INTRODUCTION

Cannabis preparations obtained from the plant Cannabis sativa have been employed in the treatment of numerous therapeutic conditions such as nausea and vomiting associated with cancer chemotherapy (1), anorexia and cachexia in HIV/AIDS (2), spasticity in multiple sclerosis and spinal cord injury (3).

Studies with whole plant preparation or single cannabinoids (smoked marijuana, encapsulated Cannabis extract etc) have often been inspired by positive anecdotal experiences of patients employing CCR (usually without legal sanction) (4). The relaxant (7) and analgesic activities have been reported (8). The leaves have been recommended as antiphlematic and also as a remedy for catarrh accompanied by diarrhoea (9-11).

CCR is currently being advocated as a therapeutic remedy for diverse ailments. Bioavailability after administration of crude Cannabis extract is usually high (12). The purpose of the present study is therefore to investigate whether CCR has any haematotoxic effect.

MATERIALS AND METHODS

Source of plant materials

The fresh leaves of Cannabis sativa L. (Family: Moraceae) were collected in July 1999, from the Crude
Drug and Research Unit of National Drug Law Enforcement Agency (NDLEA) in Enugu, Enugu State of Nigeria.

**Preparation of plant material**

Whole leaves of the plant were rinsed thoroughly in running tap water, sun-dried in air for 48 h and pulverized to coarse powder using mortar and pestle. One thousand grams (1000 g) of the powdered leaves was extracted with 1 litre of chloroform for 8 h using a soxhlet extractor (Gallenkamp, England). The crude chloroform extract was evaporated to dryness under reduced pressure, using a rotary evaporator (Gallenkamp, England) at an optimum temperature of between 40 and 45°C, to yield 173.25 g of crude resin tar.

**Treatment of animals**

Twenty Spraque-Dawley albino rats of both sexes (180-250 g, 3 months old) were obtained from The Animal House of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, a week prior to the pharmacokinetic study. The animals were fasted for 24 h prior to the study and maintained on rat chow (Top Feed Ltd. Sapele, Nigeria). Clean water was provided *ad libitum*.

The rats were divided into 4 groups of five animals each (WA = positive control group, WB = given 20 mg/kg of CCR, WC = given 40 mg/kg of CCR and WD = negative control or untreated group). CCR was suspended in ethanol: propylene glycol (9:1) and administered inter peritoneally (i.p.) to the rats in the various dosed groups. The administration of the drug suspension was repeated at the same hour everyday for 21 days. The animals were observed for physical and emotional changes.

**Haematological Evaluation**

**Sample Collection**

Blood for the total leukocyte, differential leukocyte, erythrocyte counts and packed cell volume (PCV) determination was collected before commencement of treatment (day 0) and on day 8 and 14 post treatment from the retro-bulbar plexus of the medial canthus of the eye, by carefully inserting a micro-capillary tube into the medial canthus of the eye to puncture the plexus and enable outflow of blood into the sample bottle containing EDTA. This was shaken gently to prevent clotting. A 0.02 ml volume of the blood sample was added to 0.38 ml of the leukocyte diluting fluid (containing 10 % glacial acetic acid tinged with gentian violet) in a test tube for the total leukocyte count. Another 0.02 ml of blood was added to 4.0 ml of erythrocyte diluting fluid (containing sodium citrate, formaldehyde and distilled water) for the red blood cell counts. A micro-capillary tube was newly filled with the blood sample for PCV determination.

**Procedure for the haematological determination**

For the total leukocyte counts, a drop of the diluted sample was used to load the Neubauer chamber, and all cells within the chamber were enumerated. The dilution was 1:20; the number of enumerated cells for each sample was multiplied by 50 to obtain the absolute leukocyte count per micro litre of blood (13).

For the erythrocyte counts, a drop of the blood sample diluted 200 times with erythrocyte diluting fluid was used to load the Neubauer chamber, and all red blood cells in the five groups of sixteen small
squares in the central area of the chamber were enumerated. The number of cells enumerated for each animal was multiplied by 10,000 to obtain the erythrocyte counts per micro litre of blood.

The filled micro capillary tube for PCV determination was sealed at one end and centrifuged for five minutes using a microhaematocrit centrifuge. After the packing of the blood cells, the PCV was read as a percentage using a microhaematocrit reader.

For the differential leukocyte counts, a drop of the blood sample was placed at one end of a clean grease-free slide. The blood was carefully smeared to make a thin blood film, which was dried in air and thereafter stained by the Leishman technique (13). The slides were later examined under oil immersion with light microscope. Two hundred cells were enumerated by the longitudinal counting method and each cell type was scored using differential cell counter. Results for each type of leukocyte were expressed as a percentage of the total count, which was thereafter converted to absolute values per micro litre of blood.

Data Analysis

Data on the total leukocyte counts; absolute values of the differential leukocyte counts, erythrocyte counts and PCV were expressed as group means with standard deviations for each determination. Means of the four groups were compared for significant difference of each day of the determination using single factor analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Haematological Parameters

The obtained haematological indices are presented in Figures 1 to 7. These results indicate that the erythrocytic and leucocytic counts were not significantly different in all the treated animal groups for the first two weeks of treatment. However, on the third week, a significant increase for the groups WA and WB in terms of the pack cell volume (PCV), red blood count (RBC) and total leucocyte count, absolute lymphocyte counts, monocyte count, neutrophiles and eosinophil counts.

Results of the PCV showed that there was a significant change in week 3 between the dosed groups WB and WC as against the positive control group WA and the untreated group WD ($P < 0.05$) (Figure 1).

The results of WBC total count, total RBC count, differential counts (that is absolute lymphocyte count, monocyte count and neutrophile count respectively) all followed the same pattern described above (Figures 2 to 7). However, the elevations observed in the treated groups at week 3 were significantly dose-related ($P<0.05$). The eosinophil counts, however, did not indicate any significant differences in the first two weeks of treatment. In contrast to the other haematological indices, eosinophils disappeared from the blood of the treated groups in the third week (Figure 7). Eosinophils serve as detoxifiers and the disappearance of eosinophils in the blood of the dosed groups in week two and their total disappearance in week three signifies that they have been fully consumed in the detoxification process. Therefore there was a toxic effect that cleared the eosinophils from the blood.

The presence of basophils is usually only incidental and therefore their presence in rats in the different dosed groups did not follow any trend. These general increases in haematological indices may be attributed to haemoconcentration induced by dehydration bearing in mind that though Cannabis is an appetizer, one of its side effects is dehydration (14).
Figure 1: A graph of Total RBC per µL of blood against Experimental period.

Figure 2: A graph of Total RBC per µL of blood against Experimental period.

Figure 3: A graph of Absolute Lymphocyte Count per µL of blood against Experimental Period.

Figure 4: A graph of Absolute Monocyte count per µL of blood against Experimental Period.

Figure 5: A graph of Absolute Neutrophil Counts per µL of blood against Experimental Period.

Figure 6: A graph of Absolute Eosinophil Counts per µL of blood against Experimental Period.
CONCLUSION

It can be concluded from these studies that chronic administration of CCR at high doses (above 20 mg/kg) to rats has slight haematotoxic potential.

Acknowledgement

We wish to acknowledge the Crude Drug and Research Unit of National Drug Law Enforcement Agency (NDLEA) in Enugu, Enugu State of Nigeria for providing us with the Cannabis plants for this research. We also wish to acknowledge Dr. G. B. Okide (late) who was the brain behind these series of Cannabis research though he is not around any more to see to the end of the work.

REFERENCES

use of thin-layer chromatography

*Correspondence author
Journal of Pharmaceutical and Allied Sciences
ISSN:1596-8499

Journal of Pharmaceutical and Allied Sciences
TLC) as a qualitative analytical technique especially in the detection of drugs and separation of compounds has enjoyed wide acceptance in pharmacy.

The selective absorption of different donor species on chromatographic stationary phases containing electron acceptors has been employed in the qualitative detection of such donor species (1-6). This effect of selective absorption could be useful in estimating the degree of association of the acceptor with a given donor and also in separating mixtures of donor (7-11). Chloranilic acid (CA) and other electron acceptors have been widely employed in the spectrophotometric determination of many drug substances (12-27). Earlier reports (28-31), conclusively

**Spotting on the TLC Plates**

Each of the drug samples (in chloroform) was individually transferred to the activated chromatographic plates with the aid of a thin 1.2 mm diameter capillary tube (Marian Field), such that each drug sample was spotted twice. For each sample, a different capillary tube was used.

**Development and Detection on TLC Plates**

1. Each of the plates spotted was placed vertically in four different tanks. The tanks were then quickly covered with the glass lids and allowed to stand undisturbed until the solvent fronts reached a point 15 cm away from the point of spotting. The glass plates were removed, air-dried and