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# Effects of aspirin (acetylsalicylic acid) and Cataflam (potassium diclofenac) on some biochemical parameters in rats

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**Summary**
The effects of graded doses of aspirin (acetylsalicylic acid) and cataflam (potassium diclofenac) on serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, 5'Nucleotidase, methaemoglobin, total and conjaged bilirubin were investigated in wistar rats. Results showed a significant increase ($P < 0.05$) in the levels of alanine animotransferase, aspartate amino transferase, methaemoglobin, total and conjugated bilirubin upon treatment of animals with both drugs. Aspirin significantly decreased ($P < 0.05$, $P < 0.00$) the activity of alkaline phsophatase but increased the activity of 5'ucleotidase while cataflam significantly increased the activity of alkaline phosphatase ($P < 0.001$) and 5'nucletodase ($P < 0.05$). These effects were however dose dependent and the biochemical implications of these results are discussed.

**Keywords**: *Aspirin, cataflam, biochemical parameters).*

**Résumé**
Les effets des doses graduelles d'aspirine (Acide acetylesalicyclic) et du cataflam (dichlofenac de potashsium) sur l'aspartate aminotransferase, l'alanine aminotransferase, l'alkaline phosphatase, le 5'Nucleotidase, la methawmoglobin, le taux total de la barbiturine, et de la barbiturine conjugue du serum a ètè investiguè chez les rats de souche wistar. Les resultats ont montrès une augmentation significative (P. <0.05) sur le niveau de l'alanine transferase, de l'aspartate aminotransgerase, du methaemoglobine, du taux total et conjuguè de la barbitturine chez les animaux pendant le traitement avec les 2 medicaments. L'aspirine a decrut de maniere significative (P<0.05, P <0.001) l'activitè de la phosphatase, mais accrut l'activite de la 5'nucleotidase, alorsque la cataflam a significativement accrut l'activitie de la 5' nucleotidase, alorsque la cataflam a significativement accrut l'activitè de la phosphatase alkaline (P<0.001) et la 5' Nucleotidase (P<0,05), Ces effects ont ètè dependante de la dose et les implications bochemique sont discutè ici.

## Introduction

Most analgesic drugs are purchased across the counter without formal prescription and are subject to abuse. Aspirin (acetylsalicylic acid) is the most widely used analgesic and long standing clinical trials have established aspirin as the standard medication against which non-narcotic analgesic agents are compared [1]. The major clinical usefulness of aspirin has been in the control of mild and moderately severe pain. Aspirin is thought to be harmful but it is strongly bouncing back into use. Recently, a large multi-centre study from USA (the 'Stroke Prevention in Atrial Fibrillation study') has produced evidence that aspirin may reduce the risks of embolic stroke in patients with atrial fibrillation [2].

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Cataflam (potassium diclofenac a non steroidal anti-inflammatroy drug with pronounced analgesic and anti-pyretic properties is fast gaining wide popularity as is evidenced by its prescription and non-prescription use. The drug is known to inhibit the synthesis of prostaglandins which play a major role in the causation of inflamation, pain and fever [3].

These drugs, when administered in normal therapeutic dose, do not result in scrisus side effects. However, evidence abound to support the fact that prolonged intake of aspirin or an overdose may result in hepatotoxicity, gastric bleeding and ulceration, epigastric pain, vomiting, foetal malformations lengthening of gestation period, heamorrhage, among other deliterious side effects (4,5,6,7,8,9). Adverse reactions to Cataflam include occasional epigastric pain, nausea, vomiting, diarrhoea, and rarely gastronintestinal bleeding or ulceration [3].

Reports on the biochmeical examination of aspirin and cataflam are scanty, hence this study is set up to examine the effect of graded doses of these drugs on some biochemical parameters of liver function since it is the primary site of metabolism of drugs.

## Materials and method

*Experimental animals*

Fifty adult albino wistar rats weighing 150 – 200 g were obtained from the animal house of the Department of Biochemistry, College of Medicine, University of Calabar (temperature 26 ± 2.0°C). The rats were divided into five groups of 10 rats each. Each of these groups was again divided into two sub groups, A and B of 5 rats eachsince two separate drugs were being studied. The rats were housed in well ventilated cages and allowed normal daylight cycles. Animal feed obtained from pfizer feed Mil, Aba, Nigeria and water were provided *ad libitum*.

*Drugs*

Aspirin (May and Baker, Lagos, Nigeria) and Cataflam (Ciba-Geigy Ltd, Basle, Switzerland) were purchased from Kamel Pharmacy, Calabar, Nigeria. The drugs were suspended in 0.9% saline solution and administered orally using an orogastric tube.

*Treatment of animals*

Sub groups $A_2$-5 were administered 100, 200, 300 and 800 mg/kg body weight of aspirin, respectively, for 4 days while sub-groups $B_2$-5 were also administered with similar doses of cataflam for a period of 4 days. Subgroups $A_1$ and $B_1$ were control animals which were administered normal saline (0.9%) since normal saline was used in dissolving the drugs.

*Collection of samples*

At the end of the fourth day of treatment, all rats were anaesthesized in a chloroform chamber. Blood was

obtained by cardiac puncture into well cleaned and labelled sample tubes and allowed to stand for an hour to allow time for clotting. The serum was aspirated into different tubes for subsequent assay.

*Reagents/chemicals*

Alanine aminotransferase (EC:2.6.1.2.) and aspartate amniotransferase (EC:2.6..1) were determined according to the method of Reitman and Frankel [10]. Alkaline phosphatase (EC 3.1.3.1) was determined according to the mehtod King and King [11] while 5'Nucleotidase (EC:3.1.3.5) was assayed by method of Campbell [12]. Bilirubin was analysed by the method of Malloy and Evelyn [13] and the percentage methaemoglobin was obtained using the modified method of Udosen *et al* [14].

Table 1 shows the effect of graded doses of Asprin on the activities of aspartate aminotransferase, alanine amnotransferase, alkaline phosphatase and 5'Nucleotidase; serum level of total and conjugated bilirubin, as well as percentage methaemoglobin in Wistar rats following 4 days treatment period.

The activity of alanine aminotransferase showed a dose-dependent increase from 44.54 ± 0.88 IU/L in the control group of 83.04 ± 1.08 IU/L in the group treated 800 mg/kg body weight of Aspirin. The increase in activity was significant at all experimental doses. The activity of aspartate aminotransferase also showed a dose dependent increase from 93.28 ± 1.85 in the control group to 160.08 ± 1.13 IU/L in the group treated 800 mg/kg body experimental doses of 200 mg, 300 mg and 800 mg/kg weight of Aspirin.

The activity of alkaline phosphatase showed a dose-dependent decrease from 90.53 ± 5.02 IU/L in the control group to 10.65 ± 1.54 IU/L in the group treated with Aspirin dose of 800 mg/kg body weight while the activity of 5'Nucleotidase showed a dose-dependent increase from 8.70 ± 0.42 IU/L in the control group of 12.30 ± 0.14 IU/L in the group treated 800 mg/kg body weight. The decrease in activity of alkaline phosphatase was significant ($P < 0.05$) at 100 mg and 200 mg of the drug. At doses of 300 mg and 800 mg/kg body weight, the decrease was also significant ($P < 0.001$). However, the increase in activity of 5'Nucleotidase was also significant at experimental doses of 100 mg, 200 mg ($P < 0.05$), and 300 mg and 800 mg/kg body weight ($P < 0.001$).

The level of total and conjugated bilirubin showed a dose-dependent increase following Aspirin administration, the increase was significant ($P < 0.001$) for conjugated bilirubin at all experimental doses of the drug while the increase in total bilirubin was significant ($P < 0.001$) only at doses of 300 mg an d800 mg and 800 mg/kg body weight. The perentage methaemoglobin groups also recorded a gradual dose-dependent increase which was significant ($P < 0.001$) at doses of 300 mg and 800 mg/gk body weight.

Table II shows the effect of graded doses of Cataflam on the activity of alanine aminotransferase, asparte aminotransferase, alkaline phosphatase and 5'Nucleotidase; serum level of total and conjugated bilirubin as well as the percentage methaemoglobin in experimental animals.

**Table 1:** The effect of aspirin on some biochemical parameters in wistar rats

| Parameters | Control | 100 mg/kg body weight | 200 mg/kg body weight | 300 mg/kg body weight | 800 mg/kg body weight |
|---|---|---|---|---|---|
| Alanine aminotransferase (IU/L) | 44.54 ± 0.88 | 49.10,** ± 1.12 | 58.66** ± 1.24 | 62.37** ± 2.01 | 83.04** ± 1.08 |
| Asparatate aminotransferase (IU/L) | 93.28 ± 1.85 | 95.43* ± 0.93 | 119.04** ± 1.89 | 133.62** ± 2.04 | 160.08** ± 1.13 |
| Alkaline phosphatase (IU/L) | 90.53 ± 5.02 | 61.54** ± 1.47 | 62.72** ± 1.02 | 56.37* ± 2.05 | 10.65** ± 1.54 |
| 5'Nucleotidase (IU/L) | 8.7 ± 0.42 | 9.01* ± 0.23 | 10.70* ± 0.42 | 11.24 ± 0.06 | 12.30** ± 0.14 |
| Total Bilirubin (Umol/L) | 7.69 ± 0.17 | 7.18 ± 0.51 | 8.89* ± 0.68 | 9.58** ± 0.68 | 1.77 ± 0.17 |
| Conjugated Bilirubin (Umol/L) | 1.71 ± 0.34 | 4.38** ± 0.34 | 6.33 ± 0.17 | 7.52** ± 0.51 | 8.55** ± 0.34 |
| Percentage Methaemoglobin | 78.3 ± 0.30 | 78.6 ± 0.70 | 79.8± 3.00 | 83.8* ± 5.30 | 87.2** ± 1.40 |

*Results are presented as Mean ± standard deviation.*
*** significantly different from control value (*P < 0.05, ** P < 0.001)*

**Table 2:** The effect of Cataflam on some biochemical parameters in wistar rats

| Parameters | Control | 100 mg/kg body weight | 200 mg/kg body weight | 300 mg/kg body weight | 800 mg/kg body weight |
|---|---|---|---|---|---|
| Alanine aminotransferase (IU/L) | 44.54 ± 0.88 | 47.05* ± 1.10 | 55.84** ± 2.15 | 61.14** ± 0.05 | 72.26** ± 1.36 |
| Asparatate aminotransferase (IU/L) | 93.28 ± 1.85 | 97.78* ± 2.00 | 124.80** ± 3.57 | 138.42** ± 1.65 | 157.34** ± 2.84 |
| Alkaline phosphatase (IU/L) | 85.79 ± 5.13 | 79.89 ± 8.85 | 109.46** ± 5.13 | 136.08** ± 5.13 | 278.08 ± 10.25 |
| 5'Nucleotidase (IU/L) | 9.10 ± 0.40 | 9.33* ± 0.04 | 9.49* ± 0.04 | 9.54* ± 0.03 | 9.71* ± 0.14 |
| Total Bilirubin (Umol/L) | 7.69 ± 1.03 | 9.92** ± 0.51 | 10.26** ± 0.68 | 11.97** ± 0.86 | 13.00** ± 0.68 |
| Conjugated Bilirubin (Umol/L) | 1.71 ± 0.17 | 4.10** ± 0.17 | 4.45** ± 0.51 | 4.62** ± 0.17 | 5.47** ± 1.03 |
| Percentage Methaemoglobin | 77.8 ± 1.00 | 82.10* ± 1.00 | 87.20** ± 2.00 | 89.4** ± 0.90 | 90.80** ± 1.00 |

*Results are presented as Mean ± standard deviation.*
*** significantly different from control value (*P < 0.05, ** P < 0.001)*

The activity of alanine aminotransferase increased dose dependently from 44.54 ± 0.88 I/L in the control group to 72.26±1.36 IU/L in the group treated with 800 mg/kg body weight. The increase was significant *(P< 0.05)* at doses of 200 mg, 300 mg and 800 mg/kg body weight of the drug. The activity of aspartate amniotransferase also showed a dose dependent increase from 93.28±1.85 IU/L in the control group to 157.34±2.84 IU/L in the group treated with 800 mg/kg body weight of the drug. The increase was significant *(P<0.01)* at drug doses of 200 mg, 300 mg and 800 mg/kg body weight.

The activity of alkaline phosphatase increased dose dependently from 85.7 ±5.13 IU/L in the control group to 278.08±10.25 IU/L in the group treated 800mg /kg body weight. The increases were significant *p<0.001)* at doses of 200 mg, 300 mg and 800 mg/kg body weight. There was also a slight dose dependent increase in the activity of 5' Nucleotidase from 9.10± 0.40 IU/L in the control group to 9.71±0.14 IU/L in the group treated 800 mg/kg body weight. The increases were significant at doses of 300 mg and 800 mg /kg body weight.

There was a dose dependent increase in the serum levels of total and conjugated bilirubin. The increase in conjugated bilirubin was significant *(p<0.05)* at all experimental doses at doses of 300 mg and 800mg/kg body weight. The percentage methaemoglobin was significantly *(p<0.05)* increased at all experimental doses of Cataflam.

**Discussion**

In this study Aspirin and Cataflam administered at doses of 100 mg, 200mg, 300mg and 800mg /kg body weight resulted in significant increases in the activities of alanine and aspartate aminotransferases. Increases in the activity of these enzymes have been found in patients with acute renal disease, acute pancreatitis, or when liver damage occurs secondary to heart failure or pulmonary infarction [14, 15]

Aspirin administered at doses of 100 mg, 200 mg 300 mg and 800 mg/kg body weight resulted in a significant decrease while there was a significant *(p<0.05)* increase in the activity of 5' Nucleotidase. The alkaline phosphatase activity of 85.51 – 95.05 IU/L previously reported by McComb et al [16]. The Aspirin dose of 800 mg/kg body weight reduced the alkaline phosphatase activity to 9.11 – 12.19 IU/L which fell within the range 7 – 15 IU/L observed by Kerkut [17]. This decrease in activity is normally associated with pathological changes in the liver and may probably be due to a relatively slow, but constant inhibition of the enzyme by an overdose of Aspirin [18]. Conversely, the drug Cataflam at experimental doses of 200 mg, 300 mg and 800 mg/kg body weight in activity of alkaline phosphatase (Table II). Marked elevation of this enzymes has been observed in experimental and clinical biliary obstruction and raised plasma levels of alkaline phosphatase provide a measure of extrahepatic or intrahepatic billary obstruction as in primary biliary cirrhosis which may be due to stimulation by cholestasis of excessive enzyme synthesis in the liver cells lining the bile cannaliculi [19].

Aspirin and Cataflam administration also significantly increased *(P<0.001)* the activity of 5'Nulcleotidase whose increased activity is of significance when the level of alkaline phosphatase is increased as is observed with administration of Cataflam. However, this increased activity of the enzymes may be due to hepatocellular disorder arising from toxic effects of Cataflam and Aspirin.

At various experimental doses of Aspirin and Cataflam, the level of methaemoglobin was significantly increased in comparison to the control group. This increase is remarkable and may be of clinical interest since excess methaemoglobin may be present in blood due to its increased production of diminised ability to convert it back to haemoglobin. Increased levels of methaemoglobin in blood has been reported to predispose to cyanosis [19] and may pose a serious threat to life during anoxia.[20]. Since results from this study show that the drugs increase methaemoglobin level, care should be taken during chemotherapeutic use to avoid development of acquired methaemoglobinaemia.

The level of total and conjugated bilirubin increased in a dose dependent manner following administration of Aspirin and Cataflam, with Aspirin showing greater increase in total bilirubin. The increase in total and conjugated bilirubin levels may be due to the fact that the drugs may have induced the development of hepatic cholestasis which may have impaired its secretory mechanism. This may also explain the increased activity of alkaline phosphatase following treatment with Cataflam. Moreso, the drugs may compete with bilirubin for binding to plasma albumin and bilirubin could be displaced from plasma albumin, thereby increasing its level in the blood and the risk of being deposited in the brain resulting in cerebral damage [21]. From the results observed in this study, chemotherapeutic usage of these drugs should not be abused because of the possible effects on the liver and other biochemical parameters.

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