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Acute Toxicity Study of *Cosmos caudatus* on Biochemical Parameters in Male Rats

(Kajian Ketoksikan Akut *Cosmos caudatus* ke Atas Parameter Biokimia di Dalam Tikus Jantan)

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ABSTRACT

Cosmos caudatus *or* 'ulam raja' *is a local plant with antioxidant properties and has the potential to treat free radicals-associated diseases. However, its toxic effects need to be elucidated. Acute toxicity study was carried out in male rats. Rats aged 3 months, weighing 150-200 g were divided into 4 groups, i.e. rats being fed on distilled water (control),* Cosmos caudatus *extract dose 50 mg/kg (CC50),* Cosmos caudatus *500 mg/kg (CC500) and* Cosmos caudatus *2000 mg/kg (CC2000), respectively. A single treatment was given to the respective groups and the animals were sacrificed after 7 days. Biochemical parameters before and after treatment were measured. Liver enzymes, i.e. alkaline phosphatase (ALP) and alanine transaminase (ALT), were found to be increased post treatment in CC500 and CC2000 groups. Creatinine levels were lower in CC500 and CC2000 groups post treatment compared with control group while albumin levels were lower in CC2000 group than all the other groups. In conclusion,* Cosmos caudatus *may cause acute hepatotoxicity at high doses.*

Keywords: Acute toxicity; biochemical parameters; Cosmos caudatus

ABSTRAK

Cosmos caudatus *atau ulam raja merupakan tumbuhan tempatan yang mempunyai aktiviti antioksidan dan berpotensi untuk merawat penyakit yang dikaitkan dengan radikal bebas. Walau bagaimanapun, kesan toksiknya perlu ditentukan. Kajian ketoksikan akut telah dilakukan ke atas tikus jantan. Tikus berusia 3 bulan, dengan berat badan 150-200 g dibahagikan kepada 4 kumpulan, iaitu tikus yang dirawat dengan air suling (kawalan), ekstrak* Cosmos caudatus *pada dos 50 mg/kg (CC50),* Cosmos caudatus *500 mg/kg (CC500) dan* Cosmos caudatus *2000 mg/kg (CC2000), masing-masing. Satu dos rawatan telah diberikan dan tikus dikorbankan selepas 7 hari. Parameter biokimia sebelum dan selepas rawatan telah ditentukan. Enzim hepar iaitu alkalin fosfatase (ALP) dan alanin transaminase (ALT), didapati meningkat selepas rawatan untuk kumpulan CC500 dan CC2000. Aras kreatinin didapati lebih rendah di dalam kumpulan CC500 dan CC2000 selepas rawatan berbanding kumpulan kawalan manakala aras albumin didapati rendah dalam kumpulan CC2000 berbanding kumpulan-kumpulan lain. Kesimpulannya,* Cosmos caudatus *mungkin menyebabkan hepatotoksisiti akut pada dos yang tinggi.*

Kata kunci: Cosmos caudatus*; ketoksikan akut; parameter biokimia*

INTRODUCTION

Plants have often been one of the essential sources of medication. In Malaysia, the healthcare market is booming with natural products derived from locally sourced plants. One of the local herbs, *Cosmos caudatus,* which is also known as *Ulam raja*, has the potential to be used in treating free radicals-associated diseases since it is found to possess high antioxidant capacity (Shui et al. 2005). There are a number of diseases associated with oxidative stress caused by free radicals. For instance, various age-related diseases, including cataracts, atherosclerosis, neoplastic diseases, diabetes, diabetic retinopathy, chronic gastrointestinal tract inflammatory diseases, aging of skin, diseases associated with cartilage, Alzheimer's disease and other neurological disorders (Stohs 1995).

Traditionally, *Cosmos caudatus* is used for improving blood circulation and to strengthen muscles and bones, but the mechanism of its medicinal function is still not clear (Anon. 2007). *Cosmos caudatus* (CC) also exhibits antimutagenic as well as antifungal properties (Ragasa et al. 1999). In addition, Vimala et al. (2006) showed that the antioxidant activity of this herb is more than 70%. Another study has also proved that it had extremely high antioxidant capacity of about 2400 mg L-ascorbic acid equivalent antioxidant capacity (AEAC) per 100 g of fresh sample (Shui et al. 2005).

Cosmos caudatus has been found to contain phenolic, flavonoids, flavones and flavanones and has also been shown to have strong antioxidant activity (Mustafa et al. 2010). This shows its great antioxidant potential and thus capacity to scavenge free radicals and to prevent oxidative

damage to body tissues and cells, which may be partly attributed to some of its believed medicinal functions.

With its great antioxidant activity, it may help in prevention of diseases caused by free radicals. However, the use of it is questionable in terms of potential health risks and toxicity. Subsequently, it is important to look into these issues to avoid unwanted complications. In this study, the acute toxic effects of CC were determined. The data obtained will be beneficial in future work using CC especially if CC is to be used for medicinal purposes.

Materials and Methods

Experimental animals

Forty male Wistar rats, aged 3 months, weighing 150-250 g were purchased from Laboratory Animal Research Unit (LARU), Universiti Kebangsaan Malaysia. They were kept at room temperature (27°C) with a 12 h light dark cycle. They were kept 2 per cage and fed with rat chow and distilled water.

Study design

The acute toxicity study was designed based on fixed dose method as described by Organisation for Economic Cooperation and Development (OECD) (OECD 420; 2004/73/EC) (CDER 1996). The rats were randomly divided into four groups: Control group (C), group supplemented with *Cosmos caudatus* extract at the dose of 50 mg/kg (CC50), group supplemented with CC at the dose of 500 mg/kg (CC500) and group supplemented with CC at the dose of 2000 mg/kg (CC2000).

Treatment

The aqueous extract of CC with the concentration of 1 g/mL was prepared by the School of Chemical Sciences & Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia using water extraction method which was previously described (Huda-Faujan et al. 2007). For 50 mg/kg and 500 mg/kg doses, 0.25 mL and 2.5 mL of the CC extract was diluted with 10 mL of distilled water, respectively. No dilution was done for the 2000 mg/kg dose. Each rat received a total of 0.2 mL/100 g body weight of its respective treatment solution via oral gavage. Treatment was given once at day 0 and the rats were kept for 7 days.

Biochemical parameters and full blood count

Blood samples were collected twice, at the initiation of the treatment and upon completion of treatment. The blood samples were sent to Pathological and Clinical Laboratory (M) Sdn Bhd for liver enzymes (alanine transaminase (ALT) and alkaline phosphatase (ALP)), renal profile (creatinine) and albumin analysis.

Statistical analyses

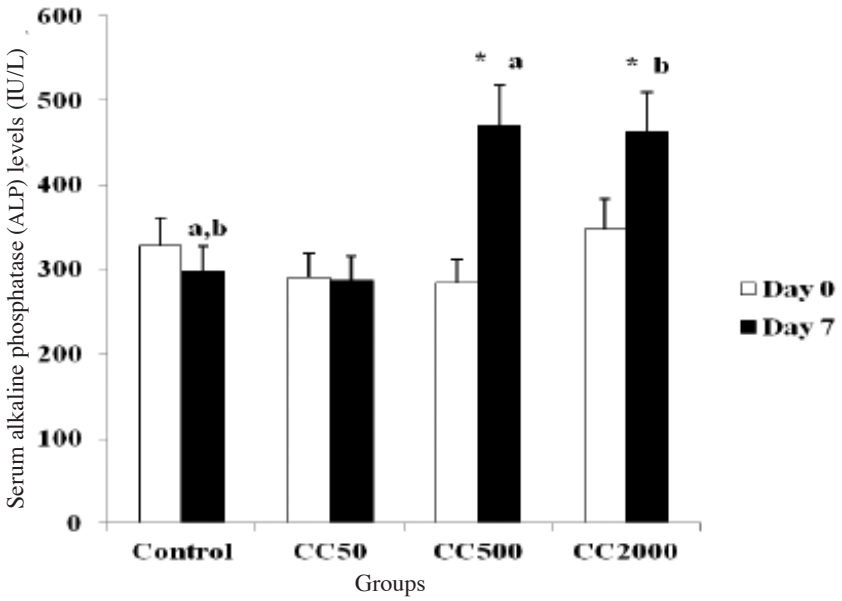
Statistical analysis was carried out using the Statistical Package for Social Sciences version 17.0 (SPSS Inc, Chicago, IL, USA) software. The data was tested for normal distribution using the Kolmogorov-Smirnov test. For normally distributed data, paired t-test and ANOVA was used. Tukey's HSD test was used as the post-hoc test for ANOVA. For the data that was not normally distributed, Wilcoxon Rank Sign test and Kruskal Wallis with Mann-Whitney test was used. All data was presented as mean ± standard error of the mean (SEM).

This study has been conducted with the approval and in accordance to the guidelines of Universiti Kebangsaan Malaysia Animal Ethics Committee with the approval code: PP/FAR/2010/NORAZLINA/20-JANUARY/284-JANUARY-2010-MAY-2010.

Results and Discussion

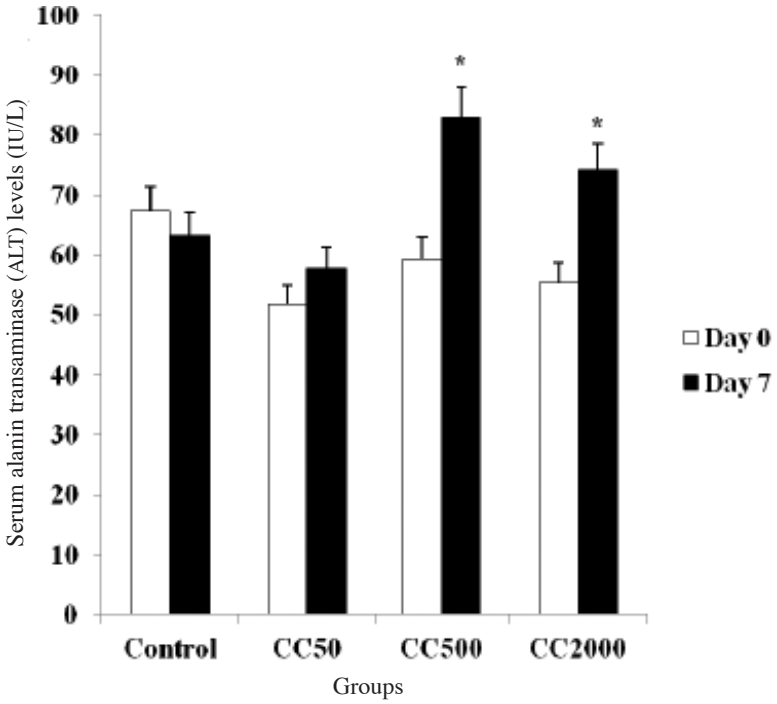
All rats in this study lived up to 7 days throughout the study. There was no death recorded. An autopsy done after the study showed no gross changes in any organs. In terms of biochemical parameters, alkaline phosphatase (ALP) levels in CC500 and CC2000 groups were increased ($p<0.05$) after treatment as compared with pre-treatment. It was also significantly higher in both groups compared with the control group post treatment (Figure 1). Alanine transaminase (ALT) levels in CC500 and CC2000 groups were increased ($p<0.05$) after 7 days. However, no significant differences were observed between different groups (Figure 2). The rise in liver enzymes after treatment suggests that *Cosmos caudatus* may have given rise to acute liver inflammation at high doses. Albumin level in group CC500 was increased ($p<0.05$) after treatment. However, it was reduced ($p<0.05$) after treatment in CC2000 group compared with all the other groups (Figure 3). The reduction of serum albumin levels in CC2000 group after treatment may also indicate liver impairment at high doses.

To date, no data are available on the effects of CC on liver enzymes. However, there is one study which reported that extracts of green tea, which contain polyphenols such as catechins, caused an increase in liver enzymes such as ALP, ALT and AST at high dose (Takami et al. 2008). *Cosmos caudatus* also contains polyphenols such as flavonoids and at high dose may cause similar effects on the liver enzymes. On contrary, other studies have found that compounds found in plants such as phenolic (Shin et al. 2010), lutein (Sindhu et al. 2010) and flavanones (Pari & Gnanasoundari 2006) exert hepatoprotective effects. Similar compounds can be found in CC (Faridah 2005; Mustafa et al. 2010) and may be potentially hepatoprotective. Nevertheless, the hepatotoxicity observed in this study may be due to the dose-dependent effect of one of the compounds as mentioned above. Further studies are needed to elucidate this.



*-denotes significant difference between before and after treatment within the same group (p<0.05)
Groups which share the common alphabet indicate significant difference (p<0.05)

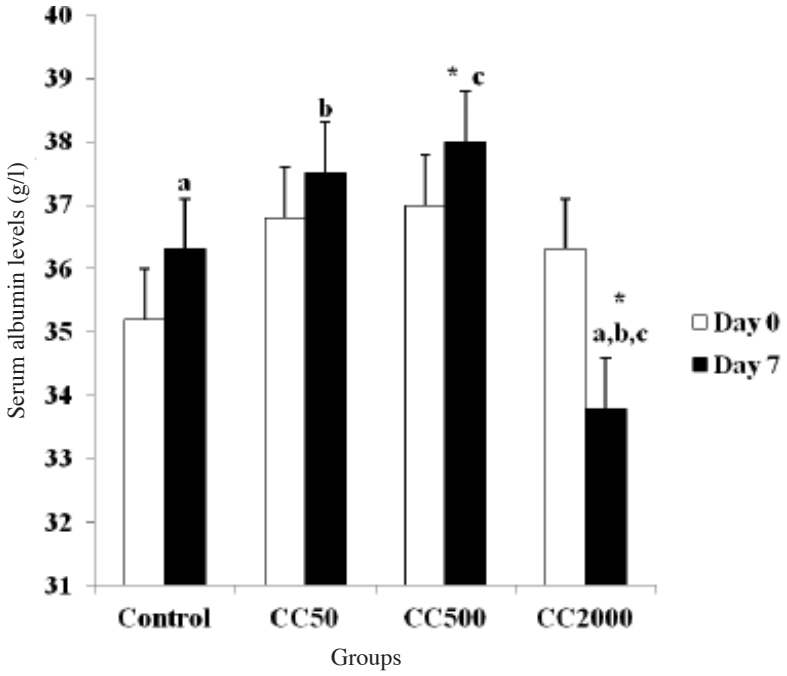
FIGURE 1. The effects of different doses of *Cosmos caudatus* on serum alkaline phosphatase (ALP) levels in acute toxicity study



*-denotes significant difference between before and after treatment within the same group (p<0.05)

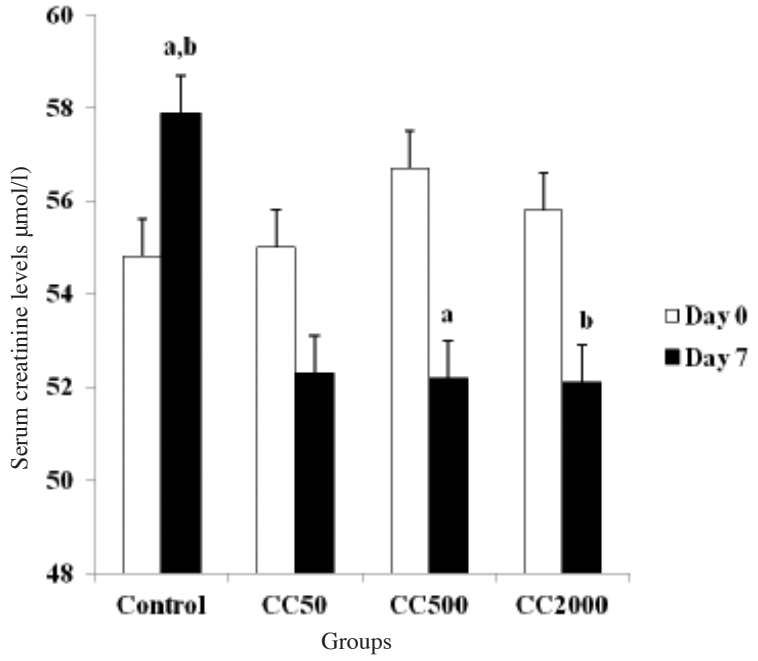
FIGURE 2. The effects of different doses of *Cosmos caudatus* on serum alanin transaminase (ALT) levels in acute toxicity study

For creatinine levels, no significant difference was seen before and after treatment in all groups. However, the levels were significantly lower (p<0.05) in CC500 and CC2000 groups compared with control group post treatment (Figure 4). This suggests that *Cosmos caudatus* may have improved the renal function which may be due to the antioxidant properties of its compounds. Others have shown that plants containing phenolic, flavonoid



*-denotes significant difference between before and after treatment within the same group (p<0.05)
Groups which share the common alphabet indicate significant difference (p<0.05)

FIGURE 3. The effects of different doses of *Cosmos caudatus* on serum albumin levels in acute toxicity study



Groups which share the common alphabet indicate significant difference (p<0.05)

FIGURE 4. The effects of different doses of *Cosmos caudatus* on serum creatinine levels in acute toxicity study

(Jain & Singhai 2010) and lutein (Preethi & Kuthan 2009) exert nephroprotective effects. *Cosmos caudatus* contains similar compounds and may possess similar antioxidant attributes.

CONCLUSION

Lower doses of *Cosmos caudatus* is safe to be consumed. Although *Cosmos caudatus* may improve renal function but it may cause acute hepatotoxicity at higher doses.

Further study on the active compounds of *Cosmos caudatus* is recommended to determine the substance responsible for the effects.

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