Acute and Oral Subacute Toxicity Study of Ethanolic Extract of *Cosmos Caudatus* Leaf in Sprague Dawley Rats

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***Abstract*—*Cosmos caudatus*, commonly known as 'Ulam Raja' by the locals, belongs to the botanical family Asteraceae. The plant is traditionally used in Malaysia for many beneficial claims such as for reducing body heat, as an anti-aging agent, improving blood circulation, promoting fresh breath, strengthening bone marrow and treating infections associated with pathogenic microorganisms. This study investigated the acute and subacute toxicity effects of *C. caudatus*, as per OECD Guidelines 423 for acute and 407 for subacute protocols. Haematological assessments as well as the body and organs weights of the rats were measured. For acute toxicity study, no evidence of toxicity attributable to the treatment was observed on behavioural pattern, haematological evaluation and organ weight in both treated and control groups. A significant weight gained (p<0.05) in rats that received 5000 mg/kg extract was observed, however, gross examination of internal organs revealed no detectable inflammation. For subacute toxicity study, there was a significant increased (p<0.05) in water intake (ml/day) in 250 and 500 mg/kg treated groups as compared with control while food intake and weight gain are comparable in all groups. A significant decreased (p<0.05) of lung and liver weights was observed in all treated groups while kidney weight of rats treated with 500 mg/kg of extract decreased significantly as compared with control. Haematological evaluation showed no significant difference except for packed cell volume (PCV) that decreased significantly (p<0.05) among the treated groups. In addition, red blood cells (RBC) of both groups of rats that received 250 and 500 mg/kg decreased significantly (p<0.05) as compared to the control group. Meanwhile, white blood cells (WBC) and mean corpuscular haemoglobin concentration (MCHC) were significantly different (p<0.05) among those rats that received 125 and 500 mg/kg extracts, respectively, as compared with control. The finding therefore revealed that different concentrations of the extract had induced different toxicity effects among the rats especially on subacute toxicity study. It is recommended that a comprehensive study to be conducted to ascertain the toxicity effects of *C. caudatus* on other biological parameters.**

***Index Terms*—*Cosmos caudatus*, feeding pattern, haematology test, organ weight, toxicity test.**

I. Introduction

Plant drugs, popularly known as herbal remedies, have been traditionally used in the treatment of a variety of diseases, where it is widely practiced in the Malaysian communities. Herbs and herbal formulations have continued to receive attention from the public because of the strong belief that these products are safe for the treatment of ailments [1]. This assumption may have influenced the indiscriminate use of these formulations to a large extent amongst the rural populace. These formulations are usually administered over a long period of time without proper dosage monitoring by the experts and lack of awareness of the toxic effects that might result from such prolonged usage [2]. The risk associated with the potential toxicity of such therapy demands that the practitioners be kept abreast of the reported incidence of renal and hepatic toxicity resulting from the ingestion of medicinal herbs [3].

C. Caudatus Kunth belongs to the family Asteraceae. It is an edible plant having 20 - 26 species worldwide and locally known as 'Ulam Raja' by Malaysian. It is an annual herb bearing purple, pink, or white ray florets, grows up to about 1 - 8 ft tall, hairless or sparsely hairy and leaves are finely dissected, 10 - 20 cm long [4]. It is found worldwide especially in tropical areas including Mexico, United States (Arizona and Florida), Central America, South America, Malaysia and Thailand [5]. In Malaysia, the plant is used traditionally for reducing body heat, improving blood circulation, as an anti-aging agent, strengthening bone marrow (because of its high calcium content), to treat infections associated with pathogenic microorganisms and to promote fresh breath [6].

Even though the leaves of *C. caudatus* are used for various medicinal treatments, to our knowledge no literature exists on its toxicity profile. This study therefore designed to evaluate the acute and subacute toxicity effects of the ethanolic leaf extract of *C. caudatus* especially on haematological parameters.

II. Materials and Methods

A. Plant Sample Collection and Identification

The fresh leaves of *C. caudatus* were collected from Taman Pertanian Universiti, University Pertanian Malaysia, Selangor, Malaysia. The taxonomical identification was carried out by officer in charge of herbarium, Mr. Sani bin Miran of the Faculty of Science and Technology, University Kebangsaan Malaysia, and voucher specimens were deposited in the herbarium of the Faculty of Science and Technology, University Kebangsaan Malaysia with voucher number of 30016.

B. Extraction of Test Material

The fresh leaves of *C. caudatus* were washed, air dried, and ground using cryogenic grinding to form fine powder. Freeze drying method was utilised to remove excess water

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from the powder. An amount of 5.0 kg of the powder was then soaked in 80% ethanol (1.5 L) for three days at room temperature. The extraction procedure was repeated twice using the same powder. The filtrates from each extraction were mixed and the excess solvent was evaporated under reduced pressure, using a rotary evaporator (Buchi, Rotavapor, Switzerland), to give a dark green crude ethanolic extract.

C. Animals

The experiment was performed on healthy male and female Sprague Dawley rats of eight weeks old and body weight of 160-200 g. They were purchased from CheNur Sdn. Bhd. The female rats were nulliparous and non-pregnant. The rats were fed with standard laboratory diets, given water *ad libitum* and maintained under laboratory conditions of temperature 22 ℃ (±3 ℃), with 12 h light and 12 h dark cycle. The experimental procedures involving the handling and treatment of animals were approved by the University Technology MARA (UiTM) Experimental Ethics Committee [Ethical number: 16/2011].

D. Procedure of Acute Oral Toxicity Test

The acute oral toxicity of the crude ethanolic extracts of *C. caudatus* was evaluated in rats using the procedures described by Organization for Economic Co-operation and Development 423 guidelines [7]. A total of 18 female animals were divided into three dosage groups with 6 animals per dose. The control group was given 10 ml/kg of normal saline. The second and third groups were given with a single dose of 2000 mg/kg and 5000 mg/kg of *C. caudatus*, respectively. Gavage dosing was performed using a curved, ball-tipped intubation needle affixed to a 5 ml syringe. All solutions were prepared just prior to dosing and were kept chilled and tightly capped.

Body weight, food, and water consumption were monitored daily. Animals were fasted approximately 12 hours prior to dosing. Following administration of a single dose of herbal preparation, the animals were observed for behavioural changes and general toxicity signs. Results were recorded for the first 30 minutes and at hourly intervals for the next 24 hours and thereafter for a total of 14 days. Body weight was recorded on Day 0 (before dosing), Day 7 and Day 14. At the end of the experiment, all of the animals were sacrificed for gross necropsy findings.

E. Procedure of Subacute Oral Toxicity Test

Repeated dose oral toxicity study was carried out according to OECD Guideline 407 [8]. The animals were divided into four groups of 12 animals each (6 males and 6 females). Group 1 received 10 ml/kg body weight of normal saline and served as control. Groups 2, 3 and 4 received extract doses of 125, 250 and 500 mg/kg body wt, respectively. Mortality, body weights, food and water consumption as well as observation for general toxicity signs of the animals were evaluated daily for 28 days.

F. Haematological Parameters

Diethyl ether was used to anaesthetize the animals before blood samples were collected through cardiac puncture into ethylenediaminetetraacetic acid (EDTA) tubes. The blood samples were analyzed for red blood cells (RBC), white blood cells (WBC) and haemoglobin (Hb) content using standard techniques [9] while packed cell volume (PCV) was estimated according to [10].

G. Organs Weight

Qualitative data on the weights of vital organs which included heart, lungs, liver, kidneys and ovary were assessed. Each of the organs was carefully dissected from the sacrificed animals and put them into a Petri dish which contained 10% normal saline. The isolated organs were dried with cotton wools and weighed on a sensitive balance. The weight of each organ was standardized to 100 g body weight of each rat.

H. Statistical Analysis

Differences in all haematological parameters, body and organ weights, water and food intake, and for all treated and control rats were determined using a One-way Analysis of Variance (ANOVA). A p value of 0.05 or less ($p<0.05$) were taken as significant. All data were expressed as mean ± standard error of the mean.

III. Results

A. Acute Oral Toxicity

The acute toxicity study showed that animals fed by oral gavages tolerated the limit dose of 5000 mg/kg body weight of ethanolic extract of *C caudatus* leaves. There were no visible signs of acute toxicity during the 14 days of observation. Absence of death at all doses up to 5000 mg mg/kg showed that the LD_{50} of the extract is greater than 5000 mg extract/kg body weight.

There was a significant increased in weight gain of rats after 14 days of extract treatment at 5000 mg/kg as compared with control. The eating, drinking habit and behaviour of all the animals used were normal. The results obtained on the average water and food intake and weekly weight gain are presented in Table I.

The results on the organs weights were presented in Table II. The macroscopic examinations did not show any change in organs colour and organs weights of all the animals.

Table III shows the results of haematology test. There were no significant differences ($p>0.05$) in the values obtained for red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) among all the groups.

B. Subacute Oral Toxicity

No behavioural changes and death were observed at the end of the treatment period. Similarly, no significant differences in food intake and weight gain were observed between control and treated groups during this period. However, there was a significant increased ($p<0.05$) in water intake (ml/day) in 250 and 500 mg/kg treated groups as compared to control (see Table IV).

Table V shows weights of lung and liver which decreased significantly in all the treated groups and kidney weights in 500 mg/kg group also decreased significantly as compared

with control. The macroscopic examinations did not show any change in colour of the organs of the treated animals compared with the control.

Table VI shows the results of haematology test. Haematological evaluation showed no significant differences except for packed cell volume (PCV) that decreased significantly ($p<0.05$) for all treated groups and red blood cells (RBC) decreased significantly ($p<0.05$) for rats that received 250 and 500 mg/kg of extract, respectively, as compared to control group. Meanwhile, white blood cells (WBC) and mean corpuscular haemoglobin concentration (MCHC) were significantly different ($p<0.05$) for 125 and 500 mg/kg groups as compared to control.

TABLE I: FEEDING PATTERN OF RATS IN ACUTE TOXICITY STUDY OF *C. CAUDATUS*

Parameter	Control	2000 mg/kg	5000 mg/kg
Average water intake (ml/day)	24.34 ±0.38[a]	28.30 ±3.99[a]	29.70 ±1.70[a]
Average food intake (g/day)	14.14 ±0.85[a]	12.66 ±0.38[a]	13.93 ±0.21[a]
Average weekly weight gain (g)	8.08 ±0.24[a]	10.05 ±1.13[ab]	11.43 ±0.88[b]

Each value represents the mean ± SEM (N = 6)
Superscripts [a b] Within row showed significant difference at ($p<0.05$)

TABLE II: ORGANS WEIGHTS (100G BODY WEIGHT) AFTER ACUTE TREATMENT WITH *C. CAUDATUS* LEAF EXTRACT

Treatment Groups	Organ weight (g)				
	Heart	Lung	Liver	Kidney	Ovary
Control	0.40±0.02[a]	0.75±0.03[a]	3.50±0.17[a]	0.75±0.04[a]	0.06±0.00[a]
2000 mg/kg	0.36±0.03[a]	1.08±0.21[a]	3.56±0.23[a]	0.81±0.04[a]	0.08±0.01[a]
5000 mg/kg	0.41±0.01[a]	0.85±0.04[a]	3.76±0.18[a]	0.81±0.03[a]	0.07±0.01[a]

Each value represents the mean ± SEM (N = 6)
Superscripts [a b] within column showed significant difference at ($p<0.05$)
All values were standardised to 100 g of body weight

TABLE III: HAEMATOLOGICAL VALUES OF RATS GIVEN ACUTE TREATMENT WITH *C. CAUDATUS* LEAF ETHANOLIC EXTRACT

Treatment Groups	RBC ($x10^6$)	Hb (g/dl)	PCV (%)	WBC ($x10^3$)	MCV (μm^3)	MCHC (%)
Control	5.4±0.4[a]	14.3±0.6[a]	44.4±4.2[a]	5.5±1.8[a]	81.6±5.2[a]	33.6±3.3[a]
2000 mg/kg	6.2±0.8[a]	15.4±0.6[a]	47.7±3.3[a]	8.5±2.9[a]	80.2±4.7[a]	32.9±2.1[a]
5000 mg/kg	6.0±0.2[a]	14.0±0.4[a]	44.7±1.7[a]	8.3±1.6[a]	74.9±4.8[a]	31.6±1.6[a]

Each value represents the mean ± SEM (n = 6)

TABLE IV: FEEDING PATTERN OF RATS IN SUBACUTE TOXICITY STUDY OF *C. CAUDATUS*

Parameter	Control	125 mg/kg	250 mg/kg	500 mg/kg
Average water intake (ml/day)	29.85 ±1.88[b]	32.34 ±0.99[ab]	36.43 ±2.24[a]	35.30 ±1.78[a]
Average food intake (g/day)	19.41 ±0.76[a]	18.54 ±0.59[a]	20.26 ±1.07[a]	19.70 ±0.92[a]
Average weekly weight gain (g)	24.71 ±3.53[a]	24.69 ±2.43[a]	27.27 ±3.38[a]	24.79 ±2.99[a]

Each value represents the mean ± SEM (n = 6)
Superscripts [A B] within row showed significant difference at ($p<0.05$)

TABLE V: ORGANS WEIGHTS (100G BODY WEIGHT) AFTER SUBACUTE TREATMENT WITH *C. CAUDATUS* LEAF EXTRACT

Treatment Groups	Organ weight (g)			
	Heart	Lung	Liver	Kidney
Control	0.38±0.01[a]	0.87±0.04[a]	4.26±0.12[a]	0.79±0.01[a]
125 mg/kg	0.38±0.01[a]	0.71±0.04[b]	3.03±0.10[c]	0.77±0.02[ab]
250 mg/kg	0.36±0.01[a]	0.72±0.04[b]	3.48±0.12[b]	0.79±0.02[a]
500 mg/kg	0.38±0.01[a]	0.66±0.02[b]	3.40±0.21[bc]	0.73±0.02[b]

Each value represents the mean ± SEM (N = 6)
Superscripts [a b c] within column showed significant difference at ($p<0.05$)
All values were standardised to 100 g of body weight

TABLE VI: HAEMATOLOGICAL VALUES OF RATS GIVEN SUBACUTE TREATMENT WITH *C. CAUDATUS* LEAF ETHANOLIC EXTRACT

Treatment Groups	RBC ($x10^6$)	Hb (g/dl)	PCV (%)	WBC ($x10^3$)	MCV (μm^3)	MCHC (%)
Control	7.3±0.3[a]	15.3±0.2[a]	65.5±2.7[a]	10.2±1.4[a]	92.6±7.5[a]	23.7±1.0[b]
125 mg/kg	6.7±0.3[ab]	15.0±0.3[a]	54.0±2.4[b]	5.2±0.9[b]	82.0±4.2[a]	28.5±1.8[a]
250 mg/kg	5.7±0.4[b]	14.9±0.1[a]	56.7±3.0[b]	7.6±1.4[ab]	102.6±7.7[a]	26.9±1.5[ab]
500 mg/kg	6.0±0.4[b]	14.6±0.3[a]	52.0±3.3[b]	5.1±0.9[b]	90.2±9.0[a]	29.1±1.7[a]

Each value represents the mean ± SEM (n = 6)
Superscripts [A B] within column showed significant difference at ($p<0.05$)

IV. Discussion

The results obtained from the acute toxicity study showed that the ethanolic leaf extract of *C. caudatus* demonstrated high safety margin since the animals tolerated up to 5000 mg/kg body weight of the extract orally. According to the chemical labelling and classification of acute systemic toxicity, based on oral LD_{50} value, which were recommended by reference [11] the crude extracts of *C. caudatus* were assigned as class 5 (LD_{50} > *2000* mg/kg), which was designated to have the lowest toxicity class (no label; *unclassified*). The high safety margin of the extract through oral route justified its widespread use by traditional healers.

There was a significant weight gained among rats that was given 5000 mg/kg of the extract as compared to control. This however, was not related to food and water intake as these parameters did not increase significantly. It might be related to neurological damage [12], [13] or toxic effects on intestinal glycosidases [14]. In subacute toxicity study, there was a significant increase (p<0.05) in water intake (ml/day) in both 250 and 500 mg/kg treated groups as compared with control. However, since the eating pattern and average weekly weight gain were comparable between treated and untreated animals, the extract could be claimed to be non-toxic to the animals.

In subacute toxicity study, the gross examination of internal organs revealed no detectable inflammation or changes in colour compared with the control. The organs weights showed no significant difference except for the lung and liver that were significantly decreased (p<0.05) in all treated groups whereas kidney weights in 500 mg/kg group also significantly decreased as compared to control. It had been reported that reduction in body and also internal organ weights are considered sensitive indices of toxicity after exposure to toxic substance [15]. Liver and kidney weights were considered useful in toxicity studies because of its sensitivity to predict toxicity and correlates well with histopathological changes. There is little interanimal variability and thus, it is frequently a target organ of toxicity. In addition, liver is known as primary detoxification organ. Lung weights is less useful in toxicity studies because of variability from poor or indistinct demarcations for trimming off the airways; a lower frequency of finding weight changes that correlated with toxicity; and less sensitivity to predict toxicity compared to histopathology [16]. Therefore, it could be claimed that liver and kidney could serve as target organ in subacute oral toxicity effect of the extract.

Haematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of physiological and pathological status in man and animals [17]. In subacute toxicity study, haematological evaluation showed no significant difference except for packed cell volume (PCV) that decreased significantly (p<0.05) among all treated groups and red blood cells (RBC) which also decreased significantly (p<0.05) for both groups of rats that received 250 and 500 mg/kg extract as compared to control group. Meanwhile, white blood cells (WBC) and mean corpuscular haemoglobin concentration (MCHC) were significantly difference (p<0.05) for 125 and 500 mg/kg groups as compared to control. The reduction in PCV and RBC values indicated that the extract was toxic to circulating cell and possibly had interfered with RBC production [18]. MCV and MCHC value are calculated RBC indices that are used in anaemia diagnosis in most animals [19]. From this result, the extract significantly altered the MCHC which were indicative of the effect of the extract on the Hb concentration per RBC. It can be concluded that ethanol leaf extract of *C. caudatus* may possess the potential to induce anaemia. The observed reduction in WBC count suggests the decline in the functioning of immune systems.

However, since the values obtained in this experiment were within the reference range for WBC, the extract could be claimed to be non-toxic to immune systems [20].

V. Conclusion

Our results had demonstrated that the ethanolic extract of *Cosmos caudatus* possesses the lowest toxicity effects as indicated in our rat model. No deaths or signs of toxicity were observed in the rats that received the extract up to an oral acute limit dose of 5000 mg/kg. However, since the extract gave effects on selected organ weights and most of haematological parameters, therefore, it is recommended that a comprehensive study should be conducted to ascertain the toxicity effects of *C. caudatus* extract on other biological parameters.

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