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REMEDIAL ROLE OF VITAMIN C AGAINST CYPERMETHRIN INDUCED REPRODUCTIVE TOXICTY IN FEMALE ALBINO RATS

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ABSTRACT

The frequent application of pyrethroids as pesticides in agriculture and in homes has led to concerns of their effects on man and animals. The paucity of information on the effects of pyrethroids on female reproduction prompted this study using cypermethrin as an example. The aim of this study was to assess the acute and sub-acute reproductive toxicity of cypermethrin and the protective role of vitamin C in pregnant albino rats. Thirty-six pregnant animals (Day 0 = day of mating, average body-weight = 190g) were randomly divided into 6 groups. Group 1 (Control) received olive oil+distil water, Group 2 (50 mg cypermethrin) every 2 days and Group 3 (50 mg/kg cypermethrin) every 2 days +(20mg/kg vitamin C)five days a week until Day 20. Group 4 received olive oil+distilwater, Group 5 (150 mg/kg cypermethrin) and Group 6, 150 mg/kg cypermethrin+20 mg vitamin C, on Day 15 only. Blood samples were collected on Days 6, 12, and 18 (Groups 1-3) and Day 17 (Group 4-6) for progesterone and estrogen enzyme immunoabsorbent assay. Maternal (Groups 1-6) and fetal (Groups 1-3) liver samples were collected at the end of the experiment for histological analysis. Cypermethrin did not significantly affect ($p < 0.05$) the level of progesterone and estrogen (Groups 1-6). Maternal and fetal liver from cypermethrin- but not cypermethrin+vitamin C-treated females showed cyto-vacuolation and swelling. Cypermethrin but not cypermethrin+vitamin C-treatment reduced neonatal birth-weights and induced embryonic resorption and still-births compared with controls. It was concluded that cypermethrin has a dose-dependent toxic effects on pregnant female albino rats that can be ameliorated with vitamin C.

INTRODUCTION

Regular application of pesticides to combat crop pests and insects in the domestic environment is a major health concern in many countries (Tinoco and Halperin, 1998). Production and application of pesticides in agriculture have also risen with the demand for food and other agricultural products surpassing their tolerance levels for man and animals in recent years (Salih and Jaafar, 2013; Pretty, 2008). Pyrethroids are among the most widely used synthetic pesticides and are known for their extreme toxicity as endocrine disruptors (Eil and Nisula, 1990; Go et al., 1999). Cypermethrin, a Type II pyrethroid and a known stomach and contact insecticide (EPA, 2002) is reported to have adverse reproductive effects in male and female mammals (rats, mice and rabbits) as well as their foetuses (McCarthy et al., 2006;

Sangha et al., 2011; 2013; Hu et al., 2013; Li et al., 2013).

The toxic effects of cypermethrin on mammals have been reported (Tiwari et al., 2010; Al-Shaikh, 2012; Bhatti et al., 2014;). Recently, Sallam et al (2015) reported that cypermethrin induced reproductive toxicity in pregnant rabbits. However, the effects of cypermethrin on steroid hormone levels in the pregnant albino rats and amelioration of such effects by antioxidants, vitamin C have not been reported. Such a study will add to the wealth of information on reproductive effects of pyrethroids generally and cypermethrin in particular. It will also be of interest to humans chronically exposed to such hazardous chemicals in the industry.

The aim of the present study therefore is to assess the acute and subacute reproductive toxicity of cypermethrin and the protective role of vitamin C in the pregnant albino rats.

MATERIALS AND METHODS

Thirty-six multiparous albino rats (*Rattus norvegicus*) randomly selected from the breeding colony of the Department of Pharmacology, University of Port Harcourt were used for this study. They were acclimatized to existing laboratory conditions and fed commercial chow of known nutritional composition and water *ad libitum*.

Research-grade cypermethrin purchased from Haihang Industrial Company, Ltd, China was dissolved in olive oil at two concentrations, 50 and 150 mg/kg body weight. 99.5% L-ascorbic acid (Vitamin C) was prepared in distilled water at a dose of 20mg/kg.

Mating and treatment

Following acclimatization, one female was paired with a male in a cage overnight. Mating was confirmed the following day by the presence of sperm cells in vaginal smear or presence of vaginal plug. Animals were then grouped into 6, each group housed in a cage. Group 1 (control I) received both 0.2ml of olive oil vehicle given every two days and 0.5ml of distilled water, administered for 5 successive days per week. Groups 2 and 3 received 50mg/kg body weight of cypermethrin every two days which was followed in group 3 by 20mg/kg ascorbic acid for 5 successive days per week. Both treatments were given until Day 20 of gestation beginning from Day 0.Group 4 (control II) received 0.5ml of olive oil and 0.5ml of distilled water. Groups 5 and 6 received a single dose of 150mg/kg of cyp on Day 15 which was followed in group 6 by 20mg/kg ascorbic acid. All treatments were given by oral gavage. Blood samples were collected via the retro-orbital plexus on days 6, 12 and 18 (groups 1-3) and on Day 17 (Groups 4-6) for the hormonal analysis. Estrogen and progesterone enzyme immunoassay kits (Bio-Intec No 10005 and 10009) were used for the hormonal assay.

Liver samples were collected from both dams and their litters (groups 1-3) on parturition and from only dams (Groups 4-6) on Day 17 of gestation through laparatomy under chloroform anaesthesia.

Statistical Analysis

Data from hormonal assay were analyzed by a two-way ANOVA which considered treatment and dosage while data from litter size and weight were analyzed by a one-way ANOVA. Where a significant F was obtained, differences between means were assessed with SNK.

RESULTS

The results showed that cypermethrin (50 or 150 mg/kg) or cypermethrin at either dose,

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followed by 20mg/kg ascorbic acid did not significantly affect the levels of estrogen or progesterone levels (Figures 1 -4)

Rats treated with 50mg/kg cypermethrin followed by 20mg/kg ascorbic acid had significantly higher litter size than control group ($p<0.05$) (figure 5). Results also show that litters from dams treated with 50mg/kg cypermethrin had significantly lower average weight than the controls ($p<0.05$) (figure 6). Ascorbic acid treatment following cypermethrin did not fully restore the litter weight to the control level

Histological sections of both maternal and foetal liver samples from the group that received 50mg/kg cypermethrin followed by 20mg/kg ascorbic acid showed no abnormalities (plate 1c) but liver sections from 50mg/kg cypermethrin-treated dams indicated mild inflammation in the form of cytovacuolation and central vein congestion (plate 1b) which were more pronounced in foetal liver samples (plate 2b). These inflammatory reactions were also observed in foetal liver samples from dams treated with both 50mg/kg cypermethrin and 20mg/kg ascorbic acid (plate 2c). Liver samples from 150mg/kg cypermethrin-treated dams also showed histological degradation with cell swelling, cytoplasmic vacuolation, narrowing of the central vein (plate 3b) but concomitant treatment with ascorbic acid did not show such histological changes (plate 3c).

Uterine examination during laparotomy indicated possible embryonic loss in animals treated with 50mg/kg cypermethrin throughout gestation but this was not observed in other groups. There were cases of still birth in both the cypermethrin-treated and vitamin C- treated groups.

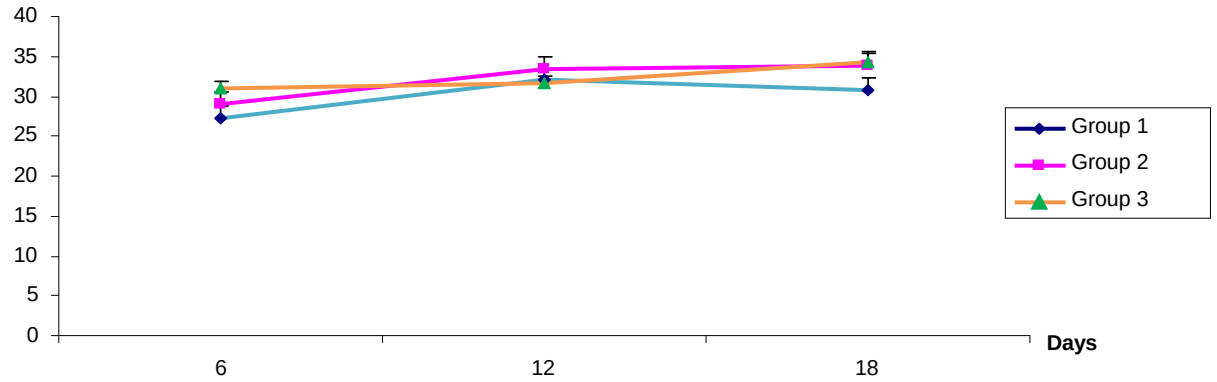

Fig 1: Mean Serum levels of progesterone (ng/ml ± SEM) for each trimester in pregnant albino rats treated throughout the gestation with olive oil + distilled water (Group 1), Cypermethrin (Group 2) and Cypermethrin + Ascorbic Acid (Group 3)

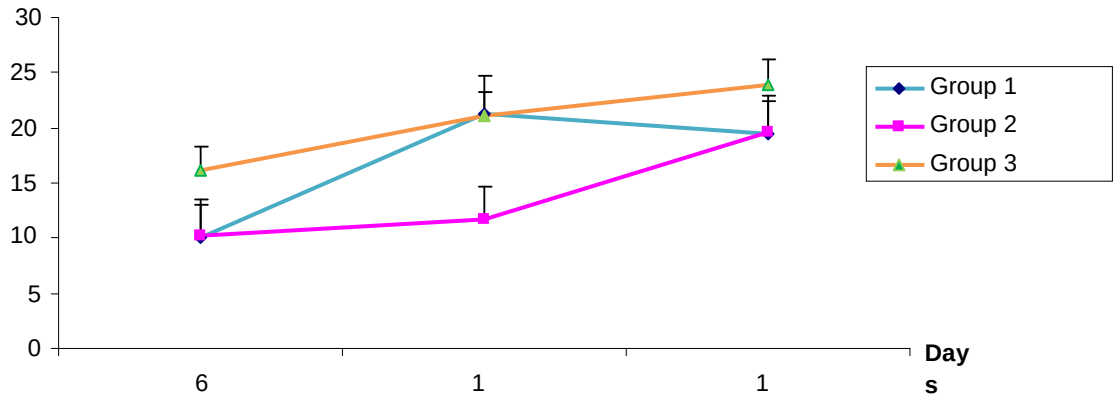

Fig 2: Mean Serum levels of Estrogen (pg/ml ± SEM) for each trimester in pregnant albino rats treated throughout the gestation with olive oil + distilled water (Group 1), Cypermethrin (Group 2) and Cypermethrin + Ascorbic Acid (Group 3)

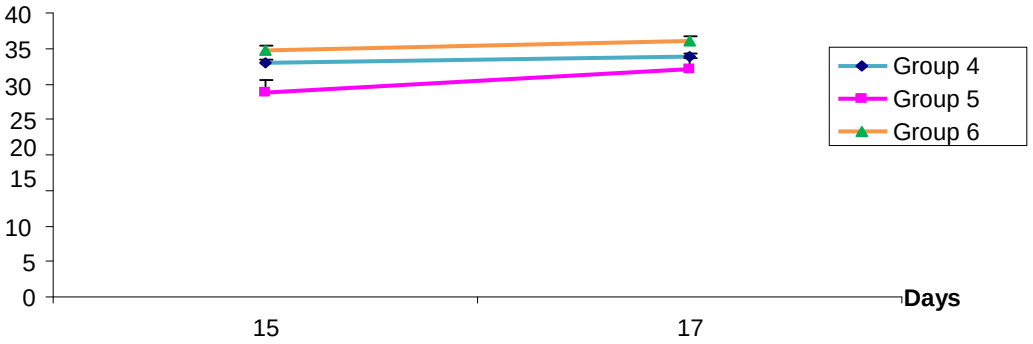

Fig 3: Mean Serum levels of progesterone (ng/ml ± SEM) in pregnant albino rats treated at mid gestation with olive oil + distilled water (Group 4), Cypermethrin (Group 5) and Cypermethrin + Ascorbic Acid (Group 6)

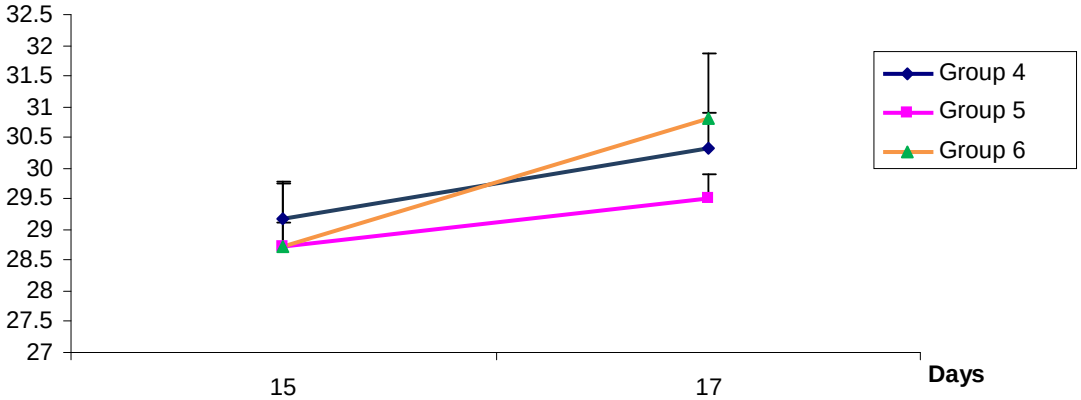

Fig 4: Mean Serum levels of Estrogen (pg/ml ± SEM) in pregnant albino rats treated at mid gestation with olive oil + distilled water (Group 4), Cypermethrin (Group 5) and Cypermethrin + Ascorbic Acid (Group 6)

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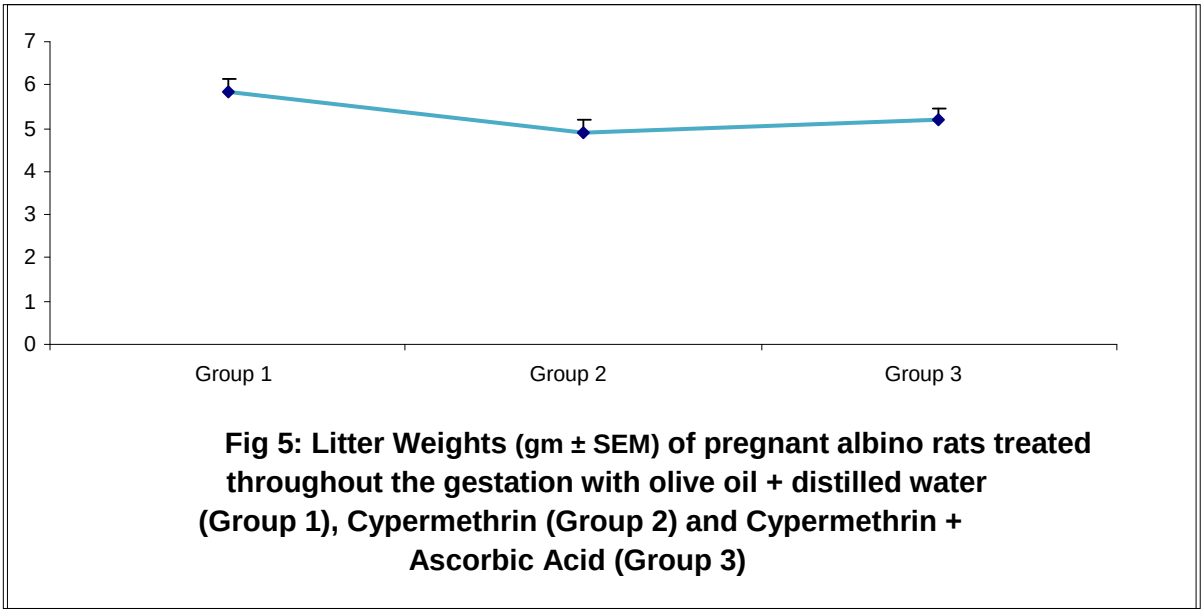

Fig 5: Litter Weights (gm ± SEM) of pregnant albino rats treated throughout the gestation with olive oil + distilled water (Group 1), Cypermethrin (Group 2) and Cypermethrin + Ascorbic Acid (Group 3)

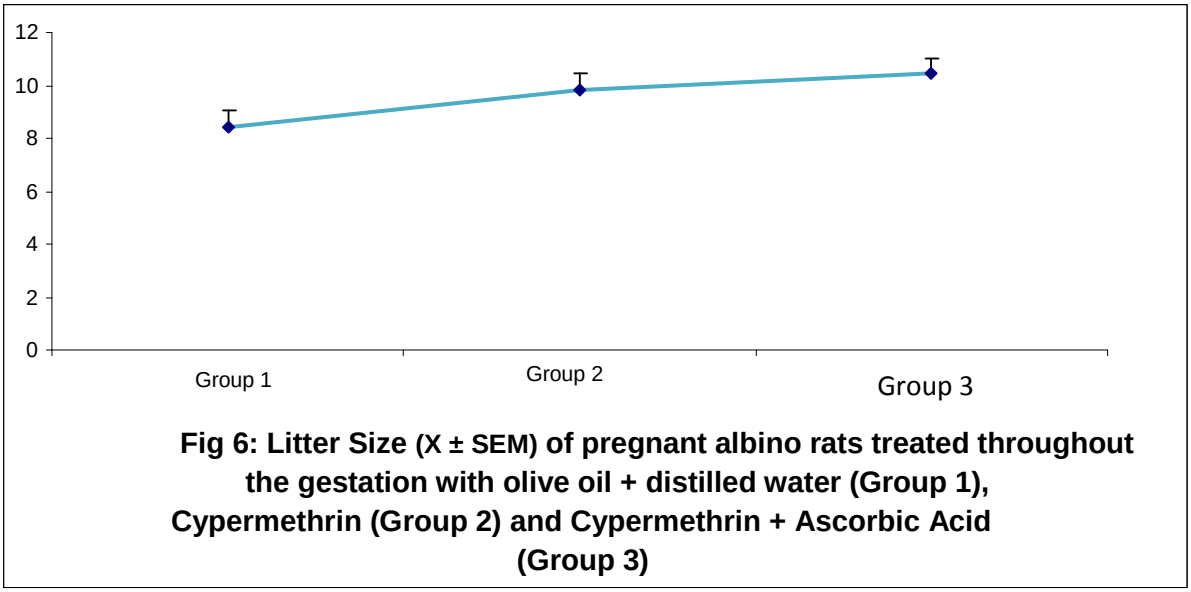

Fig 6: Litter Size (X ± SEM) of pregnant albino rats treated throughout the gestation with olive oil + distilled water (Group 1), Cypermethrin (Group 2) and Cypermethrin + Ascorbic Acid (Group 3)

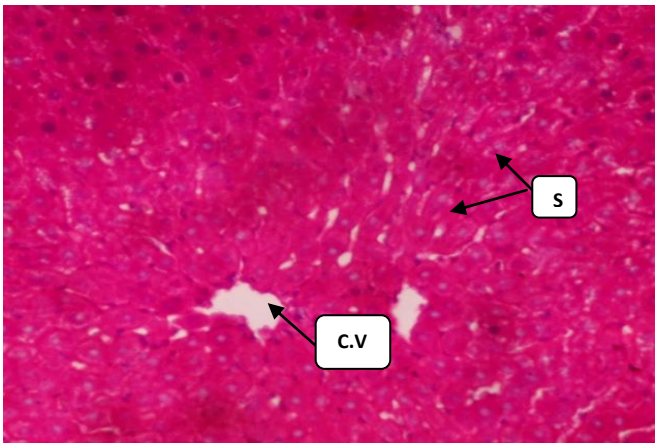

Plate 1a: Group 1 (Control I)

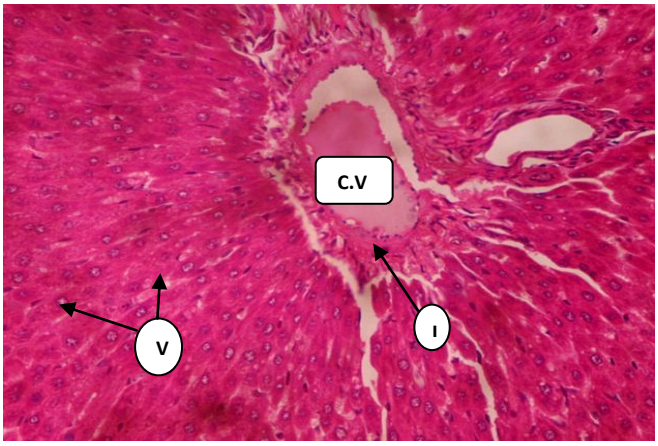

Plate 1b: Group 2

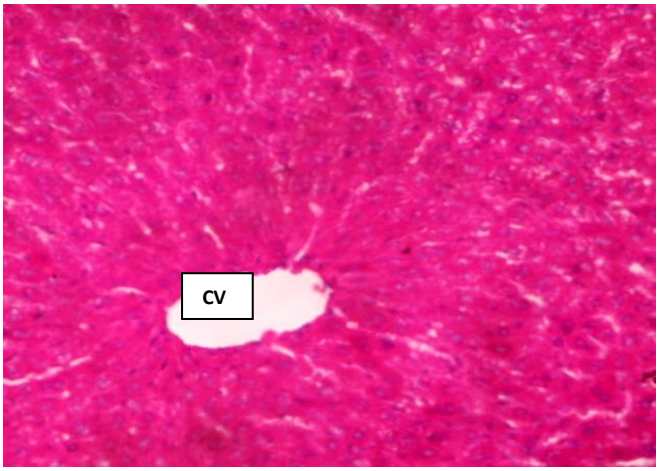

Plate 1c: Group 3

Fig 1. Photomicrograph of maternal liver sections stained with H&E (×400): plate 1a – Normal histological structure of control rats showing the central vein (CV) and the sinusoids (S). Plate 1b – mild cytoplasmic vacuolation (V) and inflammation (I). Plate 1c – No obvious change in the histological structure when compared with the control.

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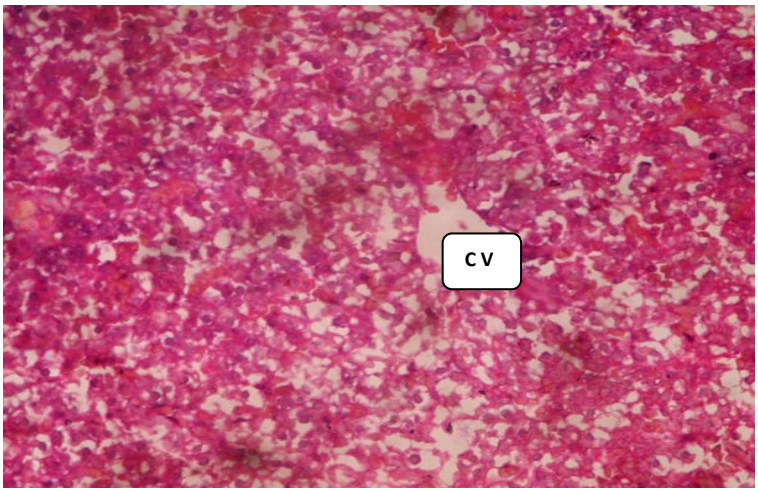

Plate 2a: Group 1 pup

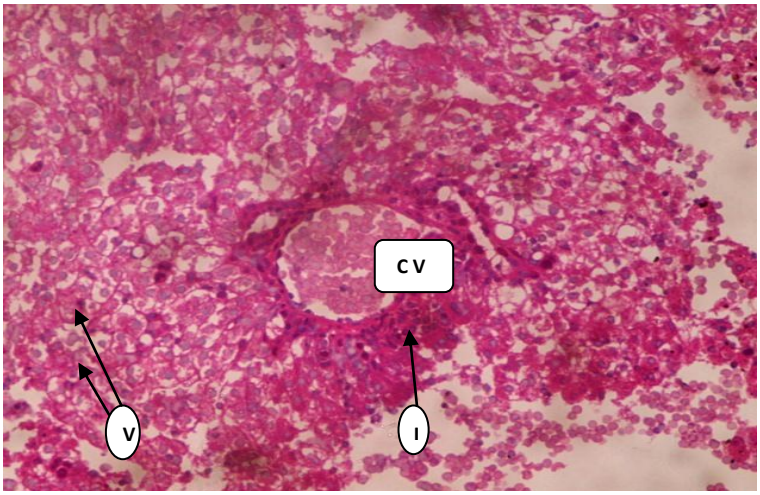

Plate 2b – Group 2 pup

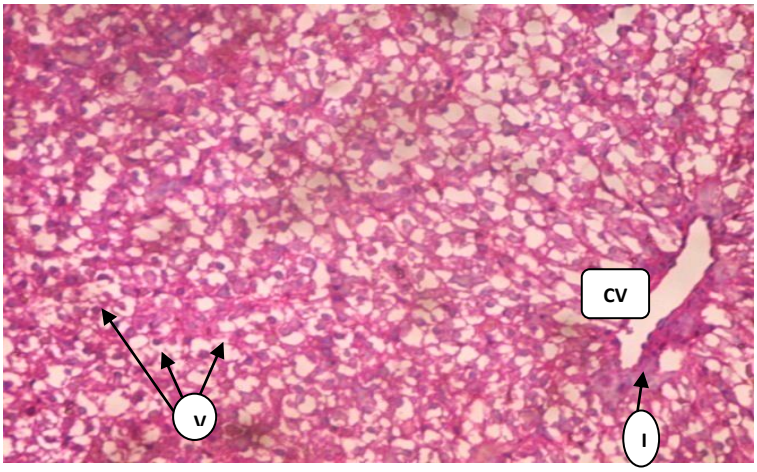

Plate 2c - Group 3 pup

Fig 2. Photomicrograph of foetal liver sections stained with H&E (×400): plate 2a – Normal histological structure of control pups showing the central vein (CV). Plate 2b –cytoplasmic vacuolations (V) and presence of inflammatory cells (I) surrounding the central vein. Plate 2c – cytoplasmic vacuolations (V) and mild inflammation (I) around the central vein.

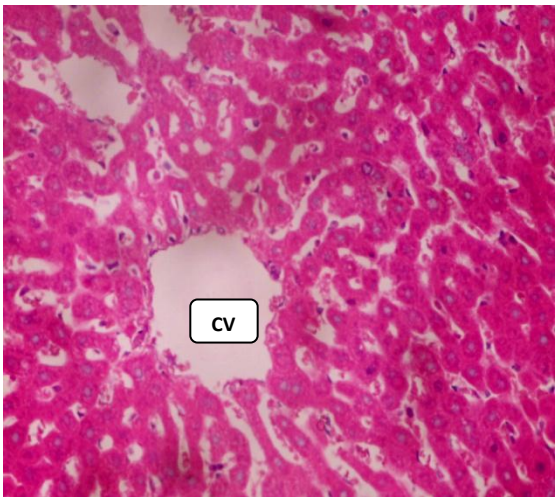

Plate 3a: Group 4 (Control II)

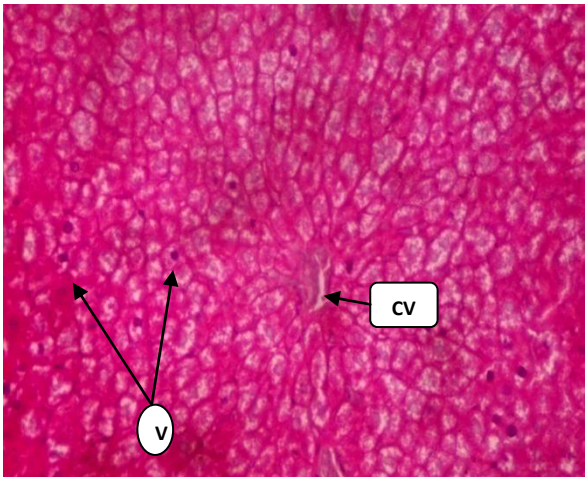

Plate 3b – Group 5

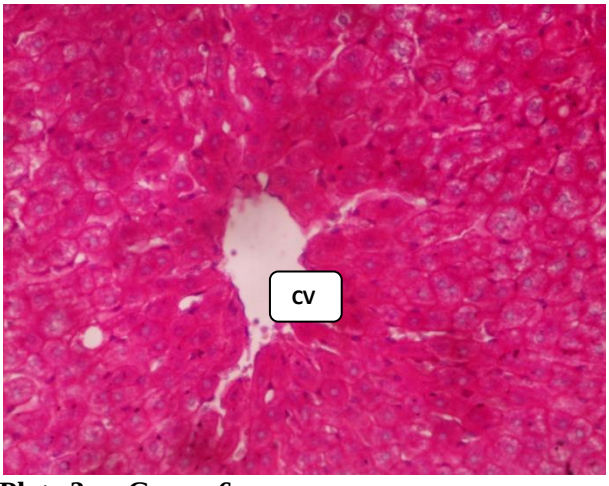

Plate 3c – Group 6

Fig 3. Photomicrograph of maternal liver sections stained with H&E (×400): plate 3a – Normal histological structure of control rats showing the central vein (CV) and the sinusoids (S). Plate 3b – cell swelling with narrowing of the central vein and cytoplasmic vacuolation (V). Plate 3c – No obvious alteration in the histological structure when compared with the control.

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DISCUSSION

The results of the present study show that cypermethrin induces developmental toxicity by the occurrence of still births, embryonic loss and systemic hepatic inflammation and cytovacuolation of litters from treated dams. Hepatic vacuolation in rats was reported by Sykes, *et al*., (1976). Enlarged sinusoid spaces, leukocyte infiltration, and other inflammatory changes induced by cypermethrin have also been reported in rats (Adjrah *et al*., 2013) and mice (Mamun *et al*., 2014) and have been demonstrated in this experiment. These findings agree with those reported in pregnant rabbits (Ullah, 2006) and in pregnant rats (Assayed *et al*., 2010). In contrast, EPA (2002) found no toxic effects of cypermethrin in pregnant rats with doses as high as 70 mg/kg.

The result also indicated that cypermethrin induced hepatic inflammation in the dams in a dose-dependent manner which agrees with the report of Kanbur *et al.* (2008) that the degree of oxidative stress and lipid peroxidation induced by pyrethroids are related to the dose administered, the duration of exposure and the administration of the indicated compounds, either alone or as a combination with other pesticides. The histopathological changes observed in the liver could be due to oxidative damage induced by cypermethrin because of its lipophilic nature which aids its penetration of cell membrane to cause membrane lipid peroxidation (Nasuti *et al*., 2013). This explanation agrees with Sadowska *et al*. (2010) that pyrethroid exposure is associated with oxidative stress, through lipid peroxidation, protein oxidation and depleted multiple antioxidant enzymes.

Administration of ascorbic acid prevented the inflammatory changes observed in the dams in the cypermethrin-treated groups but not in the foetuses. The stillbirth recorded in both groups suggests a direct toxic effect on the pups and that the toxicity of cypermethrin in the young rats is more pronounced than in the adults. Earlier reports indicate that the pathway for degrading cypermethrin is not well developed in young rats (WHO, 1989; Kaylan et al., 2007). If this is also true for rat foetuses, then cypermethrin would be expected to induce greater damage in the foetuses.

Mean litter weight of pups from dams treated with5Omg/kg cypermethrin was lower than that of control pups. This could be due to one of several factors: the higher litter size in the cypermethrin group resulted in corresponding decreased litter weight to be accommodated in the limited uterine pace the pups occupied. Other factors affecting litter size in rats include age, fertility and seasonal rhythm (Banigo, 1991, unpublished) may also be operational here. It is also possible that cypermethrin disrupted placental unction and nutrient uptake or utilization by the pups.

Progesterone and estrogen profiles from cypermethrin (50 and 150 mg/kg) treated dams were similar to those of controls indicating that cypermethrin has no significant effect on the female steroid hormones in pregnant rats. The result is in accordance with the report of Sahar *et al.* (2011) that there was no significant difference between humans exposed to synthetic pyrethroid and control in serum estrogen and progesterone, but reduced testosterone levels. Hu *et al*. (2013) had reported that male rats treated with

cypermethrin at 50mg/kg//day for 15 days by oral gavage exhibited a significant reduction in testicular daily sperm production and serum testosterone levels. Li *et al.* (2013) added that adult male rats treated with cypermethrin at the doses of 30 and 60mg/kg/day by oral gavage showed significant reduction in serum testosterone and vacuolization of sertoli cells. Therefore the difference between male and female rats in their response to pyrethroid merits further investigation.

In conclusion, cypermethrin has no significant effects on the estrogen and progesterone following subacute administration of 50mg/kg and single administration of 150mg/kg in gestating albino rats. Vitamin C appeared to reverse the cypermethrin-induced decrease in litter weights at birth and degeneration in the structure of the liver. Regular cypermethrin application may cause morphological and physiological damage in pregnant dams and foetuses in utero.

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