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DERMAL TOXICITY STUDY OF AN ANTIDERMATOPHYTIC OIL-MOISTENED DICHLOROMETHANE-METHANOL (1:1 V/V) STEM BARK EXTRACT OF *POLYSCIAS FULVA* HIERN (ARALIACEAE) IN GUINEA PIGS ANIMAL MODEL

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ABSTRACT

Polyscias fulva, a medium size tree which grows in the tropical forests of West and Central Africa, is a popular medicinal plant used to treat malaria, mental illness, venereal infections, pulmonary tuberculosis as well as dermatoses. To the best of our knowledge there is no documented evidence corroborating its safety. Thus this work aimed to determine dermal toxicity profile of the antidermatophytic oil-moistened dichloromethane-methanol (1:1 v/v) stem bark extract of *Polyscias fulva.* Guinea pig (male and female) animal model was used for both acute and sub-acute dermal toxicity studies. For acute toxicity, single doses (0.5, 4.25 and 8 g/kg body weight) of oil-moistened plant extract were administered to animals while in sub-acute dermal toxicity, doses (13, 256.5 and 500 mg/kg bw) of plant extract were administered daily during 28 days. The possible toxic effect of the plant extract was assessed based on the animal behaviors, the organ morphology and histology, the hematological and biochemical parameters. The single and repeated dermal toxicity tests on guinea pigs did not show any overt sign of toxicity on growth patterns. The lethal dose fifty (LD_{50}) of the oil-moistened extract was higher than 8000 mg/kg. Moreover, the hematological and biochemical parameters, liver and kidney histopathology analysis collectively indicate that dermal application of the oil-moistened extract from the stem bark of *Polyscias fulva* is not associated with any toxicologically relevant effects in neither male nor female guinea pigs. The overall results of this study indicate that the oil-moistened dichloromethane-methanol (1:1 v/v) stem bark extract of *Polyscias fulva* could provide satisfactory preclinical evidence of safety to launch a clinical trial on a standardized formulation of the plant extracts.

Keywords: *Polyscias fulva*, oil-moistened extract, acute toxicity, sub-acute toxicity, dermal application

INTRODUCTION

Polyscias fulva is a fast growing deciduous tree up to 30 m tall, often with a straight, slender trunk to about 9 m before developing branches, like the spokes of an umbrella. The bark is grey and smooth and the leaf scars are prominent. The flowers are green-yellow, honey scented. The fruit is small, black, more or less oval and often ribbed (Bedir *et al*., 2001)**.** *Polyscias fulva* is widely distributed throughout sub-Saharan Africa at an altitude range of 1,180-2,500 m, with annual rainfall of 1,500-2,000 mm.

In Burundi, *Polyscias fulva* stem barks, branch, trunk powder is sniffing against pulmonary tuberculosis while its powdered bark is taken orally to facilitate delivery (Baerts and Lehmann, 1989). In the Democratic Republic of Congo, the *Polyscias fulva* bark infusion is used against fevers. In Cameroon, decoction of *Polyscias fulva* bark is orally administered to cure venereal infections (Focho *et al*.,

2009). Furthermore, its stems barks and leaves, pounded are used for local application against dermatoses.

The dichloromethane extract from the bark of *Polyscias fulva* was revealed to possess a weak antiplasmodial against *Plasmodium falciparum* (IC_{50}=9.8 µg/ml) and antitrypanosomial activities against *Trypanosoma rhodesiense* (MIC= 100 µg/ml) (Ndaya *et al*., 2002). Furthermore, the oil-moistened dichloromethane-methanol (1:1 v/v) extract from the stem bark of *Polyscias fulva* showed interesting *in vivo* antidermatophytic properties (Njateng *et al*., 2013). However, to the best of our knowledge, no report is available on it safety profile. It was on this basis that we carried out the toxicity studies of the oil-moistened dichloromethane-methanol (1:1 v/v) extract of its stem barks.

MATERIALS AND METHODS

Plant material: The stem bark of *Polyscias fulva* (Hiern) was collected in April 2008 at Bazou (Nde Division, West Region, Cameroon). Botanical identification was done at the Cameroon National Herbarium in Yaoundé by Mr Tadjouteu Fulbert, where a voucher specimen was kept under the reference number 43546/HNC.

Experimental animals: Experiments were performed using young adult guinea pigs (3 months old) of both sexes (400 ± 50 g) bred in the Animal House of the Department of Biochemistry, University of Dschang, Cameroon. The animals were fed with grasses and dietary supplement rich in proteins, vitamins and calcium. Food and water were given *ad libitum* throughout the experimental period. Animals were exposed to natural room temperature (22±2 C). The bioassay was conducted in accordance with both the internationally acceptable guidelines for evaluating the safety and efficacy of herbal medicines (OECD, 1981; OECD, 1987) and the ethical guidelines of the Committee for Control and Supervision of Experiments on Animals (Registration no. 173/CPCSEA, dated 28 January, 2000), Government of India, on the use of animals for scientific research.

Preparation of plant extract: The stem barks of *P. fulva* were dried at room temperature (20-24 °C) for fifteen days and powdered to coarse particles. Four kilograms of powder was macerated in 10 L of dichloromethane-methanol (Merck) (1:1 v/v) mixture for two days with frequent stirring and this process was repeated twice on the residue. After filtration, the filtrate was evaporated to dryness under vacuum at 45 °C using a rotary evaporator (Büchi R200). The extract was further concentrated by allowing it to stand overnight in an oven at 30 °C. The yield of the extract (6.58%, w/w) was calculated with respect to the initial weight of the dry plant powder. The crude extract was then used for the preparation of the palm kernel oil-moistened extract to be used for the toxicological studies.

Acute dermal toxicity study: This test was performed on guinea pigs in compliance with the OECD guideline 402 (OECD, 1987). Hair was clipped from the back of each guinea pig (weighing 350–360 g), approximately 10% of the total surface area, and then each animal was caged individually and left undisturbed for 24 h. They were divided into five groups (5 males and 5 females): one control (palm kernel oil (8 g/kg)), three oil-moistened *Polyscias fulva* extract treated groups (0.5, 4.25, and 8 g/kg body weight (b.w.) and a satellite group (8 g/kg oil-moistened *Polyscias fulva* extract). The shaved area was immediately covered with gauze and protected using a non-irritating adhesive plaster to prevent ingestion of the extract by the animal. The guinea pigs were then returned to their cages. Following application of the test substance, the animals were observed frequently during the first day. Twenty four hours after application, the covering was removed carefully and cage side observation was made daily. Observation included evaluation of skin and fur, eyes, respiratory effects (salivation, diarrhea and urination), and central nervous system effects (tremors and convulsion, changes in the level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength and stereotyped or bizarre behaviors). Body weights and food consumption were recorded daily for 14 days. By day 14, the guinea pigs were humanely sacrificed and gross pathological examinations were done afterward the organs were removed and weighted. The satellite group was observed for 14 days more days to detect any possible persistence toxic effect.

Repeated dermal toxicity test

Animal treatment: Guinea pigs (350–360 g) were randomly distributed into a control and four test groups of ten animals each (5 males and 5 females). Fur was clipped from the animal dorsal area (approximately 10% of the total body surface) 24 h prior to the test and repeated at a weekly interval (OECD, 1981). Shaved area of each experimental animal was rubbed once a day with the test material: palm kernel oil (500 mg/kg) for control group and oil-moistened *Polyscias fulva* extract at 13, 256.5, and 500 mg/kg bw respectively for test groups, for a period of 28 days. The test materials were applied evenly on the shaved area and kept in contact with

the help of porous gauze dressing and a non-irritating adhesive tape. Signs of toxicity (described above) were recorded daily, including onset, degree and duration of toxicity. A satellite group for each sex received the higher dose (500 mg/kg) and after the treatment period, they were left for 14 days observation to detect persistence or not of the toxic effect.

Sample collection: On the 28th day of experiment, urine was collected from individual metabolic cages containing animals subjected to overnight fasting (Honda *et al*., 2009). Blood samples were collected by cardiac puncture from ketamine-diazepam anaesthetized guinea pigs into heparinised (for heamatological analyses) and non heparinised tubes (for biochemical analyses). Gross pathological examinations including relative weight of different organs (liver, kidney, lung, heart and spleen) were performed (Ozolua *et al*., 2009). Fifteen percent homogenate of each organ were prepared in normal saline solution and centrifuged at 3,000 rpm for 10 min; the supernatants were used for protein quantification (Chanda *et al*., 2009).

Hematological and Biochemical analyses: Malassez chamber was used to quantify the total red blood cells (RBCs) and white blood cells (WBCs). Hematocrit was estimated using standard methods (Ekaidem *et al*., 2006). The serum was assayed for creatinine, aspartate amino-transferase (AST), alanine amino-tranferase (ALT), total cholesterol, high density lipoprotein (HDL), and triglycerides using commercial kits (Hospitex diagnostics std, Roma, Italia). Urine creatinine was assayed using the same commercial kit. Serum and organ total proteins were determined by the Biuret method (Gornal *et al*., 1949) while proteins in the urine were titrated using the method of Bradford (1976).

Histopathological analysis: Immediately after collecting the blood samples, vascular perfusion was performed for hepatic and kidney tissues fixation using isotonic saline solution followed by 10% phosphate buffered formalin solution. Sections of 5–6 mm of the liver and kidney tissues of animals of each group were routinely stained with haematoxylin and eosin (H & E), and examined under a light microscope (Olympus CH02) to register any possible alterations compared to the normal structure.

Statistical analysis: The values were expressed as mean ± standard deviation (S.D). For each parameter, the One-Way ANOVA was used to detect significant differences between the groups. When significant differences existed, the Waller–Duncan test ($p < 0.05$) was used to compare the means.

RESULTS

Acute toxicity: The dermal toxicity profile of the oil-moistened dichloromethane-methanol (1:1 v/v) extract from the stem bark of *Polyscias fulva* was studied. It was noted that at doses up to 8 g/kg body weight, no abnormality in treated guinea pigs was observed compared to controls in relation to sensitivity to noise and pinch, coat, color of eyes, salivation, the state of saddles and vivacity. They were alert, painless to touch, able to feed themselves well and were active in their locomotor activity. The weight of the animals regardless of the sex and group was increasing over the duration (14 days) that followed the administration of the extract (Fig. 1).

In addition, we observed no change in hair, mucous membranes, no abnormality regarding secretions and autonomous activities (lacrimation, piloerection, breathing, posture and response to handling, stereotypes (grouping, excessive rotation on itself) or no bizarre behavior of such self-mutilation, walking backwards. Also, no death was recorded meaning that the lethal dose one hundred (LD_{100}) of the oil-moistened extract from the stem bark of *Polyscias fulva* was higher than 8000 mg/kg in guinea pigs. This was confirmed by the organ macroscopic examination where no particular sign of toxicity was observed. Also, there was no significant difference (P 0.05) in the relative organ weights between the test and control groups (Table 1).

Repeated dose dermal toxicity test

Signs of toxicity and growth pattern: Repeated dermal application of the oil-moistened dichloromethane-methanol (1:1 v/v) bark extract of *Polyscias fulva* to guinea pigs for 28 days at different dose levels produced neither observable physical clinical signs of toxicity nor mortality. There was no local skin irritation in any guinea pigs throughout the test period. The mean body weight gain for male and female guinea pigs in all the test groups was not significantly different from their control counterparts (Fig. 2).

Effect of extract on relative organ weights: In the same sex, the relative organ weights of guinea pigs were not significantly ($P>0.05$) affected by the oil-moistened extract irrespective of the dose (Table 2).

Hematological and biochemical parameters: No significant changes were noted in red blood cells, white blood cells and hematocrit for both treated

male and female guinea pigs compared to the control. However, a significant (P>0.05) increase of basophils in test groups compared to their control counterparts in both sexes were noted even in the satellite group (Table 3). Only hepatic proteins were significantly (p<0.05) affected in male by the administration of the test substance (Table 4).

Liver and kidney histopathology: The histopathology analysis of necropsy samples on liver and kidney of guinea pigs of either sex revealed no observable (macroscopic and microscopic) deleterious effect at all the tested doses.

DISCUSSION

Safety should be the overriding criterion in the selection of medicinal plants for use in the production of phytomedicines in healthcare systems. This is why toxicological evaluation is carried out in various experimental animals to predict toxicity and to provide guidelines for selecting a 'safe' dose in humans (Olson *et al*., 2000). The present study is part of a program intended to produce phytomedicine from stem bark of *P. fulva* for antidermatophytic uses. In our previous work, we demonstrated the antidermatophytic properties of this oil-moistened extract with a therapeutic dose of 0.005 g/kg (Njateng *et al*., 2013).

In the present study, in both acute and subacute toxicities, the oil-moistened extract administration provoked no death in treated animals compared to control. In acute toxicity, up to 8 g/kg, there was not death, then based on Hodge and Sterner scale, the *P. fulva* oil-moistened extract can be considered as almost harmless by dermal route application (Berezovskaya, 2003). This is also justified by the absence of extract effect on mobility and the softening of deposits during the acute experiment.
Also, the daily dermal administration of the *P. fulva* extract to the guinea pigs did not induce visible toxic manifestation on organ weight, biochemical parameters and all studied haematological parameters except for basophils that increased in both sex with the administratered dose, even in satellite group. The organ-to-body weight ratio is an index often used in toxicological evaluations (Mythilypriya *et al*., 2007) and was not significantly modified by repeated dose dermal treatment of guinea pigs. This lends credence to the absence of injuries on the liver, lung, heart, spleen and kidney as confirm by the histopathological study of liver and kidney. In the majority of cases, except for brain, the organs change weight proportionally to the total body weight (Mythilypriya *et al*., 2007) as it was the case in this study. No significant change was noted in serum ALT and AST levels in both sexes. Liver damage and its recovery are usually assessed by measuring the level of serum transaminases particularly ALT. Indeed changes in their serum level are biological markers of liver dysfunction and/or damage (Al-boushi *et al*., 2009; Bidie *et al*., 2010). Creatinine results from metabolism of creatine in the skeletal muscles. In normal physiological conditions, it is filtered through the kidney and rejected in urine. Any increase of creatinine in the serum coupled with a decrease of its level in the urine is an indicator of kidney (cortex and/or the glomeruli) damage. According to James and Kathleen (1992), the measure of serum or urine creatinine level can also permit to appreciate muscle mass. The results obtained in this study did not show any significant changes in the serum and urine of the treated animals, compared to the controls. Therefore, the *P. fulva* oil-moistened extract has not affected kidney function and muscle mass of guinea pigs (Bidie *et al*., 2010).

As far as the protein levels are concerned, there was no significant difference between treated animals and controls except in the case of hepatic protein level in males. Endogenous proteins ensure not only the transportation of xenobiotics in blood toward target organs, but also their biotransformation in the liver in order to activate, excrete or detoxify them (Koolman and Röhm, 2004). In this study, instead, an increase in that protein level with the increase in the dose was found. This may be due to increase in hepatic protein synthesis necessary for the xenobiotic metabolism (Ozolua *et al*., 2009).

The degree to which elevated blood lipids contribute to heart diseases is determined by their distribution among the various lipoproteins classes. High cholesterol level in the blood is the major cause of cardiovascular disorders (Nduka, 1999). High serum levels of triglycerides and low density lipoproteins (LDL) are associated with coronary artery disease (Eisenhauer *et al*., 1998). We noticed no significant difference between the lipid indices in treated animals compared to their control counterparts, meaning that *P. fulva* oil-moistened extract may not have any cardiovascular side effect.

The haematopoietic system is one of the most sensitive targets for toxic substances (Harper, 1973) and is an important index of physiological and pathological status in man and animals (Adeneye *et al.*, 2006). Apart of basophils that the total count significantly increased, there were no treatment related changes in the haematological parameters (hematocrit, RBC and WBC count) compared to the

control. Although basophil increased, the obtained values remain in the normal range (0 to 2%) in guinea pigs (Nakanishi, 2010), suggesting that this cannot be attribute to a toxic effect of the plant extract but that its administration may boost the defence system of the animals. But this may also suggest that a long term administration of this substance may result in toxic effect especially allergic reactions.

CONCLUSION

Acute and subacute dermal application of oil-moistened extracts of the *P. fulva* appeared to be well tolerated by the experimental animals since no discernable toxic effect was noted. Therefore the dichloromethane-methanol (1:1 v/v) stem bark extract of this plant can be considered safe at therapeutic dose. This is step forward in the possible development of an antidermatophytic phytomedicine from *Polyscias fulva* stem bark extract.

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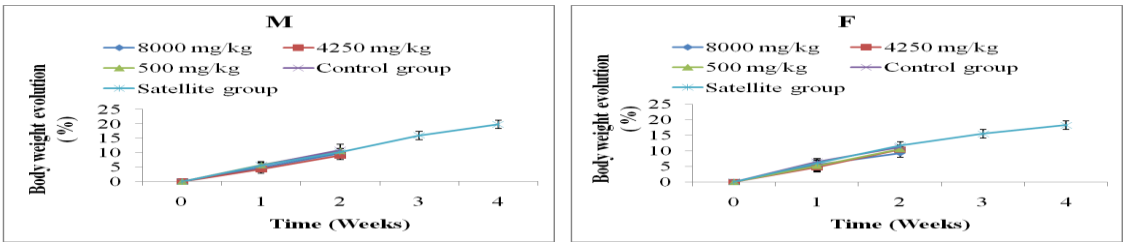

Fig. 1. Trends in mean body weight gains of male (M) and female (F) guinea pigs after single dose dermal application of the oil-moistened dichloromethane-methanol (1:1 v/v) bark extract of *Polyscias fulva* within 14 days period.

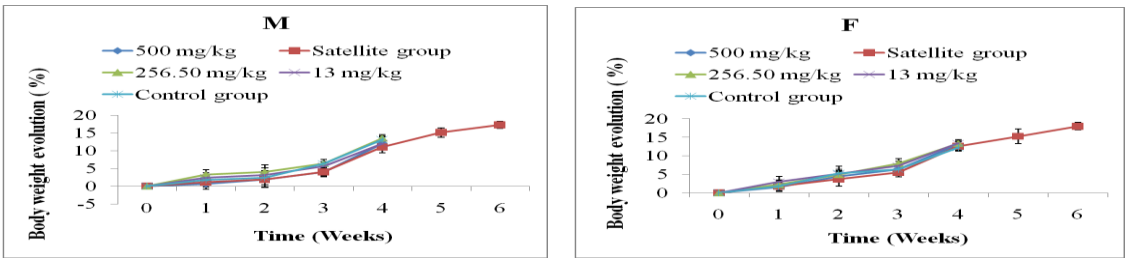

Fig. 2. Trends in mean body weight gains of male (M) and female (F) guinea pigs after repeated dose dermal application of the oil-moistened bark extract of *Polyscias fulva* within 28 days period.

Table 1. Effect of the single dose dermal application of the oil-moistened crude extract of *Polyscias fulva* on the relative organ weights of male and female guinea pigs.

Studied parameters	Control group	500 mg/kg	4250 mg/kg	8000 mg/kg	Satellite group (8000 mg/kg)
Male					
Heart (mg/g)	3.50 ± 1.10	3.33 ± 0.32	3.32 ± 0.30	3.85 ± 0.40	3,71 ± 0,80
Liver (mg/g)	30.00 ± 2.91	32.10 ± 1.60	30.35 ± 1.63	32.20 ± 2.31	30.12 ± 1.80
Spleen (mg/g)	1.91 ± 0.50	1.30 ± 0.32	1.83 ± 0.30	2.10 ± 0.90	2.00 ± 0.51
Kidney (mg/g)	8.91 ± 0.50	8.30 ± 0.32	8.33 ± 0.36	8.40 ± 0.81	8.00 ± 0.51
Lung (mg/g)	10.70 ± 2.81	9.37 ± 1.80	8.89 ± 1.60	9.61 ± 1.30	10.61 ± 2.10
Female					
Heart (mg/g)	3.44 ± 0.70	4.00 ± 0.60	3.36 ± 0.36	3.80 ± 0.20	3.77 ± 1.71
Liver (mg/g)	30.99 ± 3.50	32.90 ± 4.31	30.35 ± 2.70	32.22 ± 3.30	31.2 ± 2.20
Spleen (mg/g)	2.21 ± 0.38	2.72 ± 0.60	2.62 ± 0.30	2.00 ± 0.39	2.09 ± 0.41
Kidney (mg/g)	8.51 ± 1.50	9.63 ± 1.31	7.93 ± 1.40	8.82 ± 0.51	9.20 ± 3.90
Lung (mg/g)	12.36 ± 3.07	11.51 ± 1.23	12.80 ± 3.24	9.25 ± 1.50	10.12 ± 4.80

Data are expressed as Mean ± S.D; n = 5.
No significant difference was noted between groups (ANOVA)

Table 2. Effect of daily intake of the oil-moistened crude extract of *Polyscias fulva* on the relative organ weights of male and female guinea pigs.

Studied parameters	Control group	13 mg/kg	256.5 mg/kg	500 mg/kg	Satellite group (500 mg/kg)
Male					
Heart (mg/g)	4.00 ± 0.31	3.73 ± 0.20	3.81 ± 0.43	4.01 ± 0.30	4.10 ± 0.41
Liver (mg/g)	30.20 ± 1.50	28.83 ± 2.90	28.20 ± 5.70	28.90 ± 1.61	28.02 ± 0.71
Spleen (mg/g)	1.47 ± 0.11	1.43 ± 0.30	1.45 ± 0.13	1.50 ± 0.30	1.49 ± 0.12
Kidney (mg/g)	7.70 ± 0.40	7.50 ± 0.70	7.71 ± 0.10	7.54 ± 0.81	7.52 ± 0.40
Lung (mg/g)	9.00 ± 0.82	8.50 ± 0.91	8.21 ± 0.70	8.90 ± 0.30	8.61 ± 0.20
Female					
Heart (mg/g)	3.51 ± 0.11	3.73 ± 1.41	3.41 ± 0.32	3.72 ± 0.30	3.62 ± 0.41
Liver (mg/g)	28.22 ± 0.65	27.80 ± 2.41	28.60 ± 1.14	30.25 ± 4.90	28.91 ± 0.93
Spleen (mg/g)	1.42 ± 0.26	1.57 ± 0.20	1.54 ± 0.23	1.6 ± 0.20	1.47 ± 0.22
Kidney (mg/g)	7.72 ± 1.33	7.10 ± 0.50	7.40 ± 0.40	7.90 ± 1.22	7.13 ± 0.50
Lung (mg/g)	8.90 ± 0.61	8.85 ± 0.50	9.30 ± 1.51	9.40 ± 2.30	8.72 ± 1.0

Data are expressed as Mean ± S.D.; n = 5.
No significant difference was noted between groups (ANOVA)

Table 3. Effect of daily intake of the oil-moistened crude extract of *Polyscias fulva* on the hematological parameters of male and female guinea pigs as a function of treatment dose.

Studied parameters	Control group	13 mg/kg	256.5 mg/kg	500 mg/kg	Satellite group (500 mg/kg)
Male					
Total RBC (10^6 mm^{-3})	4.95 ± 0.07 [a]	4.10 ± 0.23 [a]	4.15 ± 0.12 [a]	4.06 ± 0.02 [a]	4.05 ± 0.08 [a]
Total WBC (10^3 mm^{-3})	6.29 ± 0.47 [a]	7.05 ± 0.05 [a]	6.79 ± 0.76 [a]	6.29 ± 1.08 [a]	6.31 ± 0.32 [a]
Neutroplils (%)	37.33 ± 2.58 [a]	39.00 ± 9.54 [a]	42.00 ± 7.00 [a]	41.02 ±1.00 [a]	38.33 ± 8.06 [a]
Eosinophils (%)	2 .66 ± 0.58 [a]	2 .60 ± 1.00 [a]	3.00 ± 1.68 [a]	3.33 ± 1.53 [a]	2.66 ± 1.53 [a]
Basophils (%)	0.00 ± 0.00[a]	0.33 ± 0.07[b]	0.66 ± 0.07 [c]	1.33 ± 0.07 [b]	0.66 ± 0.00[d]
Lymphocytes (%)	52.33 ± 3.51 [a]	50.66 ± 8.69 [a]	47.66 ±8 .50 [a]	46.00 ± 6.00 [a]	52.56 ± 4.58 [a]
Macrophages (%)	7.33 ± 2.52 [a]	9.00 ± 2.00 [a]	6.66 ± 2.08 [a]	8.33 ± 3.05 [a]	5.13 ± 2.08 [a]
Hematocrit (%)	41.45 ± 0.51 [a]	42.83 ± 3.31 [a]	40.27 ± 2.38 [a]	38.04 ± 3.58 [a]	38.42 ± 3.14 [a]
Female					
Total RBC (10^6 mm^{-3})	4.08 ±0.11 [a]	4.09 ± 0.03 [a]	4.08 ±0.09 [a]	4.01 ± 0.06 [a]	4.05 ± 0.25 [a]
Total WBC (10^3 mm^{-3})	6.26 ± 0.94[a]	6.65 ± 0.86 [a]	6.74 ± 0.92 [a]	6.69 ± 0.98 [a]	5.32 ± 0.38 [a]
Neutroplils (%)	45.01 ± 5.00[a]	49.01 ± 5.68 [a]	46.66 ± 5.51 [a]	45.00 ± 5.29 [a]	50.00 ± 9.17 [a]
Eosinophils (%)	2.40 ± 0.65 [a]	3.33 ± 0.75 [a]	2.66 ± 0.73 [a]	2.66 ± 0.58 [a]	3.00 ± 0.92 [a]
Basophils (%)	0.00 ± 0.00 [a]	0.33 ± 0.06 [b]	0.66 ± 0.06 [c]	1.00 ± 0.00 [d]	0.66 ± 0.05[c]
Lymphocytes (%)	44.66 ± 2.08[a]	40.66 ± 4.73 [a]	44.00 ± 2.65 [a]	41.00 ± 4.36 [a]	40.66 ± 8.50 [a]
Macrophages (%)	6.00 ± 0.66 [a]	6.66 ± 0.26 [a]	6.00 ± 0.45 [a]	6.33 ± 0.67 [a]	5.66 ± 0.86 [a]
Hematocrit (%)	40.33 ± 6.29[a]	39.96 ± 2.09 [a]	41.70 ± 1.59 [a]	40.69 ± 4.75 [a]	40.04 ± 5.69 [a]

Data are expressed as mean ± S.D., n = 5, RBC = Red blood cell; WBC = White blood cells. For a given row, values carrying the same letters superscript are not significantly different ($p > 0.05$, Waller–Duncan's test)

Table 4. Effect of daily intake of the oil-moistened crude extract of *Polyscias fulva* on the biochemical parameters of male and female guinea pigs as a function of treatment dose.

Studied parameters	Control group	13 mg/kg	256.5 mg/kg	500 mg/kg	Satellite group (500 mg/kg)
Male					
Serum creatinine (mg/dl)	1.32 ± 0.62 [a]	1.31 ± 0.55 [a]	1.73 ± 0.13 [a]	1.54 ± 0.32 [a]	1.65 ± 0.11 [a]
Urine creatinine (mg/dl)	3.00 ± 0.34 [a]	3.52 ± 0.59 [a]	3.76 ±0.70 [a]	2.62 ± 0.80 [a]	2.77 ± 0.66 [a]
Cardiac protein (mg/g)	114.42 ± 2.91 [a]	113.12 ± 7.76 [a]	111.89 ± 1.72 [a]	109.22 ± 7.35 [a]	110.33 ± 7.41 [a]
pleen protein (mg/g)	126.22 ± 3.28 [a]	127.79 ± 4.98 [a]	129.45 ± 3.82 [a]	129.8 ± 4.90 [a]	127.69 ± 3.15 [a]
Pulmonary protein (mg/g)	80.55 ± 4.93 [a]	78.63 ± 7.25 [a]	79.26 ± 3.31 [a]	79.64 ± 4.02 [a]	76.87 ± 9.08 [a]
Renal protein (mg/g)	116.59 ± 6.35 [a]	115.07 ± 8.61 [a]	110.98 ± 7.27 [a]	110.45 ± 6.77 [a]	111.85 ± 16.22 [a]
Hepatic protein (mg/g)	140.42 ± 3.15 [a]	149.82 ± 4.64 [b]	150.05 ± 9.07 [b]	160.42 ± 8.76 [b]	155.55 ± 3.60 [b]
Serum protein (mg/g)	67.54 ± 3.34 [a]	65.63 ± 1.94 [a]	65.73 ± 4.31 [a]	66.14 ± 7.79 [a]	66.31± 6.46 [a]
Urine protein(mg/g)	1.67 ± 0.13 [a]	1.43 ± 0.21 [a]	1.37 ± 0.24 [a]	1.57 ± 0.22 [a]	1.58 ± 0.11 [a]
ALT (U/L)	18.61 ± 4.03 [a]	20.07 ± 6.59 [a]	17.16 ± 5.26 [a]	17.99 ± 4.26 [a]	20.63 ± 6.43 [a]
AST (U/L)	14.25 ± 5.11 [a]	15.52 ±5.25 [a]	11.34 ± 5.31 [a]	13.96 ± 45.32 [a]	14.32 ± 4.68 [a]
Total cholesterol (mg/dl)	126.54 ± 14.29 [a]	130.88 ± 15.74 [a]	120.33 ± 12.75 [a]	121.59 ± 15.36 [a]	123.67 ± 13.68 [a]
HDL (mg/dl)	22.12 ± 7.18 [a]	19.44 ± 4.82 [a]	21.06 ± 6.59 [a]	19.08 ± 3.23 [a]	19.12± 8.43 [a]
LDL (mg/dl)	79.30 ± 9.87 [a]	81.82 ± 9.48 [a]	73.52 ± 8.23 [a]	77.48 ± 3.31 [a]	79.11 ± 8.03 [a]
Triglycerides(mg/dl)	167.57 ± 14.20 [a]	172.25 ± 18.10 [a]	159.93 ± 18.58 [a]	157.27 ± 10.13 [a]	175.18 ± 19.30 [a]
Female					
Serum creatinine (mg/dl)	1.97 ± 0.03 [a]	1.68 ± 0.32 [a]	1.61 ± 0.66 [a]	1.29 ± 0.45 [a]	2.15 ± 0.28 [a]
Urine creatinine (mg/dl)	2.24 ± 0.72 [a]	1.74 ± 0.33 [a]	1.70 ± 0.78 [a]	1.35 ± 0.86 [a]	2.09 ± 0.80 [a]
Cardiac protein (mg/g)	65.75 ± 6.41 [a]	61.70 ± 9.99 [a]	67.83 ± 5.62 [a]	56.62 ± 9.01 [a]	58.21 ± 0.97 [a]
Spleen protein (mg/g)	138.41 ± 5.56 [a]	131.24 ± 7.84 [a]	133.21 ± 9.67 [a]	129.62 ± 9.93 [a]	139.35 ± 9.35 [a]
Pulmonary protein (mg/g);	87.88 ± 4.38 [a]	86.08 ± 6.95 [a]	90.20 ± 6.63 [a]	89.90 ± 5.23 [a]	96.35 ± 8.13 [a]
Renal protein (mg/g);	111.00 ± 5.72 [a]	111.05 ± 1.51 [a]	110.04 ± 2.13 [a]	113.05 ± 3.74 [a]	110.36 ± 2.25 [a]
Hepatic protein (mg/g)	147.15 ± 8.01 [a]	153.33 ±5.85 [a]	155.31 ± 3.75 [a]	156.07 ± 4.35 [a]	151.63 ± 5.34 [a]
Serum protein (mg/g)	58.07 ± 4.60 [a]	64.91 ± 9.24 [a]	65.61 ± 7.91 [a]	64.91 ± 9.30 [a]	57.41 ± 7.59 [a]
Urine protein(mg/g)	1.68 ± 0.32 [a]	1.36 ± 0.12 [a]	1.31 ± 0.30 [a]	1.18 ± 0.8 [a]	1.10 ± 0.41 [a]
ALT (U/L)	13.63 ± 4.06 [a]	13.67 ± 5.81 [a]	15.99 ± 1.33 [a]	18.10 ± 3.36 [a]	13.09 ± 4.53 [a]
AST (U/L)	13.31 ± 3.50 [a]	17.99 ± 4.96 [a]	15.99 ± 6.78 [a]	17.47 ± 5.13 [a]	17.45 ± 3.02 [a]
Total cholesterol (mg/dl)	120.54 ± 21.79[a]	122.25 ± 25.05 [a]	133.75 ± 15.44 [a]	123.14 ± 62.51 [a]	135.54 ± 12.64 [a]
HDL (mg/dl)	13.44 ± 5.58 [a]	12.16 ± 4.89 [a]	10.32 ± 6.21 [a]	8.74 ± 4.89 [a]	10.00 ± 3.08 [a]
LDL (mg/dl)	79.11 ± 8.83 [a]	81.79 ± 7.43 [a]	83.58 ± 9.18 [a]	80.34 ± 7.41 [a]	85.89 ± 9.41 [a]
Triglycerides(mg/dl)	173.94 ± 22.34 [a]	166.96 ± 16.10 [a]	164.24 ± 9.72 [a]	160.60 ± 12.26 [a]	180.60 ± 21.83 [a]

Data are expressed as mean ± S.D.; n = 5. HDL = high density lipoprotein; LDL = low density lipoprotein; ALT = Alanine transaminase; AST = Aspartate transaminase. For a given row, values carrying the same letters superscript are not significantly different ($p > 0.05$, Waller–Duncan's test).

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