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Toxicity Studies on the Effects of Non-Steroidal Anti-Inflammatory Drugs in Wistar Albino Rats

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ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used drugs in medicine for various inflammatory conditions. The study was aimed at determining toxic effects of non-steroidal anti-inflammatory drugs in wistar Albino rats by assessing their Biochemical and hematological properties. A total of twenty one (21) adult Wistar rats were used for this research. Diclofenac sodium, Ibuprofen, Aspirin, piroxicam, Celecoxib and indomethazine treatment groups (n =3) was orally administered to each rat following the corresponding dose which was selected based on the LD dose in rats and body weight. Treatment of the animals was done for 14 days after the 7 days acclimatization before sacrificing them through cervical dislocation. Blood was collected by cardiac puncture, using 5ml syringes and 23G needles into blood sample containers for hematological analysis and the liver and Kidney was harvested for biochemical analysis using the principles of biochemical and hematological analysis. The hematological results show that the drug-treated groups had significant decrease in the values of Hb concentration, PCV, RBC and WBC when compared with the control group. However, Hb concentration levels were also observed to increase in Indomethazine, celecoxib, aspirin and diclofenac-treated groups when compared with the control. Biochemical results shows a significant increase of the drug-treated groups of the levels of serum ALP, total bilirubin, total protein, creatinine, albumin, urea, total cholesterol, in comparison with the control group. The results therefore showed that NSAIDs used in this study had toxic effects on vital animal tissues, resulting in hematological disorder, hepatic and renal impairments.

Introduction

The liver, gastrointestinal tract, lungs and kidneys, are organs of drug metabolism, with the liver being the chief organ of drug biotransformation [1]. Drug metabolism also known as xenobiotic metabolism is the biochemical modification of pharmaceutical substances or foreign compounds by living organisms, usually through specialized enzymatic systems. Xenobiotics metabolism involves set of metabolic pathways that modify the chemical structures of xenobiotics, which are compounds foreign to the normal biochemistry of organisms. The reactions often act to detoxify poisonous compounds; however, in some cases the intermediates formed during xenobiotic metabolism themselves can have toxic effects on living tissues [2]. Thus, the liver, kidneys and blood cells are greatly affected by drug-induced toxicity.

A wide variety of pharmacological and chemical agents is known to produce a range of acute or chronic liver and kidney diseases and hematological alterations. Non-steroidal anti-inflammatory drugs (NSAIDs) such as Ibuprofen, Piroxicam, Diclofenac Sodium, Aspirin, Indomethazine and Celecoxib are widely used drugs in medicine for various inflammatory conditions. NSAIDs act both centrally (example, analgesic, antipyretic actions) and peripherally (example, analgesic, antithrombic actions) and are one of the best therapeutic choices to prevent and treat postoperative pain [3]. These drugs are used to relieve acute visceral and musculoskeletal signs of pain (including those associated with trauma and chronic signs of pain (from conditions such as arthritis) and to decrease inflammation and central nervous sensitization associated with surgery [4, 5].

They exert their anti-inflammatory, antipyretic and analgesic effects via the suppression of prostaglandins (PGs) synthesis, by inhibiting the enzyme, cyclooxygenase (COX), which has two isoforms, COX-1 and COX-2 [6]. They inhibit both COX-1 and COX-2, the rate-limiting enzymes for the production of prostaglandins and thromboxane. COX-1 functions mainly in the control of renal haemodynamics and glomerular filtration rate (GFR), while COX-2 functions primarily affect salt and water excretion [7]. The use of these agents therefore may lead to toxic effects on body tissues or cause cellular injuries, ranging from acute to long-term chronic disorders and may include conditions considered to be degenerative. These include alterations in renal function, effects on blood pressure, hepatic injury, and platelet inhibition, gastrointestinal and cardiovascular disorders [8, 9]. However, the use of lowest effective dose of a given NSAID will reduce the incidence of the drug adverse effects and

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complications [9]. The toxicity of some compounds in these drugs can be directly related to their biliary excretion. For example, indomethacin can cause intestinal lesions. The sensitivity of various species to this toxic response is directly related to the amount of indomethacin excreted into bile.

Drug-induced aplastic anaemia may represent either a predictable or idiosyncratic reaction to a xenobiotic. This life threatening disorder is characterized by peripheral blood pancytopenia, reticulocytopenia, and bone marrow hypoplasia [10]. Indomethacin belongs to the group of xenobiotics associated with aplastic anaemia. Furthermore at least three different types of nephrotoxicity have been associated with NSAID administration [11-13]. These include acute renal failure which occur within hours of a large dose of a NSAID; analgesic nephropathy which occurs from chronic consumption of NSAID [14] and interstitial nephritis which is characterized by a diffuse interstitial edema with infiltration of inflammatory cells [13].

The study was aimed at determining toxic effects of non-steroidal anti-inflammatory drugs (NSAIDs) in wistar Albino rats by assessing their Biochemical and hematological properties.

Materials and Methods

Materials, Chemicals and Drugs

Syringes and Needles, Hand Gloves, Incubactor, Glucometer, Aucku Check active Strip Micropipette, Stop Watch, Oven, Centrifuge Model 800, Cotton Wool, HPLC, and GCMS. The chemicals included 10% Formalin, Xylene, Hemotoxylin and Eosin stains purchased at Aldrich Sigma Co.3050, Spruce Str, St. Louis Warri, Delta State. Drugs used includes; Diclofenac sodium, Ibuprofen, Piroxicam, Indomethazine, Celecoxib and Aspirin (Acetylsalicylic acid ASA).

Experimental Animals

Animal Handling

Twenty one (21) adult wistar albino rats used for this study were purchased from the animal house of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. The animals were acclimatized for seven (7) days in well aerated cages and conditioned in a breeding chamber with the natural controlled system (room temperature and a natural 12 h-12 h light-dark cycle). The rats were allowed free access to water and fed with standard commercial pelleted feed, a product of Top Feed in Sapele, Delta State. The protocol of this research was performed in accordance with the ethical standards on the care and use of animals as laid down in reference [15].

Drug Use

Preparation of NSAID and administration of drugs

All the drug solutions were freshly prepared before use and were administered orally. The drugs solution was prepared daily by grinding the drugs tablets and diluting the powder in distil water. Using 5 ml syringe, the corresponding dose was given to each rat based on body weight the dose of Diclofenac sodium, Ibuprofen, Aspirin, piroxicam, Celecoxib and Indomethazine was selected based on the LD dose in rats, when administered orally.

Ibuprofen

A 500 mg of Ibuprofen was dissolved in 100 ml of distil water to yield 0.5 ml of stock solution.

Diclofenac

A 500 mg of diclofenac sodium was dissolved in 100 ml of distil water to yield 0.5 ml of stock solution.

Piroxicam

Piroxicam capsule (500 mg) was dissolved in 100 ml of distil water to yield 0.5ml of stock solution.

Indomethacin

Indomethacin capsule (500 mg) was dissolved in 100 ml of distil water to yield 0.5ml of stock solution.

Celecoxib

Celecoxib (500 mg) was dissolved in 100 ml of distil water to yield 0.5ml of stock solution.

Aspirin (Acetylsalicylic acid ASA)

A 500 mg of aspirin tablet was dissolved in 100 ml of distil water to yield 0.5 ml of stock solution. All drugs were prepared and administered orally using the formula;

$$Volume\ of\ administration = \frac{weight\ of\ animal\ \times dose\ to\ be\ administer}{Dilution\ factor\ \times stock\ Solution}$$

Experimental Design

After the period of acclimatization, the animals were randomly divided into experimental and control groups. The wistar albino rats were grouped and drugs administered as follows;

Group A (n = 3) Control Group Wister Rats received 2ml of distil water daily within the period of the study before sacrificing.

Group B (n = 3) wistar albino rats were treated with 5 mg/kg body weight of diclofenac drug.

Group C (n = 3) wistar albino rats were treated with 10 mg/kg body weight of Ibuprofen drug.

Group D (n = 3) wistar albino rats were treated with 15 mg/kg body weight of Piroxicam.

Group E (n = 3) wistar albino rats were treated with 5 mg/kg body weight of Indomethacin drug.

Group F (n = 3) wistar albino rats were treated with 10 mg/kg body weight of Celecoxib drug.

Group G (n = 3) wistar albino rats were treated with 20 mg/kg body weight of Aspirin (Acetylsalicylic acid ASA) drug.

Sample Collection

The animals were observed in their cages for clinical symptoms daily and at the end of the 14 days treatment, the rats were sacrificed by cervical dislocation and each rat was placed on its dorsal surface, a laparotomy was carried out to expose the internal organs, and blood was collected by cardiac puncture, using 5ml syringes and 23G needle into blood sample containers and the liver and Kidney was harvested for biochemical analysis.

Determination of Biochemical and Hematological Parameters

Hematological Analysis

Serum was separated from clotted blood obtained by cardiac puncture. Total red blood cell (RBC) and white blood cell (WBC) counts were made by the haemocytometer method, hemoglobin concentration (Hb) by the cyanmethemoglobin method, packed cell volume (PCV) by capillary tube method.

Biochemical Analysis

Serum enzymes alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), urea, total protein, total bilirubin, total cholesterol, creatinine and Albumin were analysed according to the principles of biochemical analysis [15].

Results

Hematological parameters

Effect of the NSAIDS on haematological parameters of rats were observed in all the treatment groups but more

increased levels of total WBC on the group treated with indomethacin. A wide variety of pharmacological and chemical agents like Non-steroidal anti-inflammatory drugs (NSAIDs) (Ibuprofen, Piroxicam, Diclofenac Sodium, Aspirin, Indomethazine and Celecoxib) are widely used in medicine for various inflammatory conditions. They are known to produce a range of acute or chronic liver and kidney diseases and hematological alterations. Hematological parameters like Hb concentration, WBCs, PCV and RBCs were estimated in control and treated groups and are presented in Table 1.

Biochemical Parameters

Clinical Effects of Non-Steroidal Anti-inflammatory Drugs in rats

No clinical symptoms were observed with the administration of aspirin. Animals administered with indomethacin showed reduced feed intake, sluggishness, unthrifty appearance and diarrhoea. The only symptoms seen in the group given piroxicam was sluggishness.

Effect of the NSAIDS on Biochemical parameters of rats

All the NSAIDS used produced significant increases in the levels of alkaline phosphatase, urea, total cholesterol, total bilirubin, creatinine, Albumin and total protein (diclofenac sodium, Indomethazine and Aspirin) but decrease in AST, ALT and total protein (Ibuprofen and piroxicam). The Biochemical parameters of the control and the NSAIDs treated groups are shown in Table 2.

Discussion

Hematological parameters

The significant alterations in hematological parameters of rats treated with the drugs may provide evidence of toxicity. The reduction in PCV (piroxicam, Ibuprofen and diclofenac), RBC (Ibuprofen) and Hb (piroxicam) values in the above drug-treated groups may suggest drug-induced toxicity, characterized by excessive destruction of red blood cells resulting in anaemia [16]. It may also be due to loss of erythrocytes as a result of gastrointestinal bleeding. When there is a substantial loss of blood from the body, the RBC picture may indicate microcytic hypochromic anaemia [17, 18]. The findings in the present study are in agreement with those of reference [19, 20].

However, the increase in Hb values in Indomethazine, celecoxib, aspirin and diclofenac treated groups as observed may be due to the effect of these drugs which may increase the bone marrow activity. Moreover, also the increase in Hb may be due to the increase of its biosynthesis by increasing the succinyl pool as well as glycine pool. Both are required in the initial stage of the heme biosynthesis [20].

The significant decrease in white blood cells counts (WBCs) observed in Piroxicam, celecoxib and aspirin-treated groups may be due to decrease in feed intake by the rats, as was observed in the course of this experiment. This is in agreement with earlier studies which showed that decreased feed intake may have a major impact on the haematopoietic system and has been observed to decrease white blood cells, platelets and reticulocytes counts [21]. However, the observed effects of diclofenac, Ibuprofen and Indomethazine which are represented by increased levels of WBCs are generally in agreement with the results of several investigations on the animals treated with different NSAIDs [22, 23].

Biochemical Parameters

The significant alterations observed in the biochemical indices of the albino rats showed that intramuscular administration of the tested drugs at the respective dosage may cause hepatotoxicity and nephrotoxicity. The activities of AST, ALT and ALP are commonly used as biochemical indicators of liver functions. Structural and functional alterations in the liver result in elevated levels of these enzymes in circulation [24]. The levels of these aminotransferases (ALT and AST) in serum are elevated in all liver diseases. In fact, very high levels of more than 1000 units can be seen in acute hepatitis [15]. These enzymes are intracellular, thus their normal blood levels are very low, but when there is hepatocellular damage or necrosis of the liver cells [25], they leak out into the blood circulation, drastically increasing their levels in blood [24].

Thus, the increased ALP levels in the drugs– treated groups in our findings may provide preliminary evidence of liver impairments. These findings are similar to that of references [20, 26]. The significant increase of serum alkaline phosphatase (ALP) levels observed was attributed to liver impairment, as this indicates cholestatic alterations in the

Table 1. Effects of NSAIDs on hematological parameters of wistar albino rats.

Group	RBC ($\times10^6$/µL)	WBC ($\times10^3$/µL)	PCV (%)	Hb (g/dL)
Control	6.8± 0.2	10400± 378.2	44.2± 1.1	13.1± 0.4
Diclofenac	7.0 ± 0.5	21300± 680.7	42.5± 1.3	13.7 ± 0.5
Ibuprofen	6.2± 0.3	22300± 378.6	41.3± 0.8	13.1± 0.2
Piroxicam	6.8± 0.2	9300± 288.7	40.5± 0.9	13.0± 0.3
Indomethazine	7.2± 0.4	15171±438.4	46.5± 2.4	14.2± 0.3
Celecoxib	7.1± 0.6	6700± 608.3	46.3± 1.2	14.5± 0.2
Aspirin	6.9± 0.4	9050± 125.0	44.5± 1.3	14.1± 0.1

Data are expressed in mean ± SEM (n=3), Note: WBC= White blood cell, RBC= Red blood cell, PCV=Packed Cell Volume, Hb=Hemoglobin,

Table 2. Effects of NASIDs on Biochemical Parameters of Wistar albino rats.

Biochemical Indices	Control	Diclofenac	Ibuprofen	Piroxicam	Indomethazine	Celecoxib	Aspirin
Total Bilirubin (mg/dl)	0.10±0.006	0.23±0.01	0.23±0.01	0.25±0.03	0.22±0.003	0.22±0.02	0.20±0.006
Total Protein (g/dl)	0.31±0.009	0.34±0.01	0.30±0.04	0.28±0.04	0.34±0.01	0.31±0.03	0.36±0.009
Albumin (g/dl)	0.23±0.10	0.91±0.005	0.94±0.03	0.91±0.03	0.90±0.002	0.90±0.06	0.95±0.03
Creatinine(mg/dl)	0.02±0.002	0.06±0.009	0.07±0.003	0.07±0.009	0.04±0.01	0.06±0.006	0.07±0.01
ALP (u/l)	0.05±0.002	0.18±0.04	0.10±0.005	0.08±0.001	0.10±0.001	0.10±0.001	0.10±0.001
AST (u/l)	0.05±0.002	0.03±0.003	0.03±0.001	0.03±0.001	0.03±0.0009	0.03±0.0009	0.03±0.0009
ALT (u/l)	0.06±0.0009	0.02±0.0006	0.02±0.0006	0.02±0.002	0.01±0.003	0.02±0.003	0.02±0.001
Urea (mg/dl)	0.05±0.003	0.13±0.02	0.11±0.007	0.08±0.006	0.07±0.003	0.07±0.006	0.10±0.007
T.C (mg/dl)	201±1.20	239±0.6	243±3.2	205±1.9	232±3.21	211±6.1	240±0.90

Data are expressed in mean ± SEM (n=3), Note: TB=Total Bilirubin, TP= Total Protein, ALP= Alkaline phosphatase, AST=Aspartate aminotransferase, ALT= Alanine aminotransferase, TC=Total Cholesterol

liver biliary ducts, which may be due to the effect of these drugs. The ALP levels are usually elevated as a result of cholestasis or biliary obstructions and this is used as a marker of hepatobiliary dysfunctions [15]. Our findings are in agreement with reference [22].

The increase in serum total bilirubin observed in all the treated groups may be due to liver impairments, as a result of the respective doses of the drugs. Serum bilirubin is one of the markers of liver impairments, as it tests for hepatic excretory function. Bilirubin is the excretory product formed by the catabolism of heme, which is normally conjugated by the liver to form bilirubin diglucuronide and excreted through the bile, so elevated serum bilirubin is seen when there is liver impairment [27]. Serum bilirubin is also elevated when there is excessive breakdown of erythrocytes such as in haemolytic anaemia, which may be caused by the drugs. The heme formed from breakdown of haemoglobin derived from red blood cells is catabolized by the reticulo-endothelial system (RES) to produce bilirubin [28]. These findings are in concordance with the works of reference [23].

The increase serum levels of albumin noticed in the present study are not in agreement with the findings of reference [22], who observed significant decreases in serum albumin levels in wistar *Rattus norvegicus* treated with diclofenac sodium at different dosage for 7 days. The decrease Total protein levels of Ibuprofen and piroxicam-treated group may decrease due to protein-losing nephropathy, haemorrhage, dietary protein deficiency and nutrients malabsorption [29]. Thus, the decreases in serum total protein may also be due to haemorrhage in the gastrointestinal tract as a result of toxic effect of the drug, nutrient malabsorbtion and reduced feed intake. These findings are in agreement with [22].

The significant increase in levels of urea and creatinine observed in all treated groups may be due to nephrotoxic effect of the drugs, leading to reduced renal function. Urea and creatinine levels are used as marker of kidney function, but the test for creatinine is more sensitive than urea [15]. Thus, elevated serum levels of urea and creatinine may indicate kidney injury, with resultant reduced glomerular filtration. The results of this study agree with the findings of reference [26].

Urea is formed in the liver, representing the principal waste product of protein catabolism and is excreted by the kidney. These drugs may have probably caused a decrease in glomerular filtration rate, resulting in decreased excretion of urea, which may produce an increase in the concentration of the blood urea [24]. It was earlier reported that these Non-steriodal anti-inflammatory drugs inhibits cyclooxygenases, thereby suppressing the production of prostaglandins, which play an important role in maintaining glomerular filtration rate of the kidneys [6].

Creatinine is a non-protein nitrogenous substance formed from creatine and phosphocreatine during muscle metabolism. It is also removed from blood by glomerular ultrafiltration of the kidneys. As with urea, its rate of removal is influenced by glomerular filtration rate, and any abnormalities that decrease glomerular filtration rate will result in an increased serum creatinine. Previous studies have shown that an apparently minor increase in serum creatinine can reflect a marked decrease in glomerular filtration rate [29].

The significant alterations observed in serum total cholesterol in all the treated groups maybe due to the effect of the drugs. Cholesterol may increase due to hepato-biliary disease and protein-losing nephropathy. The increase in the serum levels of total cholesterol may be attributed to the toxic effect of the drugs, leading to hepatobiliary disorders and impaired cholesterol metabolism. These findings are in accord with reference [20]. The liver has a major role in controlling the plasma levels of LDL-cholesterol. It synthesizes cholesterol, removes cholesterol from lipoprotein remnants, converts cholesterol to bile acids and is the only organ that can excrete cholesterol through bile [22]. Thus, when there is drug-induced liver impairment, there will be elevated levels of serum cholesterol will be decreased [15]. It is also well known that elevated level of total cholesterol is risk factor for cardiovascular diseases, atherosclerosis, hypertension and stroke.

Conclusion

The result of the present study has shown that the drugs can cause significant alterations in the hematological and biochemical indices of rats. It can therefore be concluded that Indomethazine, Celecoxib, Aspirin, Diclofenac, Piroxicam and Ibuprofen at the various respective doses and duration of study might cause toxic effects on vital animal tissues, resulting in hematological disorder, hepatic and renal impairments. So the safe dose of these drugs might be lower than the doses used in this study. Thus, caution should be exercised in the clinical use of these drugs for therapeutic purpose, which should be limited to the lowest dose and treatment duration required to achieve the best therapeutic effect. It is recommended that further toxicological studies be performed on the NSAIDs with different dosage to ascertain the appropriate dose for safe therapeutic administration of the drugs.

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