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Sub-acute Toxicity Study of the Ethanolic Extract of Cramp Balls (*Daldinia concentrica* (Bolton) Ces. & De Not.) On Wistar Albino Rats

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ABSTRACT: The study was to determine the level of toxicity of repeated administration of ethanol extract of a medicinal mushroom, *Daldinia concentrica* in animal model. Wistar albino rats of both sexes were sorted randomly into four groups of five rats each. Graded doses of 200, 400 and 800 mg/kg of the extract were administered orally, once daily for eight consecutive days to the rats in the respective groups, while control group received 10% tween 80 in distilled water only. The animals were monitored daily for behavioural activities, while effects on body weight, organ-body weight indices and haematological parameters were evaluated at the end of the treatment period. There was significant ($P< 0.05$) and dose dependent increase in the body weight of the test groups compared with the control. The organ-body weight indices did not differ significantly ($P>0.05$), while monocyte, platelet and plateletcrit values were elevated among the 200 and 400 mg/kg body weight treatments. However, the observable changes were not sufficient to establish any toxic effect arising from the sub-acute oral administration of the crude ethanol extract of *D. concentrica* at all test doses in this study. Rather, the effects on body weight and haematological parameters suggested that the extract contains nutritional and immune boosting properties.

Keywords: Sub-acute; toxicity; *Daldinia concentrica*; ethanolic extract; Wistar rats.

Introduction

Daldinia concentrica is an ascomycetous fungus that is mostly found in tropical and temperate countries of the world (Zoberi, 1972; Jonathan, 2002). This mushroom is common throughout Europe, North America and Australia and also occurs in New Zealand. It is evident in African countries such as Bénin Republic, Cameroon, Cote D'Ivoire, Ghana, Ivory Coast, Nigeria, and Senegal in Africa likewise North America, South America and Europe on dead decaying woods (Jonathan *et al*., 2011). It belongs to the division Ascomycota, class Ascomycetes, order Xylariales, and family Xylariaceae. The mushroom, also known as King Alfred's cakes, cramp balls or coal fungus, is a hard, inedible ball – shaped fungus. The visible part of the fungus, the fruit – body is initially reddish – brown in colour, but becomes black and shiny as it ages. The flesh is purple – brownish in colour, and dark concentric rings are visible when the fungus is cut open. The fungus is an interesting genus in that it forms large stroma with a zonate inner fibrous tissues (Zoberi, 1972; Jonathan, 2002). The fruit bodies appear as a hard hemispherical cushion up to 4 cm in diameter, on dead trunks or decaying logs (Jonathan, 2002). The surface of the sporophores is black and glossy with minute spores formed by the ostioles of perithecia (Zoberi, 1972).

The chemical components of *D. concentrica* include 4:5:4′:5′-tetrahydroxy-1:1′-binaphthyl and dihydroxyperylene quinone from the fruiting body (Allport and Bu'Lock, 1958); 2,6-dihydroxybutyrophenone, 8-methoxy-1-naphthol, and 2-hydroxy-5-methylchromone from mycelial cultures of this fungus (Allport and

Bu'Lock, 1960). Novel bi-naphthyl benzophenone derivatives named daldinals, azaphilone derivatives named daldinins A, dildinins B and daldinins C (Hashimoto *et al*., 1994), concentricol (Stadler *et al*., 2001) and concentricols B, C, D (Quang *et al*., 2002) were isolated from *D. concentrica*. Alkaloids, saponins, steroids, flavonoids, anthraquinones, glycosides and tannins have also been reported in the mushroom (Subbulakshmi and Kannan, 2016).

The earliest reports on the use of *D. concentrica* in Nigeria were by Akpaja *et al*. (2003; 2005). Reports on the trado-medical use of this mushroom in Nigeria and elsewhere are well documented (Oso, 1977; Idu *et al*., 2006). It is used by traditional doctors in Yoruba land, South Western Nigeria, in the treatment of pneumonia and other bacterial infections (Jonathan, 2002). In South Eastern Nigeria, the mushroom is used in the treatment of stomach upset and weakness of the body in both men and women (Okigbo and Nwatu, 2015). The present study was to evaluate the level of toxicity of repeated administration of ethanol extract of *D. concentrica* in experimental rats.

Materials and methods

Experimental animals: Twenty Wistar albino rats (weighing between 120 - 220 g) of both sexes were purchased from the Department of Pharmacology, Faculty of Pharmacy, University of Benin. The rats were separated randomly into four groups (I – IV) of five rats each and acclimatized for a week in cages at the animal house of the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City. They were maintained under room temperature ranging between 25-26 ^{o}C, standard 12 – hour light and 12 – hour dark cycle and were fed with corn mash and water *ad libitum*. Groups I – III served as treatments while group IV served as the control. Ethical guidelines of the Faculty of Pharmacy on the use and handling of experimental animals were strictly adhered.

Preparation of mushroom extract: Fresh fruiting bodies of the test mushroom, *D. concentrica*, were thoroughly rinsed with clean water, cut into pieces and air dried to constant weight. Exactly 50 g of the dried mushroom sample was soaked in 200 ml of ethanol (96%) and left for 36 hours at room temperature (28±2 ^{0}C) with occasional shaking. The portion was filtered using Whatman No. 1 filter paper. The filtrate was evaporated to dryness in a steady air current for about twenty four (24) hours in a previously weighed evaporation dish (porcelain dish). The extract (residue) was stored in a refrigerator (4 ^{0}C) in a clean sterile container, properly corked until further use.

Preparation of dosage of extract: Three dose regimes (200, 400 and 800 mg/kg body weight) of the mushroom extract were prepared for administration to the rats in group I – III respectively. For this, a stock solution of 800 mg/ml was prepared by dissolving 80 g of the mushroom extract in 90 ml of distilled water and 10 ml of tween 80 was added to aid solubility. Subsequent doses were obtained by serial dilution of the stock.

Experimental procedures: A gavage was used to administer a single oral doses of 200, 400 and 800 mg/kg body weight to animals in groups I – III respectively for eight consecutive days, while the control, group IV received an oral dose of 0.5 ml/kg body weight of 10% tween 80 in distilled water. The initial and final body weights of the animals were recorded at commencement and last day of administration of treatments respectively. All groups were observed daily for any physical signs of toxicity including appetite, agility, eye colour, fur texture, morbidity and mortality. After the 8th day treatment period, the animals were fasted overnight. Each rat was euthanized in a chloroform fumed chamber before immediately collecting the blood sample through the abdominal aorta into universal collection bottles lined with ethylene diamine tetraacetic acid (EDTA) for haematological analysis. Vital organs including heart, liver, left and right kidneys, lung and spleen were also harvested, cleaned and weighed. The value obtained for each organ was used to evaluate the organ-body weight index.

Haematological analysis: The full blood count analyses of the respective blood samples were performed using an automated haematology analyser.

Statistical analysis: Data obtained were analysed using one way ANOVA followed by Tukey's post hoc test. P-value< 0.05 was considered statistically significant.

Results and discussion

Daldinia concentrica has been reported as a medicinal mushroom (Akpaja *et* al., 2005; Idu *et al*., 2006). However, a beneficial mushroom may also contain toxicants (Nieminen *et* al., 2006). Since toxicity is usually dose-related, correct categorization and better understanding are essential for the safe and healthy consumption of mushrooms (Diaz, 2005; Jo *et al*., 2014; Cho and Han, 2016; Erenler *et al*., 2016). In the present study, no behavioural changes or signs of toxicity were observed in the physical attributes of all experimental rats after

eight days of repeated oral dose administration of ethanol extract of *D. concentrica* (Table 1). The average body weights of the treated groups were observed to be significantly higher ($P< 0.05$) than the control in a dose-dependent manner (Table 2). This may be attributed to the nutritional properties of the mushroom extract.

Table 1: Effect of eight days repeated oral administration of ethanolic extract of *Daldinia concentrica* on some physical attributes of Wistar rats.

Physical attributes	**Treatments**			
	200 mg/kg	**400 mg/kg**	**800 mg/kg**	**Control**
Gasping	Nil	Nil	Nil	Nil
Writhing	Nil	Nil	Nil	Nil
Mortality	Nil	Nil	Nil	Nil
Appetite	Normal	Normal	Normal	Normal
Agility	Normal	Normal	Normal	Normal
Eye colour	Normal	Normal	Normal	Normal
Fur texture	Normal	Normal	Normal	Normal

Control= 10% tween 80

Table 2: Effect of eight days repeated oral administration of ethanolic extract of *Daldinia concentrica* on body weight of Wistar rats.

Dose of extract (mg/kg)	**Body Weight (g)**
200	152.33 ± 5.24^{b}
400	158.67 ± 5.93^{b}
800	187.33 ± 1.20^{c}
0 (control)	128.75 ± 4.02^{a}

Values are expressed as mean ± standard error of mean (SEM) for 5 replicates. Mean values with non-identical superscripts are significantly different ($P< 0.05$) compared with control.

It was also observed that there was no significant difference ($P> 0.05$) between the organ-body weight indices of the vital organs in the treatments compared with the control (Figure 1). Relative organ-body weights are sensitive indicators of the health status for particular organs (Ping *et al*., 2013). The heart, liver, kidney, lungs and spleen are the primary organs affected by metabolic reactions caused by toxicants (Dybin *et al*., 2002). The data suggests that the extract is nontoxic to the body weight and analysed organs (Kluwe, 1981).

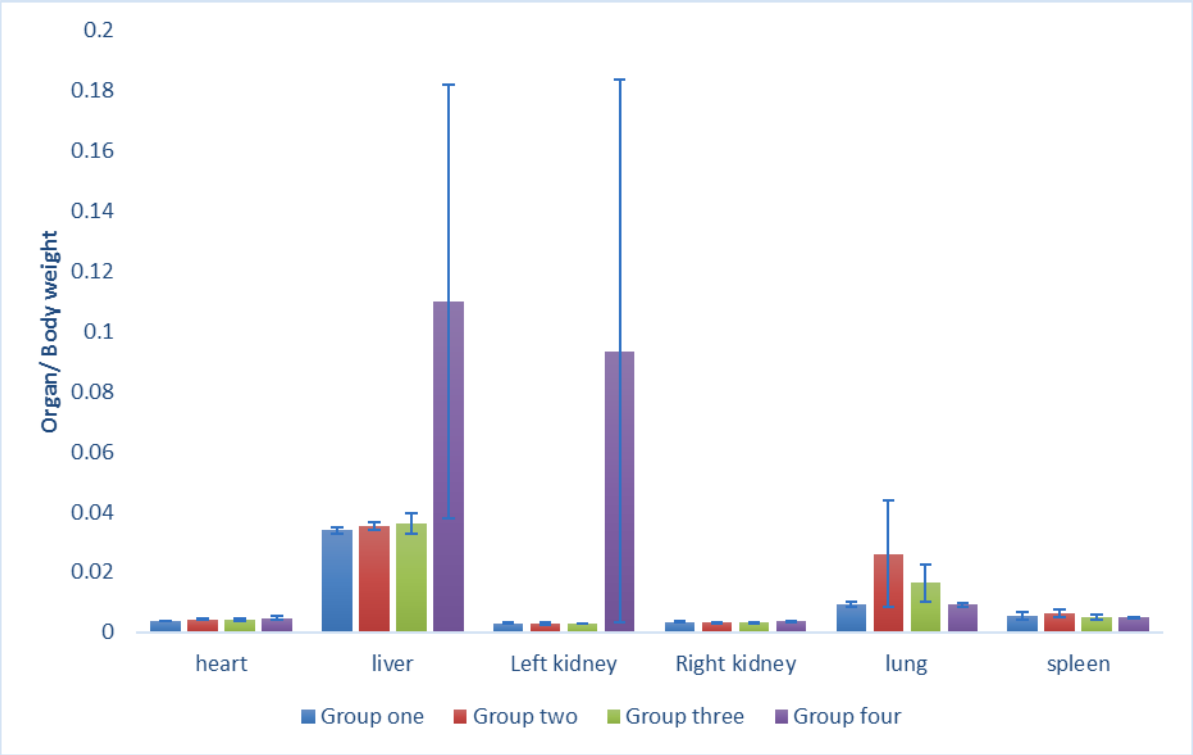

Figure 1: Effect of 8th days repeated oral administration of ethanolic extract of *Daldinia concentrica* on organ-body weight index of Wistar rats. Key: Group one= 200 mg/kg; Group two= 400 mg/kg; Group three= 800 mg/kg; Group four= Control

Analysis of blood parameters are important in the evaluation of risks associated with test compounds under investigation as the changes in the haematological system have a greater indicative value for human toxicity (Olson *et al*., 2000). Significant differences (P< 0.05) were observed in monocytes, platelets and plateletcrit values compared with the controls (Table 3). The 400 and 800 mg/kg treatments increased monocyte counts to 2.27±0.19 x 10^3/µl and 2.00±0.17 x 10^3/µl respectively compared to 1.33±0.12 x 10^3/µl in control. Although platelet counts and plateletcrit were raised in the 200 mg/kg treatment (697.33±130.16 x 10^3/µl and 0.49±0.08%) compared to respective values in control (314.67±63.99 x 10^3/µl and 0.25±0.03%), however, higher doses (400 and 800 mg/kg) had no significant (P> 0.05) effect on both parameters.

Table 3: Effect of 8th days repeated oral administration of ethanolic extract of *Daldinia concentrica* on haematological parameters of Wistar rats.

	Treatments			
Parameter	**200 mg/kg**	**400 mg/kg**	**800 mg/kg**	**0 (control)**
WBC x 10^3 (μl^{-1})	15.57±0.81	16.57±1.59	21.00±3.66	11.87±1.24
LY x 10^3 (μl^{-1})	8.97±0.84	9.40±1.18	8.97±2.10	7.23±1.03
MO x 10^3 (μl^{-1})	1.93±0.03^{ab}	2.27±0.19^{b}	2.00±0.17^{b}	1.33±0.12^{a}
GR x 10^3 (μl^{-1})	4.67±0.22	4.90±1.48	9.97±5.56	3.33±0.17
LY (%)	57.40±2.51	57.20±6.74	48.30±15.92	60.23±2.77
MO (%)	12.53±0.92	13.50±0.46	9.93±1.42	11.37±0.77
GR (%)	30.07±1.74	29.30±7.18	41.77±17.29	28.40±2.10
RBC x 10^6 (μl^{-1})	6.80±0.69	6.85±0.66	8.42±0.21	6.34±1.28
HGB (gdl^{-1})	12.30±1.27^{a}	13.70±1.08^{ab}	15.67±0.41^{b}	14.80±0.20^{ab}
HCT (%)	37.30±2.84	39.27±3.03	43.83±0.69	35.77±6.53
MCV (fl)	55.30±3.18	57.53±1.32	52.03±0.46	56.93±1.44
MCH (pg)	18.07±0.74	20.03±0.68	18.57±0.03	25.73±6.08
MCHC (gdl^{-1})	32.80±1.01	34.87±0.61	35.67±0.38	44.73±9.33
RDW (%)	17.73±0.87	16.77±0.55	18.07±1.11	21.97±4.93
PLT x 10^3 (μl^{-1})	697.33±130.16^{b}	585.00±32.51^{ab}	624.00±28.38^{ab}	314.67±63.99^{a}
PCT (%)	0.49±0.08^{b}	0.42±0.04^{ab}	0.38±0.02^{ab}	0.25±0.03^{a}
MPV (fl)	7.07±0.20	7.20±0.35	6.07±0.12	8.57±1.17
PDW (fl)	8.63±0.43	8.80±0.68	7.03±0.24	9.00±2.11

Values are mean±SEM; Tukey's mean comparisons with similar superscripts along each row are not significantly different (P> 0.05); WBC= white blood cell; LY= lymphocyte count; MO= monocyte count; GR= granulocyte; RBC= red blood cell; HGB= haemoglobin; HCT= hematocrit; MCV= mean corpuscular volume; MCH= mean corpuscular haemoglobin; MCHC= mean corpuscular haemoglobin concentration; RDW= red cell distribution width; PLT= platelet; PCT= plateletcrit; MPV= mean platelet volume; PDW= platelet distribution width.

Plateletcrit is an indicator of circulating platelets in a unit volume of blood. Increased production or amount of platelet in the blood may be as a result of acute infection, acute blood loss, inflammation, response to drugs, response to exercise or it can be caused by bone marrow disorder (Griesshammer *et* al., 1999; Schafer, 2004). Increased platelet can result in abnormal blood clots. The 400 and 800 mg/kg doses increased monocyte counts significantly (P< 0.05), which may be indicative of a boost in the immune system and growth factors production (Adeyemi *et al*., 2010). Other haematological parameters like the red blood cell, lymphocyte, granulocyte, haemoglobin, hematocrit, and mean corpuscular haemoglobin counts showed no significant difference (P> 0.05) between the test groups and control.

Conclusion

The study has revealed that oral dose administration of ethanol extract of *D. concentrica* for eight consecutive days does not have deleterious effects on the physical and behavioural features of the rats. However, mild traces of toxicity could be inferred from the platelet factor at a lower dose. It will be necessary to further extend the duration of exposure to the treatments in order to delineate the chronic toxicological properties of this extract.

References

Adeyemi OO, Akindele AJ, Nwumeh KI: Acute and subchronic toxicological assessment of *Byrsocarpus coccineus* Schum and Thonn. (*Connaraceae*) aqueous leaf extract. Int J Appl Res Nat Pro 3: 1-2. 2010.

Akpaja EO, Isikhuemhen OS, Okhuoya JA: Ethnomycology and usage of edible and medicinal mushrooms among the Igbo people of Nigeria. Int J Med Mushrooms 5: 313-319. 2003.

Akpaja EO, Okhuoya JA, Ekwerhe BA: Ethnomycology and indigenous uses of mushrooms among the Bini - speaking people of Nigeria: A case study of Aihuobabekun community near Benin City Nigeria. Int J Med Mushrooms 7: 373-374. 2005.

Allport DC, Bu'Lock JD: The pigmentation and cell-wall material of *Daldinia* sp. J Chem Soc 0: 4090-4094. 1958.
Allport DC, Bu'Lock JD: Biosynthetic pathways in *Daldinia concentrica*. J Chem Soc 0: 654-662. 1960.
Cho JT, Han JH: A case of mushroom poisoning with *Russula subnigricans*: Development of rhabdomyolysis acute kidney injury cardiogenic shock and death. J Kor Med Sci 31(7): 1164-1167. 2016.
Diaz JH: Evolving global epidemiology syndromic classification general management and prevention of unknown mushroom poisonings. Crit Care Med 33(2): 419-426. 2005.
Dybin E, Doe J, Groten J, Kleiner J, O'Brien J: Hazard characterization of chemicals in food and diet: Dose response mechanism and extrapolation issues. Food Chem Tox 42: 237-282. 2002.
Erenler A.K. Doğan T. Koçak C. and Ece Y: Investigation of toxic effects of mushroom poisoning on the cardiovascular system. Basic Clin Pharmacol Tox 119: 317-321. 2016.
Griesshammer M, Bangerter M, Sauer T, Wennauer R, Bergmann L, Heimpel H: Aetiology and clinical significance of thrombocytosis: analysis of 732 patients with an elevated platelet count. J Int Med 245(3): 295-300. 1999.
Hashimoto T, Tahara S, Takaoka S, Tori M, Asakawa Y: Structures of daldinins A-C, three novel azaphilone derivatives from ascomycetous fungus *Daldinia concentrica*..Chem Pharm Bull 42: 2397-2399. 1994.
Idu M, Osemwengie OO, Timothy O, Odia EA, Onyibe HI: A survey of indigenous flora used by folk medicine practitioners in Yobe Council Area of Adamawa State Nigeria. Plant Arch 7(2): 517-521. 2006.
Jo WS, Hossain A, Park SC: Toxicological profiles of poisonous edible and medicinal mushrooms. Mycobiol 42(3): 215–220. 2014.
Jonathan SG: Vegetative Growth Requirements and Antimicrobial Activities of Some Higher Fungi in Nigeria. PhD Thesis. University of Ibadan Nigeria. 2002.
Jonathan SG, Olawuyi OJ, Popoola OO, Aina DA: Antibacterial activities of *Daldinia concentrica*. Afr J Biomed Res 14: 57-61. 2011.
Kluwe WM: Renal function tests as indicators of kidney injury in subacute toxicity studies. Tox Appl Pharmacol 5(3): 414–424. 1981.
Nieminen P, Kirsi M, Mustonen AM: Suspected myotoxicity of edible wild mushrooms. Exp Biol Med 231(2): 221-228. 2006.
Okigbo RN, Nwatu CM: Ethnostudy and usage of edible and medicinal mushrooms in some parts of Anambra State Nigeria. Nat Res 6: 79-89. 2015.
Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G: Concordance of the toxicity of pharmaceuticals in humans and in animals. Reg Tox Pharmacol 32: 56-67. 2000.
Oso BA: Mushrooms in Yoruba mythology and medicinal practices. Copié de Eco Bot 31(13): 367-371. 1977.
Ping KY, Darah I, Chen Y, Sreeramanan S, Sasidharan S: Acute and subchronic toxicity study of *Euphorbia hirta* L. methanol extract in rats. BioMed Res Int: dx.doi.org/10.1155/2013/182064. 2013.
Quang DN, Stadler M, Hashimoto T, Asakawa Y: Chemical constituents of the Ascomycete *Daldinia concentrica*. J Nat Prod 65: 1869-1874. 2002.
Schafer AI: Thrombocytes. New Eng J Med 350: 1211-1219. 2004.
Stadler M, Baumgartner M, Grothe T, Muhlbauer A, Seip S, Wollweber H: Concentricol, a taxonomically significant triterpenoid from *Daldinia concentrica*. Phytochem 56(8): 787-793. 2001.
Subbulakshmi M, Kannan M: Cultivation and phytochemical analysis of wild mushroom *Daldinia comcentrica* and *Pheolus schweinitzii* from Tamilnadu India. Euro J Exp Biol 6(3): 46-54. 2016.
Zoberi HM: Tropical Macrofungi. Macmillan Press, London. 1972.