

Acute Toxicity of CP Women Capsules in Sprague-Dawleys Rats

Raphael Nyarkotey Obu, RND, PhD^{*}, Lawrence Aggrey –Bluwey, BSc, PGDE, MPhil²

¹Nyarkotey College of Holistic Medicine, Tema Community 7, Ghana

²Assistant Lecturer, Department of Health Administration and Education, University of Education, Winneba, Ghana

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*Corresponding author: Raphael Nyarkotey Obu, RND, PhD

Abstract

This study was design to examine the acute toxicity of the 80% -ethanolic extract of CP women capsules in healthy male and female Sprague-Dawley rats. Six samples of the product were submitted to the Department of Pharmacology & Toxicology, School of Pharmacy, College of Heath Sciences, University of Ghana, Legon, and Accra for the analysis. The investigators at the University declared in their research report that the study was conducted at the Animal Experimentation Unit of the School of Biomedical and Allied Health Sciences (SBAHS), College of Health Sciences, university of Ghana. On clinical Observation, the study conducted reports that the animals treated by the ethanolic extract of CP women capsules (5000 mg/kg) did not show any observable abnormality in movement, salivation, sleep, lethargy, there was no signs of piloerection and mortality in comparison to the control group within the first 48 hours, and daily during the 14 days of the study. With regards to Lethal Dose Fifty (LD₅₀), the study found that per monitoring the animals for 24, 48 hours and throughout the remaining 12 days, the group of rats treated by the ethanolic extract of CP women capsules did not record any deaths. Hence, the LD₅₀ of the ethanolic extract of CP women capsules, when administered orally, is greater than 5000mg/kg. In conclusion, further studies involving long term administration of aqueous extract of CP women capsules in different experimental rodents, including mice, will be needed to assess its safety for trial and use in humans. More renal function markers such as creatinine should be analyzed.

Keywords: CP Women Capsule, Toxicity, clinical observation, Lethal Dose Fifty (LD₅₀), monitoring.

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INTRODUCTION

The use of herbal medicinal products in Ghana for all forms of ailments cannot be underestimated. This notwithstanding, the public concern has to do with the toxicity of these herbal products. Hence, the Food and Drugs Authority(FDA), Ghana, as per the Public Health ACT, 2012, (ACT,851) mandates herbal producers and manufacturers to conducts toxicity and other tests prior to the product submission. ‘CP women capsules’ is an herbal supplement produced by Live Long Herbal Centre. The World Health Organization advocates the use of herbal medicines that are proven scientifically to be of good quality, safe and efficacious as affordable alternatives to orthodox medicines for the majority of the world’s populace particularly in low-income countries [1] this is an essential requirement for the registration of newly developed medicinal products [2-4]. The study was done as requested by the Food and Drugs Authority (FDA) of Ghana as part of the registration requirements of the herbal product.

OBJECTIVES

The specific objectives were

1. To determine the LD₅₀ (Lethal dose) of an 80% -ethanolic extract of CP women capsules in Sprague Dawley rats.
2. To determine the effects on physical signs of toxicity following administration of a very high dose (5000mg/kg) of an 80% -ethanolic extract of CP women capsules
3. To determine the effect of oral administration of single high-limit dose of CP women capsules on haematological and biochemical parameters of Sprague-Dawley rats

METHODOLOGY

Experimental design

The study was an acute toxicity study

Preparation of ethanolic Extract

Two kilograms of the powder were exhaustively extracted using a cold maceration with

80% v/v ethanol for 72 hours in a flat bottom flask. The extract was concentrated with a rotary evaporator at 60°C which produced a semi-solid mass of extract. The semi-solid mass was dried using a water bath at a temperature of 78-79°C and then kept in a desiccator.

Experimental animals and housing

Fourteen Sprague-Dawley rats (Hsd;SD strain), weighing 120-200 (6-9 weeks old) were obtained from the Center for Plant Medicine Research (CPMR), Mampong-Akwapim and kept at the Animal Experimentation Unit of the School of Biomedical and Allied Health Sciences (SBAHS), College of Health Science, University of Ghana, where all experimental procedures were carried out. The Department of Pharmacology & Toxicology (School of Pharmacy, University of Ghana) approved all animal procedures and techniques used in these studies. The animals were housed in groups of six in stainless steel cages (34cm*47cm*18cm) with softwood shavings as bedding. They were fed with normal commercial pellet diet (AGRIMAT, Kumasi) were given access to ad libitum and maintained under laboratory conditions (temperature 25±1°C, Relative humidity 60-70% and 12 hour light-dark cycle). All experiments were conducted during the day cycle between the hours of 7:00-15:00 GMT.

Animal Groupings and Acute Extract administration

The animals were acclimatized for a week and randomly divided into two groups of seven rats each (n=7). The rats fasted overnight before administration. One group received the 80%-ethanolic extract of CP women capsules at 5000mg/kg and the other group received a dose-equivalent volume of saline solution by oral gavage. The administration was done for a day.

48-hour clinical observations and LD₅₀ Determination

The animals were observed for clinical signs of toxidromes such as changes in movement, salivation, sleep, lethargy, piloerection and mortality within 48 hours. Observations were done 30 minutes after administration, and every 6 hours. Mortality after 24 and 48 hour post treatment were recorded and the LD₅₀ (the lethal dose) was determined.

14-day Clinical Observation

The animals were monitored and observed daily for 12 days looking out for any clinically observed toxidromes and mortality. On the 14th day of the study period, the rats were euthanized by cervical dislocation, and blood samples were collected from each animal via cardiac puncture into an ethylene-di-amine-tetra-acetic acid (EDTA) for haematological examination, and gel tubes for biochemical analysis. An automated haematology analysis (URIT-5250Vet, URIT Medical Electronics Co., Ltd., China) was used for the haematological analysis. Haematological indices

determined include red blood cell count (RBC), white blood cell count (WBC), haemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), platelet (PLT), lymphocyte count (LYM), red cell distribution width (RDW), platelet distribution width (PDW), mean platelet volume (MPV) and granulocyte (GRA). The gel tubes were centrifuged immediately after collection at 5000 rpm for 15mins using a benchtop centrifuge (High-Speed Refrigerated Centrifuge TGL-16C, China) sera were pipetted and placed in cryotubes. Serum biochemical analysis was done with a biochemical analyser (Mindray Chemistry Analyzer, BS-200, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., and China). The sera were examined for renal function (urea), and liver function (AST-aspartate aminotransferase; ALT- Alanine aminotransferase and ALP alkaline phosphatase).

STATISTICAL ANALYSIS

All data were analyzed using GraphPad Prism version 8.0 Data were expressed as mean± standard error of the mean (mean± SEM). One-way ANOVA was used to test for differences among groups, followed by Newman-Keuls multiple comparison test. P<0.05 was deemed statistically significant.

RESULTS & DISCUSSION

Clinical observations

The animals treated by the ethanolic extract of CP women capsules (5000 mg/kg) did not show any observable abnormality in movement, salivation, sleep, lethargy, there were no signs of piloerection and mortality in comparison to the control group within the first 48 hours, and daily during the 14 days of the study.

Lethal Dose Fifty (LD₅₀)

On monitoring the animals for 24, 48 hours and throughout the remaining 12 days, the group of rats treated by the ethanolic extract of CP women capsules did not record any deaths. Hence, the LD₅₀ of the ethanolic extract of CP Women capsules, when administered orally, is greater than 5000mg/kg.

Haematological analysis

Haematological parameters after a single administration of the ethanolic extract of CP women capsules at 5000 mg/kg in SD rats are presented in table 1. It was observed that, except for mean corpuscular haemoglobin concentration (MCHC), there was no significant difference between the haematological parameters measured in the control group and the group treated by the ethanolic extract of CP women capsules. MCHC is a measure of the average concentration of haemoglobin within a single red blood cell. The MCHC value in the ethanolic extract group was 33.43g/dL and significantly higher (p<0.0001) than the MCHC value in the control group. An elevated value of MCHC may either be due to more haemoglobin being concentrated in the red blood cell or to hemolysis of the RBCs. As a

result of autoimmune hemolytic anaemia, hereditary spherocytosis or due to severe burns [5].

SERUM BIOCHEMISTRY ANALYSIS

The serum biochemical markers after a single administration of the ethanolic extract of CP women capsules at 5000 mg/kg in SD rats are presented in table 2. They were grouped as renal function (urea) and liver function markers (alanine aminotransferase – ALT, aspartate aminotransferase- AST and alkaline phosphatase – ALP enzyme assays). Regarding the renal function (urea level), there was no significant difference between the control group and the group treated by ethanolic extract of CP women capsules. With regards to the liver function assessment, there was

no significant difference between the control and the ethanolic extract of CP groups. The AST value for the CP treated group that was higher than the control group than (160.9±60.00 vs. 82.80±27.55 respectively); however, this decrease was not statistically significant (p=0.4641). The ALP values on the other hand, were slightly lower in the CP-treated group than in the control group (80.70±23.30 vs. 116.2±31.39, respectively but was not significant (p=0.6316). high AST and ALP values indicate that the liver or any other organ such as the kidney that produces AST and ALP enzymes may be damaged. However, the levels observed were within the normal range and the levels observed are unlikely to be of any clinical relevance. These observations would need further investigations

Table-1: Haematological parameters after a single administration at 5000mg/kg of an ethanolic extract of CP women capsules in SD rats

Parameters	Saline	CP	P-Value
WBC (10 ³ /mm ³)	2.6±0.62	4.8±1.4	0.3288
RBC(10 ⁶ /mm ³)	6.84±0.06	6.85±0.30	0.9984
HGB (g/dl)	12.97±0.13	13.48±0.38	0.4296
HCT (%)	41.48±0.68	40.38±1.35	0.7085
MCV (um ³)	59.0±0.35	59.17±0.59	0.9685
MCHC(g/dl)	31.18±0.23	33.43±0.26****	<0.0001
PLT (10 ³ /mm ³)	1076±22.17	1004±53.88	0.3857
LYM (10 ³ /mm ³)	1.95±0.41	2.65±0.66	0.6610
MPV(um ³)	7.53±0.07	7.33±0.28	0.7477
GRA (10 ³ /mm ³)	0.88±0.11	0.93±0.22	0.8458

Values are expressed as mean +SEM (n=7). P-Value represents significance level for one-way ANOVA (followed by Newman-Keuls Multiple Comparison

Test) with * indicating significant difference from the control (distilled water) *=p, 0.05, **=p<0.001 and ****=p<0.0001

Table-2: Biochemical analysis after a single administration at 5000mg/kg of an ethanolic extract of CP women capsules in SD rats

Parameters	Saline	CP	P-Value
Urea (mmol/L)	1.88±0.68	3.04±0.51	0.3900
ALT (U/L)	82.80±27.55	160.9±60.00	0.4641
AST (U/L)	212.7±48.76	326.3±29.11	0.1143
ALP (U/L)	116.2±31.39	80.70±23.30	0.6316

Values are expressed as mean +SEM (n=7). P-Value represents significance level for one-way ANOVA (followed by Newman-Keuls Multiple Comparison Test) with * indicating significant difference from the control (distilled water) *=p,0.05, **=p<0.01 and ***=p<0.001

changes in the haematological parameters in the SD rats following a day's exposure.

- Oral administration of ethanolic extract of CP women capsules did not show any significant changes in the serum biochemistries measured in the SD rats following a day's exposure.

SUMMARY OF FINDINGS

- Oral administration of an ethanolic extract of CP women capsules at 5000mg/kg did not cause any sign of toxicity and mortality.
- The LD₅₀ of orally administered ethanolic extract of CP women capsules is above 5000mg/kg in SD rats
- Oral administration of ethanolic extract of CP women capsules did not cause any significant

CONCLUSION

The LD₅₀ (Lethal dose) of 80%-ethanol extract of CP women capsules in Sprague-Dawley rats is greater than 5000mg/kg. Further studies involving long term administration of aqueous extract of CP women capsules in different experimental rodents, including mice, will be needed to assess its safety for trial and use in humans. More renal function markers such as creatinine should be analyzed.

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