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THE EFFECT OF *Cosmos caudatus* ON PLASMA CORTICOSTERONE LEVEL FOLLOWING A CHRONIC REPETITIVE FORCED SWIMMING STRESS IN RATS

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ABSTRACT

This study examines the effect of *Cosmos caudatus* on plasma corticosterone levels following a chronic repetitive forced swimming stress (FSS). Male Sprague-Dawley strains of rats weighing 90–120 g were used. The first group was the control, administered only with saline solution. Second and third groups received quercetin (100mg/kg and 200mg/kg) respectively, while the fourth and fifth groups were treated with *C. caudatus*'s extract (100mg/kg and 200mg/kg) respectively. The rats were subjected to FSS for 15 minutes daily. The tests revealed a significant reduction in plasma corticosterone level in all treated groups (except the second group) compared to the control. At the end of the experiment, the third, fourth and fifth groups had a reduction in plasma corticosterone level which were 153.950 nmol/l ± 2.98, 145.262 nmol/l ± 2.04, 134.488 nmol/l ± 2.70, respectively. The group treated with 200mg/kg of *C. caudatus* was most significant in reducing plasma corticosterone ($p < 0.005$). The present study suggests the ability of *C. caudatus* to reduce the plasma corticosterone level.

Key words: Antioxidant, *Cosmos caudatus,* Chronic Stress, Corticosterone, Quercetin

INTRODUCTION

Malaysia has over 15,000 species of higher plants and about 1200 of these species have been reported to have potential pharmaceutical value of which some were being used as herbal medicine (Soepadmo, 1991). Nowadays, research and study about antioxidant have been growing like mushroom. Several types of spices contained high antioxidant activities which could prevent and control some of chronic disease such as cardiovascular disease and cancer (Mackeen *et al.,* 2000). According to Michael *et al.* (1995), cardiovascular disease developed following mental and physical stress. The positive effects of antioxidant on stress have been established (Michael *et al*., 1995).

Since some natural source of food have antioxidant properties, they could be utilized as anti-stress element and could therefore serve as treatment and prevention strategy for physical as well as psychological disorders. For this reason, the diseases preventing potential of natural food has become an area of scientific interest. Realizing the important of various natural products with natural anti-stress capability through antioxidant activity, the bioactivities of the plant, *C. caudatus* will therefore be examined. *C. caudatus* reside in the family asteraceae, an edible plant that occupy 20-26 species worldwide and locally known as Ulam Raja (King's salad) in Malaysia (Md Rasdi *et al*., 2010). It flowers from June to November (Guanghou *et al*., 2005). It is located world widely in tropical regions including Mexico, United States (Arizona and Florida), Central America, South America, Malaysia and Thailand (Samy *et al*., 2005). Md Rasdi *et al.* (2010) defined *Ulam*, a Malay word which describe a component which constitute food, medicine and beauty. It is widely popular Malay herbal salad.

Traditionally in Malaysia, the plant is utilized for the improvement of blood circulation, as an anti-aging agent, antipyretic, fortifying bone marrow (high calcium content), promoting fresh breath and treating infection associated with pathogenic microorganisms (Hassan 2006; Bodeker *et al*.,

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2009). Through xanthine-xanthine oxidase enzymatic assay, *C. caudatus* exhibit moderate antioxidant activity (Norhanom *et al*., 1999). Antioxidant activities of *C. caudatus* were shown by Faridah *et al.* (2003, 2006) by measuring the antioxidative and radical scavenging activities of the compound isolated from the plant. Antimicrobial studies of *C. caudatus* have demonstrated the varying degree of antimicrobial activities (Md Rasdi *et al*., 2010). Many preceding studies on *C. caudatus* revolved around the physical stress (Hassan 2006; Bodeker G. *et al*., 2009; Norhanom *et al*., 1999; Faridah *et al*., 2006; Faridah *et al*., 2003), while the effects on psychological stress have not yet been examined. With the reports of negative aspects of psychotherapeutic drug treatment already documented (Lehman, 1979), a potent anti-stress compound which exhibit the psychotherapeutic effects with minimal side effect should be progressively studied. This study was therefore aimed at elucidating the effect of *C. caudatus* on plasma corticosterone level following a chronic repetitive forced swimming stress in rats.

MATERIALS AND METHODS

Chemicals and raw materials

All chemicals used were of analytical grade and purchased from Sigma-Aldrich (M) Sdn Bhd. Cayman's Corticosterone EIA kits were purchased from i-DNA Biotechnology (M) Sdn Bhd. The samples of *C. caudatus* were cultivated at a local farm in Perak. The plants were deposited at the Faculty of Applied Sciences' Universiti Teknologi MARA, UiTM Herbarium and a sample number was taken.

Extraction

The samples were allowed to dry at room temperature and grinded into powder. The extraction used standard Soxhlet method, carried out using 150mL of methanol. The heating power was set to two cycles per hour so that six cycles of extraction were achieved within 3 hours of extraction time. The crude extract solutions obtained are concentrated and dried using vacuum rotary evaporator at temperature 80ºC or less to remove the solvents. Higher temperatures were avoided to minimize component degradation. All extracts were placed in a room temperature condition before weighing gravimetrically to determine the yields (Matsurah *et al*., 2007).

Animals

Male Sprague-Dawley strain of rats weighing 90–120g was used. Animals were housed in individual cages, in natural light cycle. Subjects were maintained on an *ad libitum* food and water regimen. They were habituated to human handling for 14 days by stroking and holding the rats gently for 5 minutes every day. The animals were randomly divided into 5 groups, containing 10 rats per group. The first group was the control, administered only with saline solution. Second and third groups were treated with quercetin (100mg/kg and 200mg/kg) respectively, while the fourth and fifth groups were treated with *C. caudatus*'s extract (100mg/kg and 200mg/kg) respectively. The quercetin and *C. caudatus*'s extract were diluted in saline solution and administered using oral gavage. The animals were treated for 21 days prior to and throughout the experiment at 5 pm.

Forced swimming stress (FSS)

Using a modified version of Porsolt-Behaviour Despair Test (Parsolt *et al*., 1977), the animals were individually placed in a glass cylinder (60 cm in height, 15 cm in diameter) filled with 45 cm high of water (25±2ºC). The water depth was adjusted so that the animals must swim or float without their hind limbs or tail touching to the bottom. All animals were then forced to swim for 15 minutes. FSS was repeated daily for 14 days at 2 pm.

Blood withdrawal

The blood samples were withdrawn using the orbital sinus bleeding at 12 o'clock in the afternoon for Basal level (D0) and after 1st (D1), 4th (D4), 7th (D7), 10th (D10), 13th (D13) exposures, proceeding a minimum of 18 hours of rest following FSS (Fig. 1). The procedures were carried out in the afternoon; purely to measure the plasma corticosterone level in resting period of nocturnal rodents which peaks in the evening (Jessica *et al*., 2008). The blood samples collected were not exceeding 1.5ml per rat. The blood collected then proceeded to centrifugation and the plasmas were isolated and kept frozen at -80ºC.

Plasma Corticosterone level

Plasmas were assayed for corticosterone using Cayman's EIA Kits and the results are expressed as nmol/l.

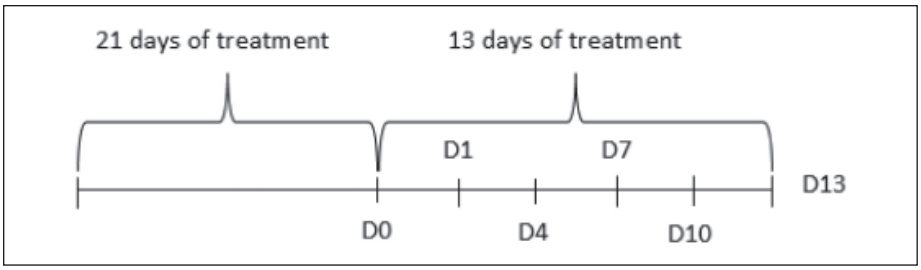

Fig. 1. Timeline of *C. caudatus*'s extract quercetin and saline solution administration to animal model for 21 days prior and 13 days throughout FSS.

Data Analysis

Data were analyzed by Statistical Package for Social Sciences (SPSS) program. Differences within and between groups were compared via multiple Comparison, using Anova and Post Hoc Test, Least Significant Difference and t-test. All Data were expressed as mean ± standard error of mean (SEM).

RESULTS AND DISCUSSION

Plasma corticosterone level

The basal level for plasma corticosterone in the control group after 21 days of saline administration was 155.463 ± 2.46 nmol/l. For the group treated with 100mg/kg of quercetin, plasma corticosterone measured was 144.812 ± 1.67 nmol/l, whilst the group administered with 200mg/kg of quercetin was 144.725 ± 1.79 nmol/l. The group treated with 100mg/kg and 200mg/kg, the plasma corticosterone level measured at 144.788 ± 2.54 and 146.212 ± 2.28 nmol/l respectively. There was no significant difference between all 5 groups of the animal in the beginning of the experiment.

Following the FSS, all animal groups showed a significant increment in plasma corticosterone level (Fig. 2). The plasma corticosterone level remained higher than the basal state until the 7th (D7) exposure to FSS. All groups showed significant reduction in plasma corticosterone level at the 7th (D7) exposure. Gradually to 10th (D10) and 13th (D13) exposures to FSS, groups treated with 200mg/kg of *C. caudatus* and 100mg/kg of quercetin returned to basal level and proceeded to be lower than the basal level at the end of the experiment. The pattern coherently followed the reduction of plasma corticosterone level of the control group.

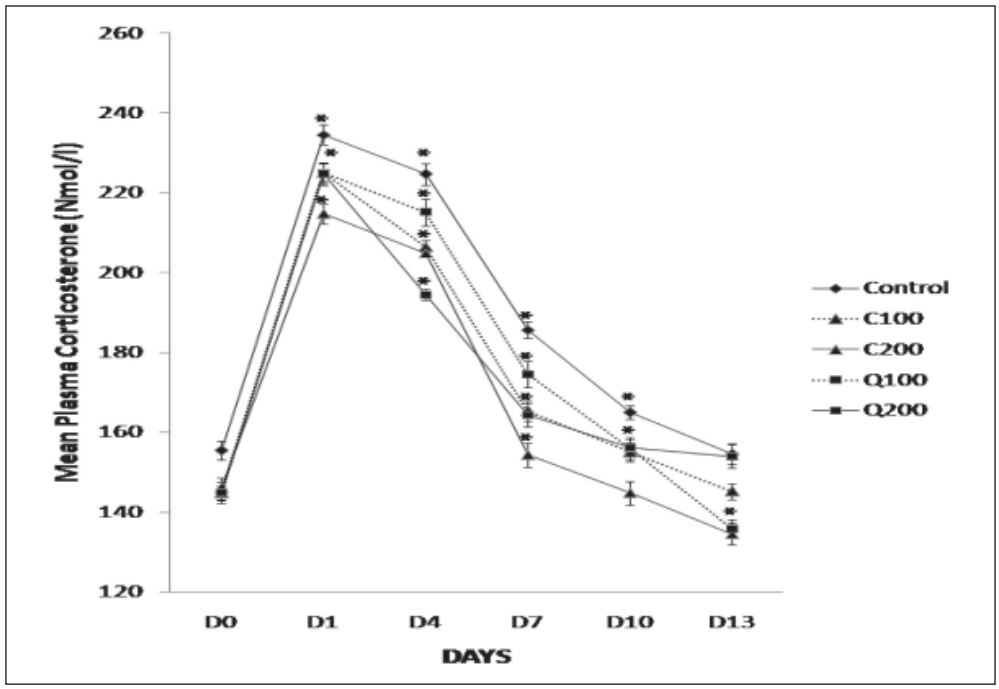

Fig. 2. Mean plasma corticosterone level at basal state and following FSS. Data were expressed as mean ± SEM. Significance compared to basal values (*p<0.005). C100 is *C. caudatus* 100 mg/kg; C200 is *C. caudatus* 200 mg/kg; Q100 is quercetin 100 mg/kg and Q200 is quercetin 200 mg/kg. Note: D0 = reading for basal level was taken.

The group treated with 100mg/kg of *C. caudatus* however did not past the basal level, whilst the group treated with 200mg/kg quercetin remained slightly elevated at the end of the study. The group treated with 200mg/kg of *C. Caudatus* manifested the lowest level of plasma corticosterone after the 1st (D1) exposure to FSS (214.662 ± 2.44 nmol/l) and lowered its level significantly close to the basal state during the 7th (D7) exposure (154.325 ± 3.03 nmol/l) and completely returned to basal level during the 10th (D10) exposure to FSS (144.737 ± 2.90 nmol/l).

This study emphasized the mechanism of adaptation towards stress. Plasma corticosterone level significantly increased after an exposure to stressors and remained elevated for the next 6 exposures. Towards the 7th (D7) exposure to FSS, plasma corticosterone level reduced significantly, indicating that the animal had become used to, or adapted to the stress (Ainsah *et al*., 1999). Congruently to the study done by Ruzymah and colleagues,in 1995, similar response proceeding repetitive stresses induced by light ether anesthesia which manifested the adaptation between the 4th (D4) to 5th (D5) exposures (Ruzymah *et al*., 1995). The elevation of corticosterone level is an established physiological response towards psychological stress, which is mediated via increased secretion of corticotrophin releasing factor (CRF) by the hypothalamus and adreno-corticotrophin (ACTH) and endorphins by the pituitary (Lim *et al*., 1983).

C. caudatus for the purpose of this study contains quercetin and its three glycosides, quercetin 3 O-β-D-arabinofuranoside, quercetin 3-O-α-D-rhamnoside, and quercetin 3 -O-β-D glucoside. The four compounds gave positive cyanidin test, a general reagent for identification of flavonoids (Faridah *et al*., 2003). Pretreatment with quercetin antagonized CRF induced anxiogenic – and depressant - like behaviour indicated that quercetin negatively modulated CRF-induced behavioural effects (Bhutada *et al*., 2010; Kawabata *et al*., 2010). It was reported that quercetin produced behavioural effects through modulation of neurotransmitter systems like GABA, nitric oxide and serotonin (Filho *et al*., 2008), which also implicated in anxiety and depression. The ability of quercetin at higher dosage (200 mg/kg) to reduce plasma corticosterone level at the end of the experiment (D13) seems to be insignificant, may be due to the development of tolerance with repeated administration of 200 mg/kg quercetin (Ainsah *et al*., 1999). Our findings are important because stress have been implicated as major factor in development of many physical as well as psychological disorders.

Stress activates the hypothalamic-pituitary-adrenal (HPA) axis and thus increases the release of glucocorticoids hormone and regulated with the negative feedback mechanisms (Ainsah *et al*., 1999)]. However the mechanism of action of *C. caudatus* on its ability to reduce plasma corticosterone is still unknown. It is worthwhile in future study to include a group of adrenalectomized rats to enable us to understand the mechanism of *C. caudatus*'s action. Nevertheless, the utilization of *C. caudatus* as an anti-stress must be used with caution as one study had demonstrated that *C. caudatus* at high dosage (2000 mg/kg) could lead to acute toxicity (Norazlina *et al*., 2013). Our study however only used low dosage (100 mg/kg and 200 mg/kg) and both have shown positive results.

CONCLUSIONS

In conclusion, the results from this study demonstrated that *C. caudatus* could probably in future serve as anti-stress or anti-anxiety agents through the mechanism of corticosterone action on CRF and ACTH regulation. The mediation effect may act through direct and indirect antagonism of CRF receptors. Nevertheless further comprehensive study involving various doses of *C. caudatus* and a longer duration of treatment are needed to consolidate the findings convincingly.

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