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PharmTech

The effect of Ulam Raja (*Cosmos caudatus*) on drug-metabolizing enzymes, lipid peroxidation and antioxidant status in mice liver.

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Abstract: Recent studies indicated that *C. caudatus*, a herbal plant, possesses strong antioxidant and free radical scavenging activities. It might also have potential tumour-inhibitory effect. This study was conducted to investigate the effects of *C. caudatus* on drug-metabolizing enzymes (DME), antioxidant status and lipid peroxidation in mice liver. The extent of liver injury was assessed by measuring lactate dehydrogenase (LDH) activity. This study involved 30 adult ICR male mice (25-35 grams) that were divided into 5 groups. Aqueous extract of *C. caudatus* (CC) were administered orally to mice for 21 days in three different doses [100, 500 and 1000 mg/kg body weight respectively]. Positive control mice were given diet containing 0.5% butylated hydroxyanisole (BHA). After 21 days, the mice were sacrificed and their livers harvested. The results showed that NADPH-cytochrome P450 reductase activity was significantly increased only in mice treated with 500 mg/kg CC compared to the control group. DT-diaphorase (DTD) activity was significantly increased only in mice treated with 1000 mg/kg CC. Superoxide dismutase (SOD) activity was significantly increased only in mice treated with 500 mg/kg CC. Catalase (CAT) activity was significantly increased in mice treated with 1000 mg/kg CC. MDA level (which indicated the extent of lipid peroxidation) was significantly reduced in all CC-treated groups. Lactate dehydrogenase (LDH) level (which indicated the extent of liver injury) was significantly reduced in all CC-treated groups. In conclusion**,** *Cosmos caudatus* supplementation might be able to protect mice livers against damage caused by oxidative stress mainly through inhibition of the lipid peroxidation process and LDH activity, as well as through some modulation of the activities of certain antioxidant enzymes.
Key Words: Cosmos caudatus, liver, drug-metabolizing enzymes, antioxidant status, lipid peroxidation.

Introduction

Plant drugs, known as herbal remedies, have often been one of the essential sources of medication. It have been traditionally used in the treatment of a variety of illnesses, popularly 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 (44).

Cosmos caudatus (CC) is an appetizer (ulam) consumed mainly by the Malay community in Malaysia. It is known as "Ulam Raja". Recent studies have shown that *Cosmos caudatus* extract possesses strong antioxidant and free radical scavenging activities (37), (7). Another study showed that it might have potential tumour-inhibitory effect (10). In addition, (46) showed that it has the potential to be used in treating free radicals-associated diseases since it is found to possess high antioxidant capacity.

C.caudatus 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 (46). It is found worldwide especially in tropical areas including Mexico, United States (Arizona and Florida), Central America, South America, Malaysia and Thailand (45). 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 (3).

It also exhibits antimutagenic as well as antifungal properties (42). In addition, Vimala et al. (56) 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 more than 2500 mg L-ascorbic acid equivalent antioxidant capacity (AEAC) per 100 g of fresh sample (46).

Cosmos caudatus has also been found to contain phenolics, flavonoids, flavones and flavanones and has also been shown to possess strong antioxidant activity (36). Therefore, it is postulated that the antioxidant potential is great and it may prevent oxidative damage to body tissues and cells by virtue of its capacity to scavenge free radicals. In this study, we aimed to observe the effects of *Cosmos caudatus* supplementation on drug-metabolizing enzymes activities, lipid peroxidation and antioxidant status in mice liver.

Materials and Methods

Chemicals

Butylated hydroxyanisole, 1-chloro-2,4-dinitrobenzene, 5,5'-dithiobis-2-nitrobenzoic acid, reduced glutathione, oxidized glutathione, pyrogallol, 2,6-dichlorophenol-indophenol, potassium ferricyanide, ethylene diamine tetra-acetic acid (EDTA), bovine serum albumin (BSA), sodium pyruvate, thiobarbituric acid, reduced nitcotinamide adenine dinucleotide (NADH) and reduced nitcotinamide adenine dinucleotide phosphate (NADPH) were obtained from Sigma Chemical Co. (USA).

Experimental Animals and Treatment

Thirty adult ICR male mice aged 8-10 weeks old (25-35g) were obtained from Laboratory Animal Research Unit (LARU), Universiti Kebangsaan Malaysia. They were randomly divided into 5 groups and experimented for 21 days.

1. Group I (n=6): Normal control group. Normal diet and sham-treated with distilled water through oral gavage

2. Group II (n=6): Normal diet and treated with 100mg/kg body-weight of the plant extract (CC100)

3. Group III (n=6): Normal diet and treated with 500mg/kg body-weight of the plant extract (CC500)

4. Group IV (n=6): Normal diet and treated with 1000mg/kg body-weight of the plant extract (CC1000)

5. Group V (n=6): A positive control group. Diet containing 0.5% 'butylated hydroxyanisole' (BHA)

Standardized aqueous extract (CC) were administered via oral gavages to mice for 21 days duration of the study and positive control group were fed with diet prepared with 0.5% BHA. The mice were housed singly in plastic cages at room temperature with a 12-hour light-dark cycle. They were fed with commercial rat chow (Gold Coin, Selangor, Malaysia) and tap water *ad libitum*. Ethical approval was obtained from the Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC) (PP/FAR/2011/AZMAN/27-JANUARY/347-FEBRUARY-2011-FEBRUARY-2013).

Materials and *Cosmos caudatus* extract

Cosmos caudatus aqueous extract was obtained from Forest Research Institute Malaysia (FRIM), (Kepong, Selangor Darul Ehsan, Malaysia). The extract was crude in nature and of brownish powder form. CC aqueous powder was dissolved in deionized water and given via oral gavages at doses of 100, 500, and 1000 mg/kg mouse weighted every day for 21 days.

Materials needed for preparing the diet for positive control, were the standardized mice pellet, 'butylated hydroxyanisole' (BHA) (Sigma Chemicals, USA) and distilled water. About 1 kg mice pellet were crushed then, only 5 g 'butylated hydroxyanisole' (BHA) were added to the crushed mice pellet. Distilled water was added too until it was mixed well. It was then doughed into small finger-like shape. The fingers were dried up for few days before it was served.

At the end of the treatment, the mice were euthanized by cervical dislocation. Livers were then harvested.

Tissue preparation

Animals will be sacrified by cervical dislocation and the entire liver is perfused immediately with ice cold 0.9% NaCl. Liver carefully removed, trimmed for free from extraneous tissue and rinsed in chilled phosphate buffer (pH7.4). Liver is then blotted dry, weighed quickly & homogenized in ice cold phosphate buffer (pH7.4) in order to produce a 10% (w/v) homogenate. An aliquot of this homogenate (0.5ml) will be used for assaying GSH while the remainder will be centrifuged at 10, 000 rpm for 20 min. The resultant supernatant is transferred into pre-cooled ultracentrifugation tubes & centrifuged at 105, 000 g for 60 min. The supernatant (cytosol fraction), after discarding any floating lipid layer & appropriate dilution, will be used for the assaying glutathione-S-transferase, DT-Diaphorase, lactate dehydrogenase & antioxidant enzymes. The pellet representing microsomal fraction, will be suspended in homogenizing buffer & used for assaying cytochrome P450, cytochrome b5, cytochrome P450 & cytochrome b5 reductases and lipid peroxidation.

Biochemical studies

Estimation of protein

Protein was determined by the method of Lowry et al. (27) using BSA as standard, at 700 nm.

Estimation of cytochrome P450 and cytochrome b5

Cytochrome P450 and cytochrome b5 was determined by using the carbon monoxide difference spectra. Both enzymes contents were estimated in the microsomal suspension by method of Omura & Sato (38).

Detrmination of NADPH-cytochrome P450 reductase and NADH-cytochrome b5 reductase

Assay of NADPH-cytochrome P450 reductase was performed according to the method of Omura & Takesue (39) with some modifications, measuring the rate of oxidation of NADPH at 340 nm. NADH-cytochrome b5 reductase was assayed according to the method of Mihara & Sato (32), measuring the rate of reduction of potassium ferricyanide at 420 nm by NADH.

Determination of activity of glutathione S-transferase and DT-diaphorase

The cytosolic glutathione S-transferase activity was determined according to the procedure of Habig et al. (18). DT-diaphorase activity was measured as described by Ernster et al. (11).

Estimation of GSH

The level of acid-soluble sulphydryl group which reflected GSH level was estimated as total non-protein sulphydryl group by method described by Moron et al. (34). Reduced glutathione was used as a standard to calculate nmol of –SH content per gram of tissue.

Determination of activity of glutathione reductase and glutathione peroxidase

Glutathione reductase was determined procedure of Carlberg & Mannervik (5). Glutathione peroxidase activity was measured by the coupled assay method as described by Lawrence & Burk (24).

Determination of activity of catalase and superoxide dismutase

Catalase was estimated at 240 nm by monitoring the decomposition of H_2O_2 as described by Aebi (1). Superoxide dismutase was assayed by method of Marklund & Marklund (29).

Estimation of lipid peroxidation

Lipid peroxidation in microsomes was estimated by the thiobarbituric acid-reactive substances (TBARS) method, as described by Ledwozyw et al. (25) and is expressed as malondialdehyde formed per mg protein.

Determination of lactate dehydrogenase

Lactate dehydrogenase was assayed by measuring the rate of oxidation of NADH, by method of Wahlefeld (57).

Statistical Analysis

Data will be expressed as mean $\pm$ SEM. All values will be analyzed for non-normality using Shapiro-Wilk test. Data will also be analyzed using one-way analysis of variance (ANOVA). Student's unpaired t-test for normal distributed values whilst Mann-Whitney test for non-normal distributed values. The statistical analyses will be performed using the SPSS statistical software package, version 19 and statistical significance will be accepted at $P < 0.05$.

Results

Body weight

Mice treated with the aqueous extract of *C.caudatus* and BHA showed a significant increased in body-weight as compared with the control group of mice. (Figure 1)

Phase-I enzymes (Cytochrome P450 system)

Different components of the cyrochrome P450 system were determined in the microsomal fraction of mouse liver. Haem proteins, cytochrome P450 and cytochrome b5, did not show any significant alterations as compared with their control values.

For NADPH-cytochrome P450 reductase, elevation in the activity was only in CC500 group (p<0.05) (**Figure 2).** No significant changes of NADH-cytochrome b5 reductase as compared with their controls. BHA induced only NADPH-cytochrome P450 reductase (p<0.05).

Phase-II enzymes

In phase-II enzymes. glutathione S-transferase and DT-diaphorase were assayed in the cytosol of liver. Only DT-diaphorase showed a significant increase (p<0.05) in the activities in *Cosmos caudatus*-treated groups of mice and it was only in CC1000 group (Figure 3). BHA significantly (p<0.05) enhanced the activities of both glutathione S-transferase and DT-diaphorase.

Antioxidant parameters

Antioxidative parameters such as reduced glutathione and glutathione reductase showed a significant decrease in their level/activity following *C.caudatus* treatment. The level of reduced glutathione was significantly reduced in all treatment group (p<0.05) (Figure 4). Glutathione reductase level was decreased in CC100 group and CC500 group (p<0.05) (Figure 5). Glutathione peroxidase did not showed any significant alterations as compared with the control values. Superoxide dismutase and catalase revealed a significant increased only in CC500 group (p<0.05) (Figure 6) whereas catalase activity was only increased in CC1000 group (p<0.05) (Figure 7). BHA significantly induced (p<0.05) the activity of superoxide dismutase and catalase.

Lipid peroxidation and lactate dehydrogenase

Malondialdehyde formation was taken as an indicator of lipid peroxidation. Lipid peroxidation and specific activity of lactate dehydrogenase both showed significant decrease (p<0.05) in all treatment group following the administration of *Cosmos caudatus* (Figure 8 & 9). BHA inhibited the level of lactate dehydrogenase and malondialdehyde (p<0.05).
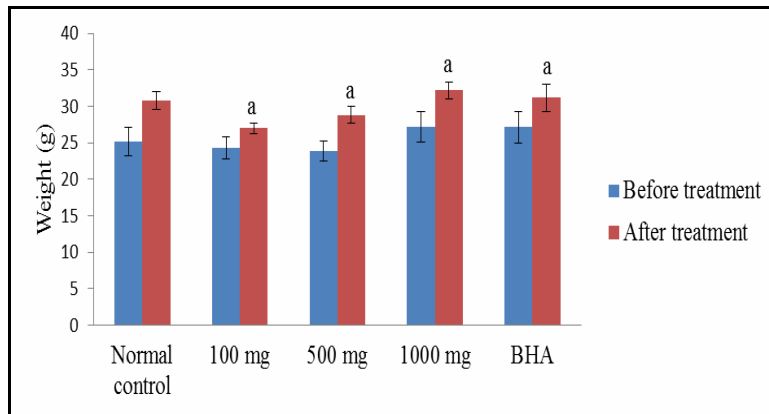

Figure 1. Comparison of mice weight (g) before & after treatment of CC. [a]*P*<0.05 compared to normal control group.
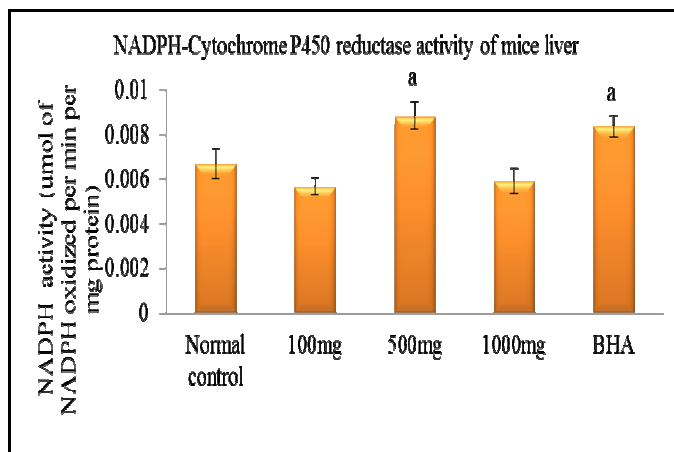
Figure 2. Effect of 21 days treatment of CC on drug-metabolizing enzymes.
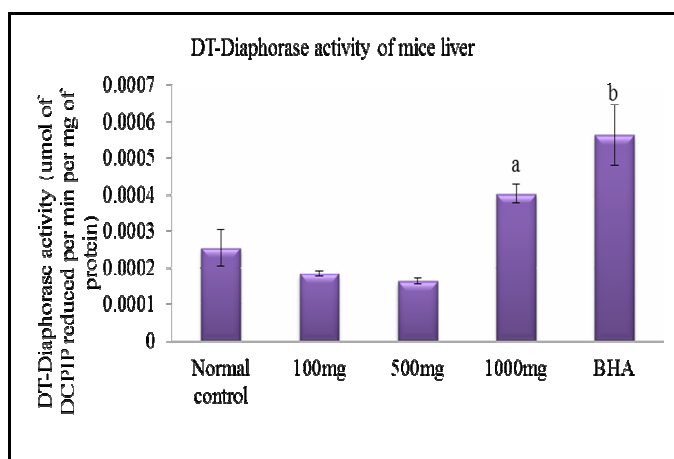[a]*P*<0.05 compared to normal control group.

Figure 3. Effect of 21 days treatment of CC on drug-metabolizing enzymes.
[a]*P*<0.05, [b]*P*<0.01 compared to normal control group.

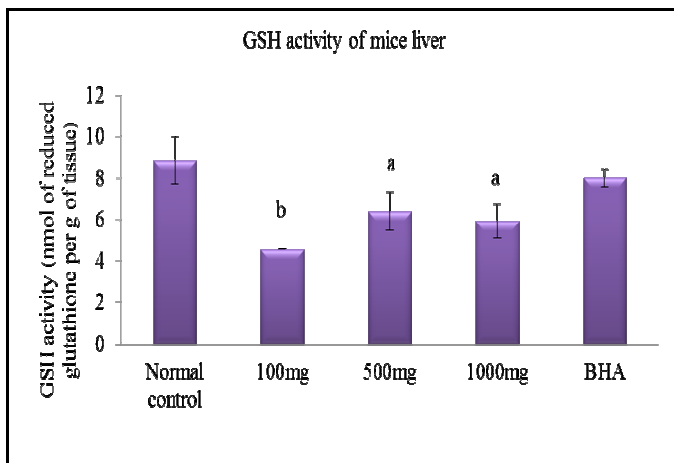**Figure 4.** Effect of 21 days treatment of CC on antioxidant status. [a]$P<0.05$, [b]$P<0.01$ compared to normal control group.

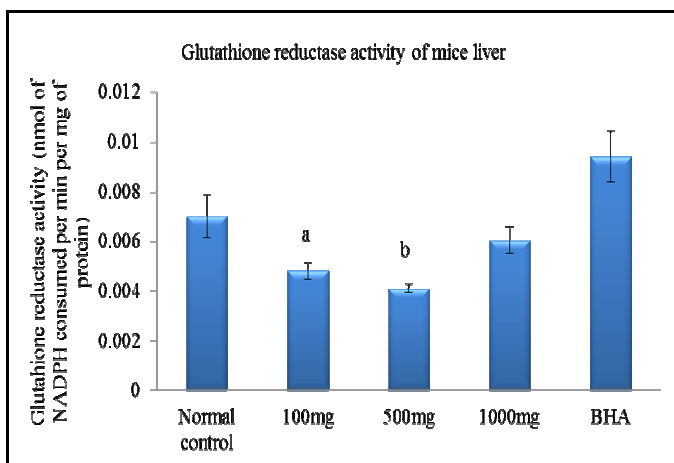**Figure 5.** Effect of 21 days treatment of CC on antioxidant enzyme. [a]$P<0.05$, [b]$P<0.01$ compared to normal control group.

Figure 6. Effect of 21 days treatment of CC on antioxidant enzymes. [a]$P<0.05$, compared to normal control group.
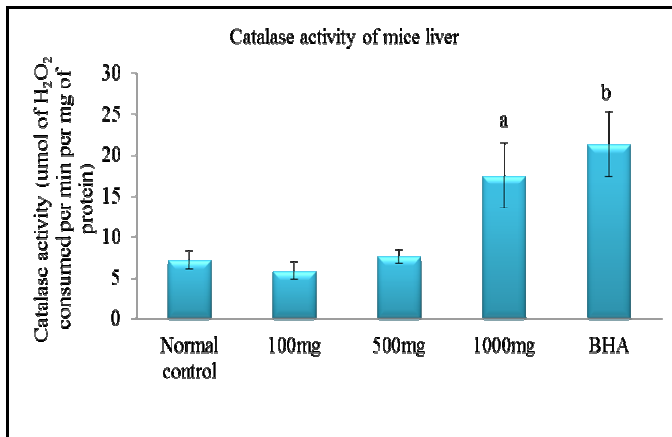
Figure 7. Effect of 21 days treatment of CC on antioxidant enzyme. [a]$P<0.05$, [b]$P<0.01$ compared to normal control group.

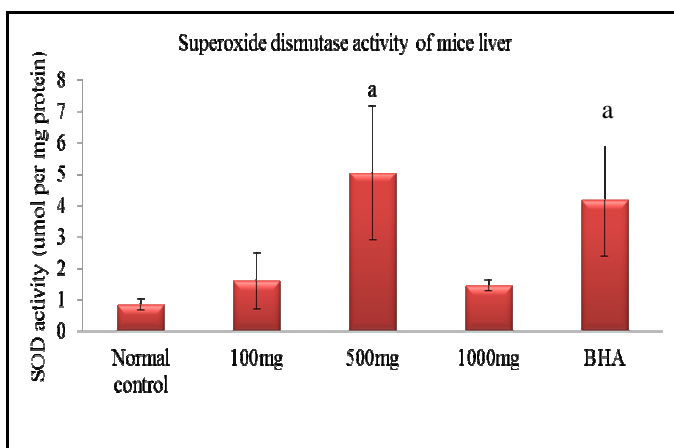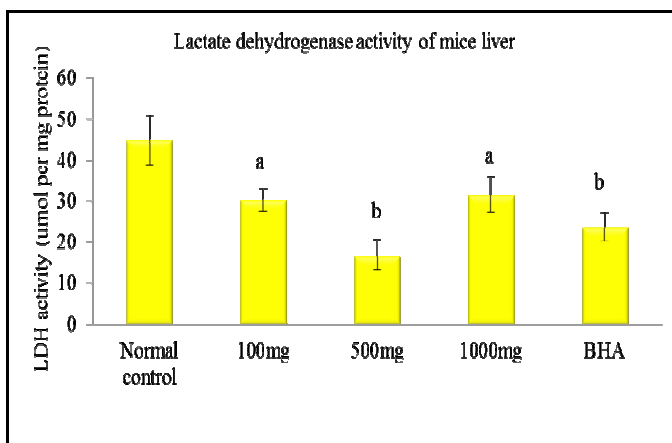**Figure 8.** Effect of 21 days treatment of CC on LDH activity. [a]$P<0.05$, [b]$P<0.01$ compared to normal control group.
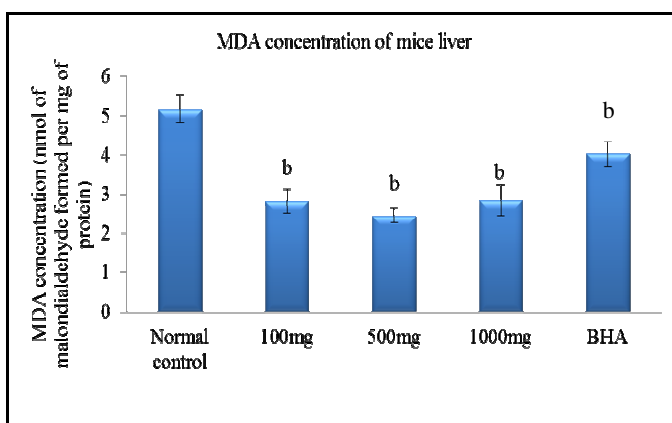

Figure 9. Effect of 21 days treatment of CC on MDA concentration. [b]$P<0.01$ compared to normal control group.

Discussion

Cancer chemoprevention is an exciting area of pharmaceutical cancer research involving the use of either natural or synthetic components to delay, inhibit or reverse the development of cancer in normal or preneoplastic conditions (52), (35), (40). The findings of this study are based on the examination of the inducibility of enzymes involved in the metabolism of xenobiotics (including carcinogens) and drugs, as this is one of the reliable markers for evaluating the chemopreventive potential of the test materials in a murine model. BHA was used as a positive control to validate the authenticity of assay protocols since it has been proved to be a chemoprotectant in diverse animal models of site-specific carcinogenicity (41), (20).

From the studies done, there were no adverse effects on the animals at the given dose levels of *Cosmos caudatus* extract (100, 500 and 1000 mg of the extract per kg body-weight of mice, for 21 days). It caused neither increase in the rate of mortality nor decrease in the body-weight of animals. At a cellular level, the liver was no indication of damage as observed by the measurement of LDH activity. Its activity was significantly reduced from the control value showing the possibility of protection against damage caused during normal metabolic process. Even the higher dose (1000 mgkg^{-1}) used had a safety margin sufficiently distant from the toxic range.

Phase I enzymes i.e. the microsomal cytochrome P450 detoxification system (consisting of cytochrome P450 isoenzymes, cytochrome b5, NADPH-cytochrome P450 reductase and NADH-cytochrome b5 reductase), which is abundant in the liver, constitutes a major electron transport chain in the membrane of endoplasmic reticulum. It plays a key role in oxidative activation, inactivation and promotion of excretion of most xenobiotics compounds and also in modulating the duration and intensity of their toxicity (15), (33). Several studies have shown that herbal extracts were able to increase the levels of all the measured components of cytochrome P450 system (cytochrome P450 isoenzymes, cytochrome b5, NADPH-cytochrome P450 reductase and NADH- cytochrome b5 reductase) (47-49).

During oxidative metabolism, in the microsomal microenvironment involving the cytochrome P450 system, the electron flows from NADPH or NADH through a flavoprotein cytochrome P450 reductase or cytochrome b5 reductase to different isomorphic forms of cytochrome P450 and cytochrome b5 (14). Some herbal extracts (47-49) by virtue of its action as inducer of cytochrome P450 may be speculative of acting as a cancer blocking agent. It may be referred in this context that a chemical found in cruciferous vegetables, indole-3-carbinol, is a potent inducer of cytochrome P450 enzyme and has chemopreventive activity in a number of animal models (54). For phase I enzyme, only NADPH-cytochrome P450 reductase resulted in elevation of the activity was only in CC500 group (p<0.05). It may be postulated that, the enzyme can be induced at CC500 and not at other doses. May be at higher dose (CC1000) it may inhibit the system and at lower dose (CC100) it is too low to stimulate the NADPH-cytochrome P450 reductase system. Further experiments may be carried out to understand this phenomena. No significant changes of cytochrome P450, cytochrome b5 and NADH-cytochrome b5 reductase as compared with their controls. No significant changes in the above mentioned enzymes may be due to the inhibition effect on the active site of the enzymes. The extract might act as nucleophile for oxidation by free hydroperoxide as a substrate (8) and thus inhibit the active site of the enzymes.

The main function of phase I metabolism is to prepare a compound for phase II metabolism and not to prepare the drug for excretion. Phase II is usually the true detoxification of drugs and xenobiotics which gives products that are generally water soluble and can be easily excreted. Glutathione S-transferase (GST) and DT-diaphorase (DTD) (phase II enzymes) are found in abundance in the liver of mice. The action of phase II enzymes on the subtrates generated by the action of phase I enzymes on various chemical compounds leads to their eventual excretion from the body (14). Glutathione S-transferase is a critical detoxification enzyme that primarily functions in conjugating 'functionalized P450 metabolites' with endogenous ligands (reduced glutathione) favouring their elimination from the body of the organisms (19). There are persuasive evidences to support the induction of glutathione S-transferase and protection against a wide spectrum of cytotoxic, mutagenic and carcinogenic chemicals (23), (43). Molecular forms of glutathione S-transferase having different substrate specificities have been identified to be present in the cytosol (28). 1-chloro, 2,4-dinitrobenzene (CDNB) is used as non-specific substrate in the assay for glutathione S-transferase. Thus, the specific activity of the enzyme measured was the sum of all of its isoforms. The protective effect of many naturally occurring chemopreventive agents against carcinogenesis have been ascribed to decrease bioavailability of potential DNA damaging entities and their destruction into excretable metabolites facilitated through induction of glutathione S-transferase (6).

From this study, there were no significant changes in GST activities. This was may be due to the absent of subtrates that being produced by the phase I enzyme system for the phase II enzyme system action that lead to their eventual excretion from the body. The reduced level of GSH may also explained the findings, as GST conjugates 'functionalized P450 metabolites' with endogenous ligands (GSH). It might be that the components from the extract and their metabolites are not the substrate for GST. Despite that, may be some of the components inside the extract inhibited the active sites of the enzyme. Eventually, no significant changes was noted.

DT-diaphorase [NAD(P)H:quinone oxidoreductase] is an important phase II enzyme and is generally induced coordinately with other phase II detoxifying enzymes (51). Induction of DT-diaphorase is a major indicator of the potency of many anticarcinogenic substances (2), (9). This enzyme protects against the toxicity of quinones and their metabolic precursors viz. polycyclic aromatic hydrocarbon, benzene, etc (9), (50). Induction of DTD facilitates bioreductive activation metabolism of quinones by two-electron oxido-reduction of quinone to hydroquinone, obliterating semiquinone radical and subsequent oxygen radical production. This is very important for maintaining homeostatic cellular environment. The stable hydroquinone resulted from two electron oxido-reduction of quinone by DT-diaphorase can be conjugated by glucuronide or sulfate and excreted and thus affords protection from reactive intermediates (9), (22), (26), (51). In this study, there is a significant elevation if DT-diaphorase enzyme activity but only at CC1000. At dose CC1000 the metabolites that were produced reached the desired concentration to stimulate DT-diaphorase enzyme. It may be postulated that, doses less than CC1000 is less potent in order to stimulate DT-diaphorase enzyme.

During the normal cell activity, various process continuously happens inside the cell to produce reactive oxygen species (ROS). Amongst the ROS are hydrogen peroxide (H_2O_2), superoxide radical anion (O_2^-) and hydroxyl radical (OH^-). When the produced ROS yielded high concentrations, it will damage the proteins, lipids and DNA. Antioxidants can prevent the situation. Cellular antioxidants consist of enzymatic forms (example superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic forms (example glutathione, 'thiol' mixture, certain vitamins and phytochemicals like isoflavone, polyphenol and flavonoids).

From the studies done, the level of glutathione reductase was reduced. It can be explained through several theories. Glutathione reductase plays a significant role in the reduction of oxidized glutathione to reduced glutathione at the expense of NADPH and regulates GSH-GSSG cycle in the cell (55). Probably the amount of GSSG is reduced inside the mice liver because the mice were normal and is not under influenced of oxidative stress. The free radicals that existed inside the mice liver is too little and glutathione reductase activity need not to increase for catalysing the reduction of GSSG to GSH. Reduced glutathione correlates well with the activity of glutathione reductase. The decreased level of reduced glutathione because glutathione reductase activity was noted to be reduced. Furthermore, it contributes in efficiently maintaining the basal level of cellular GSH (31) and the mice were normal and is not under influenced of oxidative stress and the free radicals that existed inside the mice liver is too little. The same explaination can be given for the decreased level of glutathione peroxidase. All of the mice were normal mice and they were not under influenced of oxidative stress and the free radicals that existed inside the mice liver is too little. It is also been postulated that the level of glutathione reductase and glutathione peroxidase were saturated or more than enough inside the liver of normal mice. A low concentration of H_2O_2 is brought about by glutathione peroxidase, whereas, catalase comes into play when the glutathione peroxidase pathway is reaching saturation with the substrate (13). It might require a higher dosage of the extract if the activities of those enzymes were expected to be elevated.

Superoxide dismutase (SOD) is the main antioxidants enzyme (4) that changes two superoxide molecule into oxygen and water (53). The study revealed that superoxide dismutase activity increased significantly at CC500 compared to controls. This show that the extract is proven to increase the antioxidant activity of SOD. At higher dose (CC1000) may be the dose is high enough to inhibit the enzyme activity and at lower dose (CC100) it is too low to stimulate the enzyme.

For catalase (CAT) activity, the study revealed that it increased the enzyme activity significantly at CC100 compared to controls. This show that the extract is proven to increase the antioxidant activity of catalase. Catalase helps to neutralize the toxic effects of hydrogen peroxide (H_2O_2). Catalase catalyze the changing of H_2O_2 to water and non-reactive oxygen species. It than directly prevent production of hydroxyl radical and protect cells from oxidative damage (58). No significant changes noted at lower dose may be due to the mice inside the research are the normal mice and the free radical that being produced inside the liver are in small amount.

Glutathione peroxidase (GPx) yielded no significant changes from the studies done. To protect mammalian cell from oxidative damage, glutathione peroxidase catalyze the reduction of hydrogen peroxide (H_2O_2) and organic hidroperoxide by using glutathione (GSH). (30). GSH act by donating the hydrogen. No significant changes noted may be the mice are the normal mice and the free radical that being produced inside the liver are in small amount. In addition, the substrate for glutathione peroxidase that are GSH and H_2O_2 are reduced and lead to reduction of glutathione peroxidase activity. Hence, maybe there are reduction in the expression of the related gene that coded for the production of the enzyme.

For glutathione reductase (GR), there were significant reduction at CC100 and CC500 compared to controls. Glutathione reductase, catalyze reduction of oxidized glutathione (GSSG) by depending on NADPH to maintain GSH concentration inside the cell (17). The existence of the significant reduction may be due to the smaller amount of GSSG inside the mice liver as they were normal mice and they were free from oxidative stress. As a result, the free radical that being produced is so little that the glutathione reductase activity need not to increase in order to catalyze the reduction of GSSG to GSH. Last but not least, maybe there was a 'down regulation' towards the enzyme activities.

Lipid peroxidation is refers to the oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lie methylene bridges ($-CH_2-$) that possess especially reactive hydrogens. As with any radical reaction, the reaction consists of three major steps: initiation, propagation, and termination (16). Malondialdehyde (MDA) is one of a good indicator towards lipid peroxidation and tissue injury due to oxidative stress (21).

From the studies carried out, the level of MDA for all treated group reduced significantly compared to controls. Hence, the extract has the potential to reduce oxidative stress by decreasing the lipid peroxidation.

Lactate dehydrogenase (LDH) catalyses the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD^+. It converts pyruvate, the final product of glycolysis to lactate when oxygen is absent or in short supply, and it performs the reverse reaction during the cori cycle in the liver. The presence of increased amounts of cytoplasmic cellular enzymes like LDH in the extracellular space is of benefit because they serve as indicators suggestive of disturbances of the cellular integrity induced by pathological conditions. Therefore, increased levels/activity of LDH is a useful index of tissue/cellular damage (12). From the research done, all of the treated mice yielded a significant reduction compared to controls. This show that cell breakdown, example due to free radical activities almost never happen. It is proven that *Cosmos caudatus* can protect the mice liver from oxidative damage mainly through inhibition of the lipid peroxidation process and LDH activity, as well as through some modulation of the activities of certain antioxidant enzymes. This is proven by the high antioxidant capacity that equivalent to L-ascorbic acid (AEAC) 2500 mg per 100 g fresh sample (46).

Conclusion

In conclusion, *Cosmos caudatus* supplementation might be able to protect mice livers against damage caused by oxidative stress mainly through inhibition of the lipid peroxidation process and LDH activity, as well as through some modulation of the activities of certain antioxidant enzymes.

Acknowledgement

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