasma creatinine in the male wistar albino rats administered with indomethacin orally for a period of four weeks showed significant elevations ($P<0.05$) when compared with the mean values of the control group.

Table-1: Biochemical parameters measured in the control group and experimental group one (two weeks)

Parameters measured	Control group (n= 15)	Experimental group(n=15)	Remark
AST (U/I)	6.1 ± 1.11	7.27 ± 2.03	NS
ALT (U/I)	6.47 ± 1.05	7.18± 1.46	NS
ALP (IU/L)	18.9 ± 5.6	22.62 ± 4.26	NS
Urea (mmol/L)	5.57 ± 1.47	6.30 ± 1.65	NS
Creatinine (mmol/L)	59.3 ± 4.2	60.9 ± 7.32	NS

KEYS:

AST= Aspartate aminotransferase
ALT= Alanine aminotransferase
ALP= Alkaline phosphatase
NS= Not-significant
n= number of rats

Table-2: Biochemical parameters measured in the control group and experimental group two (four weeks)

Parameters measured	Control group (n= 15)	Experimental group(n=15)	Remark
AST (U/I)	6.1 ± 1.11	15.9 ± 1.73	S
ALT (U/I)	6.47 ± 1.05	22.2 ± 3.55	S
ALP (IU/L)	18.9 ± 5.6	44.1 ± 5.90	S
Urea (mmol/L)	5.57± 1.47	12.09 ± 2.81	S
Creatinine (mmol/L)	59.3 ± 4.2	107.7 ± 5.6	S

KEYS:

AST= Aspartate aminotransferase
ALT= Alanine aminotransferase
ALP= Alkaline phosphatase
S= Significant
n= number of rats

The elevated mean values of plasma aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in the wistar albino rats administered with indomethacin orally for a period of four weeks (experimental group two) as confirmed in this study may be indicative of hepatocellular injury. These findings are in agreement with the previous work of Rudnick *et al.*, [13] who in their research work in which mouse was used as the model reported that the chronic use of indomethacin causes hepatocellular injury and

may influence the development or progression of liver injury in humans.

The results in this study went further to show significant increase ($P<0.05$) in the mean values of plasma urea and plasma creatinine in wistar albino rats administered orally with indomethacin for a period of four weeks (experimental group two) as compared to that of the control group. These significant elevations are in agreement with the previous work of Oates *et al.*, [14] who reported that these elevations may be suggestive of renal impairment caused by the inhibition of prostaglandin synthesis as a result of the chronic administration of indomethacin. The findings are also in agreement with the previous work of Abanta *et al.*, [15] who in their research work reported elevated levels of plasma urea and plasma creatinine in experimental rats after indomethacin administration for six weeks.

The mean values of the biochemical parameters measured in the experimental group one and two as well as the control group are further illustrated with a Bar chart as shown in Figure-1. The illustrated bar chart showed no significant increase in the mean values of plasma aspartate aminotransferase, plasma alanine aminotransferase, plasma alkaline phosphatase, plasma urea and plasma creatinine in experimental group one as compared to that of the control group while that of the experimental group two showed twice or more the mean values of plasma aspartate aminotransferase, plasma alanine aminotransferase, plasma alkaline phosphatase, plasma urea and plasma creatinine as compared to that of the control group.

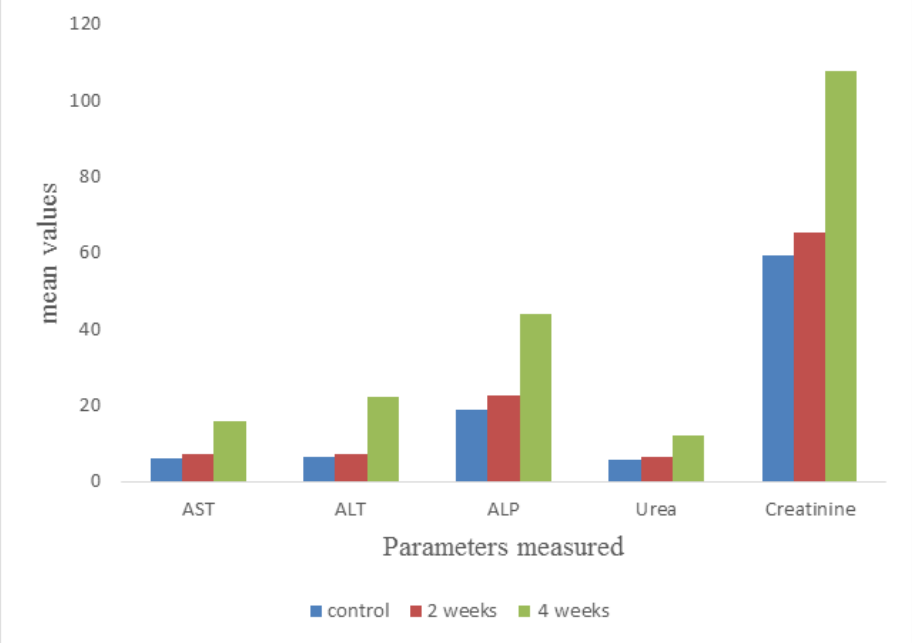

Fig-1: A bar chart showing the mean values of the biochemical parameters measured in the three groups of male wistar albino rats

CONCLUSION

In conclusion, this study has revealed that the concentrations of plasma aspartate aminotransferase, plasma alanine aminotransferase, plasma alkaline phosphatase, plasma urea and plasma creatinine are elevated in male wistar albino rats orally administered with indomethacin for four weeks while the concentrations of these parameters remained unaltered in the male wistar albino rats orally administered with indomethacin for two weeks.

RECOMMENDATION

- It is recommended that further research work should be carried out on the histopathological evaluation of the liver and kidneys of these male wistar albino rats orally administered with indomethacin for four weeks in order to ascertain the extent of damage to these organs.
- The recommended maximum dosage of indomethacine should not be exceeded unless otherwise prescribed by a medical doctor

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