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HEPATOTOXICITY FOLLOWING PARACETAMOL ADMINISTRATION IN PREGNANT SPRAGUE DAWLEY RATS

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ABSTRACT

Paracetamol is the commonest analgesic used in pregnant state. It is said to be safe when taken under relatively low dose. However, in recent times paracetamol is widely exposed to the sales of "over the counter mixtures of analgesics resulting to its indiscriminate use. This becomes a cause for concern based on the fact that is has been documented that the minimum amount for toxicity to occur in majority of adults of average size (65kg) are in excess of 10 /15g (15mg/kg or 68mg/lb), equivalent to 20- 30 tablets. The main concern with paracetamol is death resulting from hepatic toxicity (poisoning of the liver) following overdose, either accidental or deliberate which on the other hand depends on the individual sensitivity to the drug.

Toxicity of the liver cells following paracetamol administration in pregnant Sprague Dawley rats was studied. 20 Sprague Dawley rats weighing, 160 and 180g (at beginning of the experiment) were used for the study. They were divided into two group (A & B) of 10 animals each. Group A served as the control group, while group B received 7.3mg/kg/day of paracetamol from 10th day of gastation till the end of parturition. The drug was administered by gavage. The animals' weights were monitored at an interval of three days before gestation to the end of parturition. They were allowed free access to feed and water ad libitum. The maternal rats were then sacrificed for tissue processing. Three deaths were recorded amongst the paracetamol treated maternal rats during parturition and a prolonged gestational period was also observed in the same animals while five maternal rats had a normal gestational period and a safe parturition. Histopathology results of the paracetamol treated maternal rats showed normal rats that had a safe parturition at the end of the normal gestational period showed pyknotic nuclei of cells, centralobular infiltration, paranchymal degeneration and focal necrosis while two of the maternal rats that had prolonged gestational period (44 days) with signs of bleeding during parturition showed parenchymal degeneration, haemorrhage, pyknotic nuclei of cells, peace meal necrosis and chronic venous congestion. The paracetamol treated maternal rats showed a weight loss, which was statistically significant ($P > 0.05$) when compared to the control. These findings reflect liver function impairment. Hence paracetamol though considered safe at a considerable low dose especially in pregnant state, expectant mothers should be wary of its indiscriminate use.

Key- words: Hepatotoxicity, paractamol, pregnant, sprague, Dawley, Rats.

INTRODUCTION

In gynaecology several drugs are in use during pregnant state usually out of necessity despite their reported toxicities and negative side effects (1). However in recent times drug administration during pregnancy is done with utmose care. The clinical conditions necessitating the use of drugs during pregnancy include hypertensions, thromboembolism, hyperthyroidism, epilepsy, diabetes mellitus, preterm labour, arthritis, pain and fever, among others (2).

Paracetamol was discovered in 1946, as the major constituent of phenacetin a white crystalline substance used to reduce body temperature and relieve pain. It first became available in the UK in 1956 and was include in the British pharmacopoeia in 1963 (3). The precise mechanism of its action is not entirely clear, although it appears to work by inhibiting the production centrally (in the brain) of prostaglandins (substances that cause blood vessels to contract) (4) and also has weak peripheral cyclooxygenase (prostaglandin synthesis) inhibitory effect (5 & 6). About 3% of the drug is excreted unchanged in the urine, and about 80% is excreted in conjugated from usually with glucuronic acid and conjugation is done in the liver (3). Paracetamol reportedly causes chronic hepatic necrosis (3). It is particularly effective in treating common headache. When taken in combination with codeine or other drugs, it is used to control mild to moderate pains (4). It is the most widely used medicine for the purpose of relieving pain and fever, with some 4, 100 million tablets containing paracetamol being taken annually in the UK for a wide range of common ailments (4). When taken at the recommended dosage, there are virtually no adverse effects and there are almost no groups of people or conditions for which it is not appropriate (7 & 8). It causes less gastric precipitation than aspirin. The main concern with paracetamol is death resulting from hepatic toxicity (poisoning of the liver) following oversose, either accidental or deliberate (9).

Minimum amounts of paracetamol for toxicity,to occur in majority of adults of average size (65-100kg/143-220lb) are in excess of 10-15g (150mg/kg or 68mg/lb), equivalent to 20 to 30 tablets. However, in lighter individual toxic levels may be reached with fewer tablets, that is, 15 tablets (7. 5g) in a person weighting 50kg (110lb). alternatively, some individuals may be at increased risk because of a higher sensitivity to the toxic effects of paracetamol when taken in over dose (4). The individual risk of toxicity following a paracetamol overdose can be difficult to assess. Increased sensitivity to the toxic effects of paracetmol may help to explain why, in rare circumstance, certain individuals die after taking less than the estimated minimum threshold toxic dose. The risk of liver damage may also be increased in staggered overdose, such as when people are taking a number of medications containg

cetamol (9).

Although recovering of liver function is generally rapid and complete, severe hepatic failure develops three to six days after ingestion in a small minority of severely poisoned patients and is often fetal. Therefore, people attempting sucide using the drug may recover temporarily, only to die days later (4). For these reasons both paracetamol and aspirin pack size were reduced in 1998 by the UK Department of Health in attempt to reduce the risk of accidental over dose associated with "Over the counter mixtures" of pain killers/analgesics (4 & 10). From September 2002, packs were reduced to 16 tablets or capsules from general sales outlet and 32 from pharmacies. For amounts of more than100 tablets, a prescription is required. Pharmacists can, however, dispense up to 100 tablets in certain circumstance. Paracetamol packs also now carry more information on the risk of over dose on their labels (9).

Elseviers and De Broe (10) found that the use of over the counter" analgesties mixtures was a major causative factor of this problem. The causes of over the counter administration and abuse of drugs in Nigeria include the deteriorating economy, poor financial status of the people and illiteracy, which is the major predisposing factor to unawareness and ignorance of the effects of drug abuse. Consequently, many Nigeria including pregnant women are exposed to the use of the over the counter drugs especially paracetamol. This is despite the fact that little has been reported as to the effects of this in pregnant state.

The aim of this study is to determine the hepatic toxicity due to paracetamol administration in pregnancy based on the fact that it is the Commonest analgesties used in pregnant state and subject to *Over the counter mixture of pain killers/analgesics* (4 & 10). Secondly, the main concern with paracetamol is death resulting from hepatic toxicity and in rare circumstance; certain individuals die after taking less than the estimated minimum threshold toxic dose (4). Thirdly, against the background that they were documented as tolerable (safe) during pregnancy, especially when taken below certain doses (6).

Materials and method

Twenty Sprague Dawley rats were procured from the Animal House of the Univeristy of Nigeria, Enugu Campus and allowed 2 weeks of acclimatization. They were housed in cages measuring 11 by 7cm and were allowed free access to food and water ad libitum. Individual identification of the animals were done by the number of stokes marked on their tails.

Mating of the animals

The reproductive status and estrous period of the animals were determined by obtaining their virginal smears. After two complete regular cycles, timed mating of female animals was done on the night of the proestrous (N) phase of the cycle. In the morning following mating, vaginal smear were taken again. The presence of spermatozoa and squamus cells in the smear confirmed mating and fertilization of ovulated spermatozoa. The sperm positive morning was thus designated day 0 of pregnancy.

EXPERIMENTAL PROCEDURE

Twenty female Sprague dawley rats weighing between 160gand 180g were randomly divided into two groups (A and B) of ten rats each. Animals' in group A received distilled water orally and served as control. The paracetamol treated animals (group B) received doses of 7.3mg kg/day respectively by gavage. The animals were allowed food and water liberally. Each rat was weighed at an interval of three days before the experiment to the 13th day after parturition. The treatment commenced from 10th day of gestation to the end of parturition. The treatment commenced from 10th day of gestation to the end of parturition. The maternal rats in the experimental and control groups were weighed using Avery scale.The maternal rats were then sacrified by exposure to chloroform and opened up, in other to obtain their livers. They were immediately fixed in 10% formalin for about 24hrs. Photomicrographs of the liver in the prepared slides were then examined for hisotological changes. The mean weight of the animals before gestation was taken nd designated as (a). The mean weight of the animals before gestation at the unset of drug administration was taken and the mean weight for each group at the 13th day after arturition was taken and designated as (b), after which the mean weight at 13th day after parturition was substracted from the mean weight before gestation for each animal group (a- b). the statistical method employed is the ANOVA. The weight of the paracetamol treated group and the control was then tested for a statistical significant difference by using the students T test to compare two variants (Paracetamol vs. control).

RESULTS

The different in weight between the paracetamol treated rats with a mean weight difference (29 ±0.8g) and percentage weight loss (17.15%) compared to that of the control rats (-1±3.2g) and percentage weight loss (-0.58%) was statistically significant (P<0.05) (Table 1).

Examination of the Photomicrographs of the animals in the control group showed normal liver architecture (Fig 1-4). Three deaths of animals in the paracetamol treated group were recorded. Five of the paracetmol treated maternal rats that had a safe parturition at the end of the normal gestational period showed pyknotic nuclei of cell, centrolobular infiltration, paranchymal degeneration and focal necrosis. (Fig, 5-9) while two of the maternal rats that had prolonged gestational period (44 days) with signs of bleeding during parturition showed parenchymal degeneration, haemorrhage, pyknotic nuclei of cells and chronic venous congestion (Fig 10, 11).

DISCUSSION

Our result showed that there was a general destruction coupled with degeneration of the hepatic cells secondary to its indiscriminate use in pregnancy (Long term administration) at a daily dose of 7.3mg/kg/day. The dosage of 7.3mg/kg/day was chosen, as this is the dosage frequently used clinically in the treatment of body aches and pain induced by various ailmens during pregnancy (6). The morbidity of the three maternal rats observed at about 3-6days after the normal gestational period (21-23days) may have probable been due to hypersensitivity following the toxic effects of paracetamol when taken in overdose, either accidentally or deliberate (4). Secondly it could be attributed to the fact that, the individual risk of toxicity following a paracetamol overdose can be difficult to assess. Increased sensitivity to the toxic effects of paracetamol may help to explain why, in rare circumstances, certain individuals die after taking less than the astimated minimum threshold toxic dose (9). The general destruction coupled with degeneration of

destruction coupled with degeneration of hepatic cells observed could also be in agreement with the fact that minimum amounts of paracetamol for toxicity, to occur in majority of adults of average size (65-100/143-220lb) are in excess of 10-15g (150mg/kg or 65mg/lb), equivalent to 20 to30 tablets. However, in lighter individuals toxic levels may be reached with fewer tablets, that is, 15tablets (7.5g) in a person weighing 50kg (110lb). alternatively, some individuals may be at increased risk because of a higher sensitivity to the toxic effects of paracetamol when taken in over dose (4). The drastic loss in weight by the paracetamol treated maternal animals was statistical significant ($P< 0.05$)compared to the weight of the control animals. This probably could bedue to gastrointestinal tract derangement induce by paracetamol (12&13).

The liver is major vital organ in the body and the center of bodily metabolism which when severely damaged cannot be replaced thereby leads to death. The risk of liver damage may also be increased in staggered overdose, such as when people are taking a number of medications containing oaracetamol (4).therefore, we should be wary of the indiseriminate use of paracetamol as a result of "over-the counter mixtures" of painkillers (4&10).References

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Table 1: Showing effects of long term administration of paracetamol during pregnancy on the maternal body weight of Sprague –Dawley Rats.

Animal Group	Drugs Administered	Mean weight B/F Pregnancy (a)	Mean weight at 10th day of pregnancy	Mean weight at 13th day after parturition (b)	Mean weight difference (a-b)	Means weight loss (%)
A	Control	172+ 5.4	180+3.1	-1+3.2	-1+3.2	-0.58%
B	Paracetamol	169 3.1	178 5.1	140+4.3	*29+0.8	*17.15.

1. Significantly different value of control means +S.D.

(*p<0.005; All weights in grams)

LEGENDS

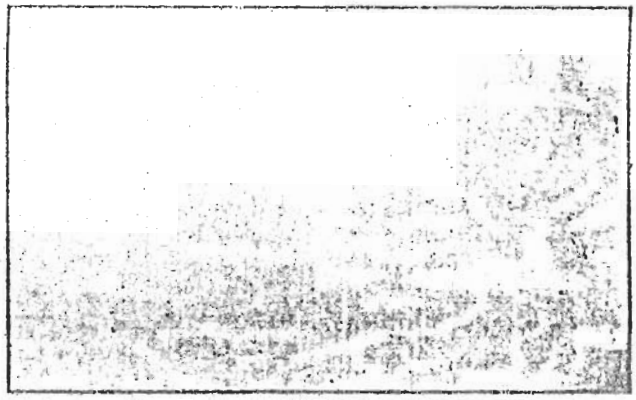

Plate 1 section of liver from control rats showing normal histological architecture. X 100

Plate 2 section from pregnant rats treated with long-term administration of paracetamol. Cords of cells around the central vein are degenerated while parts show pylznotic nuclei. X 100.

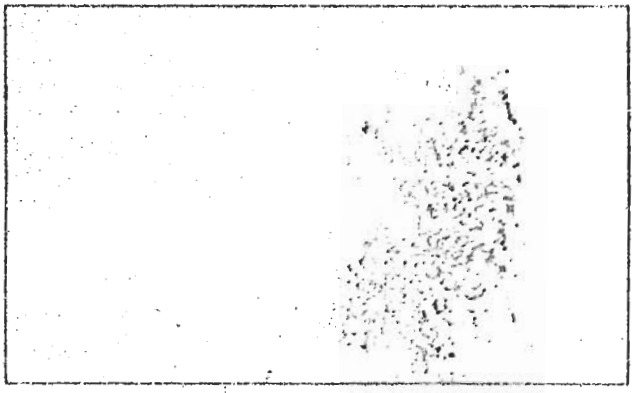

Plate 3 section of liver of long-term administration showing an area of haemorrahage and inflammatory cell infiltration. X 100.

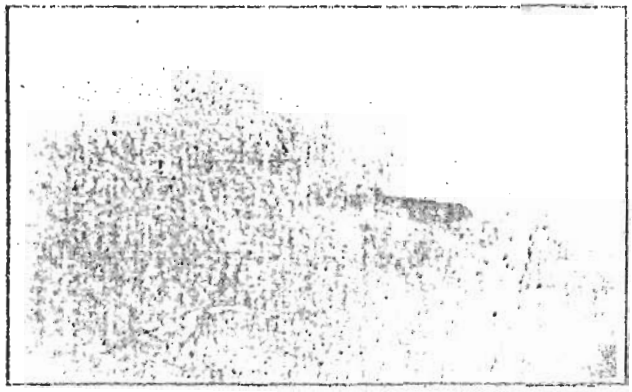

Plate 4 section of liver of long-term administration showing grossly necrotic area of liver cells. X 100.