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Research Article

Evaluation of Haematological and Serum Biochemical changes Associated with Administration of *Combretum sordidum* Exell Leaf Extract to Wistar Rats

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Abstract
The acute and sub-chronic toxicity test of the acetone extract of *C. sordidum* was carried out using changes in hematology and serum biochemistry of male Wistar rats. For acute toxicity study, 25 female adult rats were divided into 5 groups. Group A (Control) were administered with 2ml/kg of distilled water, while rats in groups B to E were administered with the extract of 500, 1000, 2000, and 3000 mg/kg body weight respectively. 25 male rats were used for sub-acute toxicity test. The extract was administered at doses of 100, 200, 400 and 800 mg/kg body weight respectively, once daily for 14 days to rats in groups B to E. Rats administered with the extract had increased mean PCV, RBC, Hb. MCV, MCH, while MCHC was constant, but the extract at a dose of 800mg/kg caused reduction in MCV and MCH. The higher doses of 400mg/kg and 800mg/kg also caused an increase in ESR, WBC, Neutrophils, Platelets and a decrease in Lymphocytes. The results show the extract induced erythropoiesis at low doses, but inflammatory response at high doses. Serum biochemistry showed an elevation in the TP, Albumin, Globulin, TC, AST and ALT with the higher doses (400mg/kg and 800mg/kg) showing more prominent increment. This confirms the on-going inflammation observed in the haemogram. Specifically, elevation of AST and ALT are strong indicators of liver injury as can be observed in this study. It was concluded that prolonged administration of low dose of *C. sordidum* extract induces erythropoiesis, but high doses (>400mg/kg) causes liver injury. Therefore, low dose administration of ≤200mg/kg is safer for medicinal purposes.

Key Words: *Combretum sordidum*, Haematology, Serum Biochemistry, Wistar rats

INTRODUCTION

Combretum sordidum Exell, locally known as Apoka pupa (Yoruba) (Aworinde and Erinoso, 2015), is a scandent shrub or a creeper with white flowers that are easily recognized by the small red scales on the under-surface of the leaves. It is from the family Combretaceae: a family of plants with 20 genera and 600 species, in tropical and subtropical regions of the world (Ekeke *et al.*, 2014). This family is highly heterogeneous and occurs mainly in the tropics, warm temperate and sub-tropical zones of the world. The members of this family are trees or small trees, shrubs or deciduous climbers, sometimes scrambling shrubs with 4- to 5-winged fruits. Forty-nine (49) known species and 8 imperfectly known species have been reported in West Africa with 25 species occurring in Nigeria (Ekeke and Agbagwa, 2014).

Phytochemical screening of the *C. sordidum* has shown the presence of saponins, flavonoids, cardiac glycosides, alkaloids, tannins and anthraquinone. Constituents of the extracts from the phytochemical analysis confirmed the reason for the use of the plant for antimicrobial action against organisms and therefore could be harnessed as a potent antimicrobial agent. Findings from more recent studies, however, have shown that the leaves of *C. sordidum* possess both antimicrobial and antioxidant properties (Olaoluwa and Ogunbor, 2015).

Closely related plants from different regions of Africa are used as traditional remedies and have been scientifically proven to possess medicinal properties. *C. racemosum* P. Beauv leaves and root bark, and *C. celastroides* subsp. laxiflorum Welw leaves used in Congolese traditional medicine for many medicinal purposes, particularly for treatment of symptoms consistent with hypertension has been shown to possess antioxidant and endothelium relaxant properties (Nsuadi *et al.*, 2012). The leaves of *C. bracteosum*, *C. padoides*, *C. vendae* and *C. woodii* used traditionally in South Africa for treatment of diarrhea have also been reported to possess antioxidant and antimicrobial properties (Ahmed *et al.*, 2014).

C. molle is used African ethno medicinal practices and has been shown to reduce fever and pain due to its anti-inflammatory and analgesic properties (McGraw *et al.*, 2001; Ojewole, 2008; Yeo *et al.*, 2012). A further study showed an alkaloid extract of *C. molle* is a potent inhibitor of Hematopoietic prostaglandin D2 synthase (H-PGDS), a member of the glutathione S-transferase super family of

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enzymes that catalyses the conjugation of electrophilic substances with reduced glutathione. The enzyme catalyses the conversion of PGH2 to PGD2 which mediates inflammatory responses. The inhibition of H-PGDS is of importance in alleviating damage to tissues due to unwarranted synthesis of PGD2 (Moyo *et al.*, 2014).

Review of work in this area has shown that there is a dearth of information on the toxicity profile of *C. sordidum*, thus a baseline study on its effect of the primary vehicle of distribution in the body; the blood, is very imperative. This study is therefore aimed at evaluating the haematological and serum biochemical changes caused by acute and sub-chronic administration of acetone extract of *C. sordidum* in a bid to understand the safety of *C. sordidum* on both human and animal body systems using the Wistar rat as experimental animal.

MATERIALS AND METHODS

The Plant: The leaves of *Combretum sordidum* Exell were collected from Ibadan. The confirmation of the plant identity was carried out at the Herbarium of Forestry Research Institute of Nigeria (FRIN), Ibadan, and the Voucher No: 109923 issued. The leaves of the plant collected were air dried and pulverized.

Extraction of the fruit: Soxhlet extraction of 1kg of the pulverized leaves of *C. sordidum* using a soxhlet apparatus was carried out with acetone (distilled) as the solvent of choice. The acetone extract obtained was concentrated on a water bath at low temperature. The result was a paste-like extract, weighing 72.2g (percentage yield of 7.22%). The extract was stored at 4_0C and freshly reconstituted in corn oil for each experiment.

Experimental animals: 25 adult female and 25 male Wistar rats were acquired from the Experimental Animal Housing Unit of the Department of Veterinary Physiology, Biochemistry and Pharmacology. Acclimatization of Wistar rats under same housing conditions was carried out for twenty one (21) days prior to the commencement of the experiment. The animals were fed with pellet sized feed (Vitafeed) and clean water was supplied *ad libitum.*

Acute toxicity test: Twenty five adult female Wistar rats were randomly and equally divided into five groups; Groups A (Control), B, C, D and E. The rats in Group A were administered with distilled water (2ml/kg), while rats in Groups B - E were administered with *C. sordidum* extract doses of 500mg/kg, 1000mg/kg, 2000mg/kg and 3000mg/kg. The rats were observed for a period of 24 hours for signs of toxicity and or death.

Sub-chronic toxicity test: Twenty-five adult male Wistar rats were equally divided into five groups as above. The test groups (B-E) were administered with the extract at doses of 100mg/kg, 200mg/kg, 400mg/kg and 800mg/kg. The extract was administered once daily at regular intervals for 14 days.

Sample Collection: On day 14, the Wistar rats were first anaesthetized by the use of ether, before blood was collected from the retro-orbital sinus of each rat in all groups. A total of about 5ml of blood was collected from each rat. 2.5ml of blood was collected in lithium-heparinized bottles for haematology and 2.5ml of blood was also collected in lithium-heparinized bottles for serum biochemistry. They were then sacrificed by cervical dislocation. The tunica vaginalis was excised and the testes were milked to the surface. The spermatic cord was then exposed, ligated and incised. Semen samples were thereafter collected from the cauda epididymis. This method of collection was as described by Saba *et al.,* 2009. The semen samples were analyzed immediately after collection.

Blood haematological parameters evaluated: The packed cell volume (PCV) was determined by Haemocytometer, while the red blood cell count (RBC) and haemoglobin concentration (Hb) were analyzed by adopting the Microhematocrit and Cyanmethaemoglobin method respectively. The MCV, MCH, and MCHC were calculated by formular. The Erythrocyte Sedimentation Rate (ESR) was determined using the Westergren method. WBC count was determined by differential cell count,

Serum biochemical parameters evaluated: The semi-automated clinical chemistry analyser, RX MONZA, was used in analysing Total Cholesterol (TC), Total Protein (TP), albumin level. Globulin level was obtained from the difference of total protein and albumin. The Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) levels were determined by the spectroscopic method outlined by RANDOX lab manual.

Statistical Analysis

All data were entered into an excel spread sheet and analysed using Graphpad Prism (Version 6). One-way ANOVA (Analysis of Variance) was used to analyze the data and Tukey was used as the post-hoc test, to statistically confirm the differences between groups. The differences in mean were considered significant at $p<0.05$..

RESULTS

Acute Toxicity Test

There was no mortality recorded in any group during and after the acute toxicity study. The rats did not show signs of toxicity as well.

Sub-Chronic Toxicity Test

Haematology

Packed Cell Volume (PCV): There was a significant ($p < 0.05$) increase in the mean PCV of Wistar rats administered with the graded doses of extracts in groups B (45.40 ± 0.24 %), C (42.40 ± 0.24 %), D(43.80 ± 0.20 %), and E (44.60 ± 0.24 %) (Table 1).

Red Blood Cell Count (RBC): The mean RBC count for the Wistar rats in the control group (08.71 ± 0.84 x 10_6/µL) was non-significantly ($p > 0.05$) higher than those for groups B (08.44 ± 0.53 x 10_6/µL), and C (08.57 ± 1.00 x 10_6/µL), while those in group D (08.86 ± 0.92 x 10_6/µL) and E (10.46 ± 0.63 x 10_6/µL) showed a non-significant ($p > 0.05$) increase in mean RBC count (Table 1).

Haemoglobin Concentration (Hb): The mean haemoglobin concentration for rats in the control group (13.08 ± 0.37 g/dl)

was significantly ($p < 0.05$) lower than those for the groups B (15.02 ± 0.07 g/dl), C (14.02 ± 0.07 g/dl), D (14.52 ± 0.08 g/dl) and E (14.78 ± 0.07 g/dl) (Table 1).

Mean corpuscular volume (MCV): There was a non-significant ($p > 0.05$) increase in mean MCV value for Wistar rats in group B (54.60 ± 3.28 *fl*), C (50.40 ± 2.23 *fl*), and D (51.40 ± 4.82 *fl*) compared to that of the control group (47.20 ± 5.16 *fl*), and rats in group E (43.20 ± 2.62 *fl*) showed a non-significant ($p > 0.05$) decrease in mean MCV value (Table 1).

Mean corpuscular haemoglobin (MCH): The mean MCH value for rats in groups B (18.20 ± 1.02 pg), C (16.60 ± 1.12 pg), D (16.80 ± 1.66 pg) showed a non-significant ($p > 0.05$) increase compared to the control rats (15.60 ± 1.66 pg), while there was a non-significant ($p > 0.05$) decrease in the mean MCH value for rats in group E (14.20 ± 0.92 pg) (Table 1).

Mean corpuscular haemoglobin concentration (MCHC): There was no significant difference in the mean MCHC value of rats in all the groups (Table 1).

Erythrocyte sedimentation rate (ESR): There was a significant ($p < 0.05$) increase in the mean value of ESR for rats in groups C (06.80 ± 0.37 mm/hr), D (10.40 ± 0.24 mm/hr) and E (12.80 ± 0.49 mm/hr) compared to that of the control rats (01.80 ± 0.37 mm/hr). Group B (02.80 ± 0.58) on the other hand, had a mean ESR value non-significantly ($p > 0.05$) higher than that of the control rats (01.80 ± 0.37 mm/hr) (Table 1).

White blood cell count (WBC): The mean WBC for the control rats (07.52 ± 0.86 x 10_3/µL), was non-significantly ($p > 0.05$) lower than that of the rats in groups B (07.84 ± 0.45 x 10_3/µL), D (07.68 ± 0.60 x 10_3/µL) and E (08.32 ± 1.03 x 10_3/µL). There was however a decrease non-significantly ($p > 0.05$) in the mean WBC of rats in group C (07.04 ± 0.99 x 10_3/µL) when compared to the control rats (07.52 ± 0.86 x 10_3/µL) (Table 2).

Lymphocytes: The rats in groups B (67.60 ± 0.24 %) and D (67.40 ± 0.24 %) showed a non-significant ($p > 0.05$) decrease in their mean lymphocyte value compared to that of the control rats (68.00 ± 0.63 %), and a significant ($p < 0.05$) decrease was observed in group E (62.80 ± 0.58 %) rats. The mean lymphocyte value for Wistar rats in group C (73.00 ± 0.32 %) on the other hand, was non-significantly ($p > 0.05$) higher than that of the control rats (68.00 ± 0.63 %) (Table 2).

Neutrophils: Relative to the control rats (31.60 ± 1.86 %), there was a non-significant ($p > 0.05$) decrease and a significant ($p < 0.05$) decrease in the mean neutrophils level for rats in groups B (31.40 ± 0.24 %) and C (25.60 ± 0.24 %), respectively. While the mean neutrophils for rats in groups D (41.80 ± 0.20 %) and E (35.80 ± 0.66 %) were significantly ($p < 0.05$) higher than that of the control rats (31.60 ± 1.86 %) (Table 2).

Platelets: The mean platelets count for the control rats (11.20 ± 0.49 x 10_3/µL) was non-significantly ($p > 0.05$) lower than that of the rats in groups B (11.80 ± 0.20 x 10_3/µL), C (12.20 ± 0.20 x 10_3/µL) and D (12.20 ± 0.20 x 10_3/µL). The rats in group E (13.20 ± 0.49 x 10_3/µL), however, showed a significant ($p < 0.05$) increase in mean platelet count (Table 2).

Table 1:
Packed cell volume and red cell indices of Wistar rats administered with acetone extract of *Combretum sodidum* for 14 days

Parameter	Group A (Control)	Group B (100mg/kg)	Group C (200mg/kg)	Group D (400mg/kg)	Group E (800mg/kg)
PCV (%)	39.60 ± 1.12	45.40 ± 0.24_c	42.40 ± 0.24_a	43.80 ± 0.20	44.60 ± 0.24
RBC (x 10_6/µL)	08.71 ± 0.84	08.44 ± 0.53	08.57 ± 1.00	08.86 ± 0.92	10.46 ± 0.63
Hb (g/dl)	13.08 ± 0.37	15.02 ± 0.07_c	14.02 ± 0.07_{ae}	14.52 ± 0.08	14.78 ± 0.07_c
MCV (*fl*)	47.20 ± 5.16	54.60 ± 3.28	50.40 ± 2.23	51.40 ± 4.82	43.20 ± 2.62
MCH (pg)	15.60 ± 1.66	18.20 ± 1.02	16.60 ± 1.12	16.80 ± 1.66_e	14.20 ± 0.92_d
MCHC (g/dl)	33.00 ± 0.00	33.00 ± 0.00	33.00 ± 0.00	33.00 ± 0.00	33.00 ± 0.00
ESR (mm/hr)	01.80 ± 0.37	02.80 ± 0.58_{cde}	06.80 ± 0.37_{bde}	10.40 ± 0.24_{bce}	12.80 ± 0.49_{bcd}

*Values with superscripts on the same row indicate statistical significance ($p<0.05$).

Table 2:
White blood cells and Platelet count of Wistar rats administered with acetone extract of *Combretum sordidum* Exell for 14 days

Parameters	WBC (x 10_3/µL)	Lymphocytes (%)	Neutrophils (%)	Platelets (x10_3/µL)
Group A (Control)	07.52 ± 0.86	68.00 ± 0.63	31.60 ± 1.86	11.20 ± 0.49
Group B (100mg/kg)	07.84 ± 0.45	67.60 ± 0.24_{ce}	31.40 ± 0.24_{cde}	11.80 ± 0.20_{cd}
Group C (200mg/kg)	07.04 ± 0.99	73.00 ± 0.32_{bde}	25.60 ± 0.24_{bde}	12.20 ± 0.20_a
Group D (400mg/kg)	07.68 ± 0.60	67.40 ± 0.24_{ce}	31.80 ± 0.20_{bce}	12.20 ± 0.20_a
Group E (800mg/kg)	08.32 ± 1.03	62.80 ± 0.58_{bcd}	35.80 ± 0.66_{bcd}	13.20 ± 0.49

*Values with superscripts on the same column indicate statistical significance ($p<0.05$).

Table 3:
Serum biochemical parameters of Wistar rats administered with acetone extract of *Combretum sordidum* for 14 days

Parameters	Group A (Control)	Group B (100mg/kg)	Group C (200mg/kg)	Group D (400mg/kg)	Group E (800mg/kg)
TP (g/dl)	03.84 ± 0.14	04.56± 0.04$_{cde}$	04.16±0.02$_{bde}$	05.26±0.02$_{bce}$	06.14±0.14$_{bcd}$
ALB (g/dl)	01.22 ± 0.06	01.26 ± 0.09$_{de}$	01.04±0.02$_{de}$	02.04± 0.02$_{bc}$	02.06±0.04$_{bc}$
Glob (g/dl)	02.62 ± 0.18	03.30 ± 0.06$_{e}$	03.12± 0.02$_{e}$	03.22 ± 0.04$_{e}$	04.08±0.14$_{bcd}$
Chol (mg/dl)	37.20 ± 2.15	56.40± 0.51$_{cde}$	37.20±0.49$_{bde}$	64.60±0.24$_{bce}$	71.80±1.56$_{bcd}$
AST (U/L)	50.60 ± 5.13	75.00 ± 0.45$_{ce}$	53.40±7.65$_{bde}$	84.60± 0.24$_{ce}$	104.2±2.48$_{bcd}$
ALT (U/L)	38.00 ± 5.10	62.00± 0.89$_{cde}$	35.00±0.32$_{bde}$	75.60±0.24$_{bce}$	92.00±1.64$_{bcd}$

*Values with superscripts on the same row indicate statistical significance ($p<0.05$).

Biochemistry

Total Protein Levels (TP): The mean TP of rats in the control group (03.84 ± 0.14 g/dl) was significantly ($p < 0.05$) lower than that of the rats in groups B (04.56 ± 0.04 g/dl), D (05.26 ± 0.02 g/dl) and E (06.14 ± 0.14 g/dl), but non-significantly ($p > 0.05$) lower than group C (04.16 ± 0.02 g/dl) (Table 3).

Albumin Levels (ALB): The mean albumin level for rats in groups D (02.04 ± 0.02 g/dl) and E (02.06 ± 0.04 g/dl) were significantly ($p < 0.05$) higher than that of the control rats, while the rats in group B (01.26 ± 0.09 g/dl), showed a non-significantly ($p > 0.05$) increase in mean albumin level. There was however a non-significant ($p > 0.05$) decrease in the mean albumin level for rats in group C (01.04 ± 0.02 g/dl) (Table 3).

Globulin Levels (GLOB): The mean globulin level for the rats in groups B (03.30 ± 0.06 g/dl), C (03.12 ± 0.02 g/dl), D (03.22 ± 0.04 g/dl) and E (04.08 ± 0.14 g/dl) were all significantly ($p < 0.05$) higher than those of the rats in the control group (02.62 ± 0.18 g/dl) (Table 3).

Total Cholesterol Levels (TC): The mean total cholesterol value for rats in groups B (56.40 ± 0.51 mg/dl), D (64.60 ± 0.24 mg/dl) and E (71.80 ± 1.56 mg/dl) were all significantly ($p < 0.05$) higher than those of the control rats (37.20 ± 2.15 mg/dl) (Table 3).

Aspartate aminotransferase Levels (AST): The mean AST for the rats in groups B (75.00 ± 0.45 U/L), D (84.60 ± 0.24 U/L) and E (104.2 ± 2.48 U/L) were all significantly ($p < 0.05$) higher than those of the control rats (50.60 ± 5.13 U/L), while the mean AST for the rats in the group C (53.40 ± 7.65 U/L) was non-significantly ($p > 0.05$) higher compared to that of the rats in the control group (50.60 ± 5.13 U/L) (Table 3).

Alanine aminotransferase Levels (ALT): The mean ALT value for Wistar rats in groups B (62.00 ± 0.89 U/L), D (75.60 ± 0.24 U/L) and E (92.00 ± 1.64 U/L) were all significantly higher ($p < 0.05$) than those of the control rats (38.00 ± 5.10 U/L). The rats in group C (35.00 ± 0.32 U/L), however, showed a non-significantly ($p > 0.05$) decrease in mean ALT value (Table 3).

DISCUSSION

Toxicological evaluation of the acetone extract of *Combretum sordidum* was carried out in this study based on the blood haematology and serum biochemistry, using Wistar rats as the experimental animals. The extract administered caused an increase in packed cell volume (PCV), red blood cell (RBC) count, haemoglobin (Hb) concentration, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) value. The elevation of PCV, RBC count, Hb concentration indicates the induction of erythropoiesis. Erythropoiesis is the process whereby erythroid precursor cells proliferate and differentiate into RBCs (Elliott, 2008).

The MCV and MCH which are respectively the measurement of the average size of a single red blood cell (PCV/RBC) and average concentration of hemoglobin in a single red blood cell (Hb/RBC), tend to show mirroring results. In this study, it was observed that there was a significant increase in mean MCV and MCH. The mean MCHC however was constant in value across all doses. An increase in MCV is characteristic of Macrocytosis; a condition in which erythrocytes are larger than normal (Veda, 2012). Macrocytosis results from the response of the bone marrow to increased cell destruction or blood loss, with release of reticulocytes into the peripheral circulation (Strzoda and Kaferle, 2009). The increase in MCV and MCH value (Macrocytosis) might be due to the increased stimulation of erythropoiesis leading to presence of immature red blood cells (reticulocytes) in circulation, a condition known as Reticulocytosis.

There was however a decrease in MCV and MCH at the highest dose (800mg/kg), which showed shrinking of the red blood cell size (Microcytosis); a process that could eventually lead to the red blood cell destruction. Inflammatory states are often accompanied by microcytic anemia (Microcytosis). The cause of this anemia is twofold. First, renal production of erythropoietin is suppressed by inflammatory cytokines, resulting in decreased red-cell production. Second, lack of iron availability for developing red cells can lead to microcytosis (Wess, 2005). Erythropoietin is known to play a role in the protection from apoptosis and inflammation due to hypoxia, toxicity or injury (Chateauvieux, 2011).

The extract administered, especially the higher doses of 400mg/kg and 800mg/kg caused an increase in erythrocyte sedimentation rate (ESR), white blood cell (WBC) count, Neutrophils and Platelets, but a decrease in Lymphocytes, all of which are indicators of the presence of inflammation. The mean ESR was observed to increase dose-dependently, with the higher doses (400mg/kg and 800mg/kg) showing a significant elevation compared to the control. Elevated ESR values indicates presence of infection, inflammation and malignancy (Lensen *et al*., 2013) and this might be due to the high dose of *C. sordidum* extract administered.

There was an increase in the mean WBC count and this elevation might be due to the presence of inflammation, since elevated white blood cell count typically reflects the normal response of bone marrow to an infectious or inflammatory process (Abramson and Melton, 2000). The neutrophil value decreased at lower doses (100mg/kg and 200mg/kg) and increased at higher doses (400mg/kg and 800mg/kg). Neutrophil count increase is an important hematological parameter indicative of infection, inflammation, malignancy and tissue damage (Takahashi *et al*., 2001). There was a slight increase in platelet count value (Thrombocytosis), and this can be either autonomous or reactive. Infection (sepsis),

trauma/stress of surgery, and inflammation are common causes of reactive thrombocytosis (Chen and Afsari, 2002). These researchers suggest that the increase in platelet count could have been caused by an inflammation. This property might prove useful in the treatment of platelet insufficiency in bone marrow or platelet destruction in the blood stream, liver or spleen. The lymphocyte level was low compared to the control, this however could be caused by autoimmune disorders or infection. The low lymphocyte level as opposed to the elevation of WBC count could be due to the fact that elevation in lymphocyte is stimulated by viral infections e.g. human immunodeficiency virus (HIV).

The extract of *C. sordidum* administered to the rats also caused an elevation in the total protein (TP), Albumin, Globulin, total cholesterol (TC), aspartate aminotransferase **(**AST) and alanine aminotransferase **(**ALT) values, with the higher doses (400mg/kg and 800mg/kg) showing more prominent increment. There was an observable increase in the mean total protein (TP), Globulin and Albumin. High level of globulin can be caused by chronic inflammation or liver disease. The TC level was elevated, with the higher doses showing a more prominent increase. Determining the factor contributing to the elevation in TC level, be it low density lipoprotein (LDL) or high density lipoprotein (HDL) is very crucial. Increase in TC due to HDL (good cholesterol) would be a beneficial property, since HDL particles mediate cellular cholesterol efflux, have antioxidant properties, and modulate vascular inflammation and vasomotor function and thrombosis (Hafiane and Genest, 2013).

The AST and ALT levels were elevated, with the higher doses (400mg/kg and 800mg/kg) showing more profound increase. Both ALT and AST levels are reliable tests for liver damage and the increase in both levels could be a strong indicator of liver inflammation or damage. In chronic liver diseases such as hepatitis C and cirrhosis, the serum ALT level correlates only moderately well with liver inflammation. Also AST and ALT levels tend to be higher in cirrhotic patients with continuing inflammation or necrosis than in those without continuing liver injury (Johnston, 1999).

In conclusion, prolonged administration of low dose of *C. sordidum* extract induces erythropoiesis and high dose (400mg/kg and 800mg/kg) adminstration causes liver inflammation as well as a decline in sperm quality, dose administration of ≤200mg/kg is therefore safer compared to high doses of the extract.

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