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BIOCHEMICAL STUDIES ON TURMERIC POWDER WITH SPECIAL REFERENCE TO ANTIOXIDANT EFFECT AND POSSIBLE ROLES IN PROTECTION AGAINST CHEMICAL CARCINOGENESIS AND TOXICITY

(With 4 Tables and 3 Figures)

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دراسات بيوكيماوية على مسحوق نبات الكركم مع التركيز على تأثيره كمضاد للأكسدة والطرق المحتملة التي يقي بها الجسم من الكيماويات المسرطنة والتسمم

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شجع شيوع استخدام مسحوق الكركم وغيره من الأعشاب الطبية في علاج كثير من الأمراض – على دراسة التأثير الكيميائي الواقي من المواد المسرطنه لهذا النبات مع التركيز على مستوى تركيز مادة الجلوتاثيون المختزل ونشاط خميرة الجلوتاثيون إس ترانسفيريز وكذلك مستوى أكسدة الدهون في أنسجة الكبد والكلى والمخ كطريقه في حماية الجسم والأنسجه المختلفة من المسرطنات ، لذا أجريت هذه الدراسة على ثلاث مجموعات من فئران التجارب البيضاء تتكون كل مجموعة من ٢٥ فاراً ، أضيف مسحوق الكركم إلى عليقة الحيوانات بتركيز ١٠ و٢٠% يوميـــاً و لمدة ٤ أسابيع متواصلة للمجموعة الأولى والثانية على التوالي و بقيت المجموعة الثالثة بدون مسحوق الكركم كضابط للتجربة . وقد أظهرت النتائج في نهاية التجربه إنخفاض ملحوظ في تركيزات مادة الجلوتاثيون المختزل في كافة الأنسجة ، أما نشاط خميرة الجلوتاثيون إس ترانسفيريز فقد زاد بدرجة كبيرة حيث وصل نشاطه إلى ١,٩ – ١,٧ – ١,٨ مرة مقدار مثيله في المجموعة الضابطة والكبد والكلى والمخ على التوالى . أيضـــاً إنخفض مستوى أكسدة الدهون بدرجة ملحوظة حيث وصلت نسب الإنخفاض إلى ٥٣% – ٢٥% – ٣٥% في كل من الكبد و الكلى والمخ على التوالي عند مقارنتها بالمجموعة الضابطة. وقد أظهرت جرعات الكركم العالية ٢٠% تأثيراً أكبر من الجرعات المنخفضة ١٠%. هذه النتائـــج تدل على أن مسحوق نبات الكركم يمكن أن يكون له دوراً مهمـــاً في حماية الجسم من كثير من الكيماويات المسرطنه و ذلك من خلال تأثيره على المؤشرات الثلاث السابقة و غيرها في أنسجة الجسم .

SUMMARY

The effects of dietary supplementation of turmeric powder on the level of reduced Glutathion (GSH), the activity of phase II metabolising enzyme Glutathione S-transferase (GST) and lipid peroxidations has been investigated. Three groups of albino rats, each of 25 rats, were supplemented with 0, 10, and 20 % turmeric powder in the diet daily for 4 weeks. The activity of GST was significantly increased -in a dose depentent manner to 1.9, 1.7 and 1.8 times in the liver, kidneys and brain homogenates respectively compared with the control. The level of GSH were significantly reduced in tissue homogenates of all treated groups. The lipid peroxidation was signficantly reduced to 53 %, 25 % and 35 % in liver, kidneys and brain homogenates respectively as compared to controls. Such results suggested the potential value of turmeric powder as protective agent against chemical carcinogenesis and other forms of electrophilic toxicity. The importance of these results can be implicated in relation to cancer chemoprevention effects of turmeric against the induction of tumours in various target organs.

Key words: *Turmeric powder, Antioxidant, Carcinogenesis, Toxicity.*

INTRODUCTION

Turmeric is the powdered dry rhizome of *Curcuma longa Linn.*plant of the family Zingiberaceae. Curcumin (diferuloylmethane) is the major yellow pigment of turmeric. They are commonly used as spice, food preservatives, yellow coloring and flavouring agents. Turmeric has been used for centuries for the treatment of inflammatory diseases (Nadkarni, 1976). It has been found that, the polyphenolic compounds present in some plants have a great anti-oxidant activity as that shown by curcumin which markedly inhibited lipid peroxidation in the different tissues of rat and mice (Sharma *et al.*, 1972; Sharma 1976 and Okuda *et al.*, 1993). Also, turmerin, the aqueous extract of turmeric, was efficient in inhibiting lipid peroxides formation and produced 80% protection against peroxidative damage of DNA in-vitro (Shalini and Srinivas, 1987 and Srinivas *et al.*, 1992). In the same manner, the in-vitro studies of Soundamini *et al.* (1998) declared that, oral administration of curcumin significantly lowered CCl_4, paraquat and cyclophosphamide-increased lipid peroxidation in the liver, kidneys and

brain of mice and those catalyzed by iron in rat tissues (Sreejayan and Rao, 1993 and Reddy and Lokesh, 1994). Ruby *et al.* (1995) compared the cytotoxic, tumor reducing, and antioxidant activities of natural curcuminoids (curcumin I, II and III) isolated from turmeric, and concluded that curcumin III was the most active as cytotoxic agent and in the inhibition of Ehrlich-ascites carcinoma (EAC) in mice. The amounts of curcuminoids I, II and III required for 50% inhibition of lipid peroxidation were 20, 14 and 11 g, respectively. A series of naturally occurring dietary constituents of curcumin were ultimately inducers of phase II detoxification enzymes (Dinkova-Kistova and Talalay, 1999), and inhibit liposomal peroxidation as a part of the anti-oxidant and anti-inflammatory activities of curcumin (Ramsewak *et al.*, 2000, Iqbal, *et al.*, 2003), as they assessed a chemotherapeutic effects. Some plant products were also documented as GSH and GST enzyme activity inducers in the oesophagus, stomach and liver and hence considered a protective agents against cancer (Aruna and Sivaramakrishnan, 1990). Curcumin depleted GSH level in rat hepatocytes (Donatus *et al.*, 1990) and sulfhydryl levels in stomach of mice, while the activity of GST in the liver was significantly increased by 1.8-fold than those of control mice when curcumin given orally at a dose of 250 mg/kg b.wt for 15 days, (Susan and Rao, 1992). The anticarcinogenic effect of 5% dietary turmeric for 7 consecutive days in Swiss mice against BP-induced forestomach tumorigenesis and 7, 13-dimethylbenz(a)anthracene (DMBA)-induced skin neoplasia, was revealed by a significant increase in GSH level by 12% and GST activity by 32% in the liver when compared to control group (Azuine and Bhide, 1992; Huang *et al.* 1993 and *1994)* . In addition, a 10% dietary turmeric in rats enhancing the hepatic xenobiotic metabolizing enzymes proved by a significant increase in GST activity (Goud *et al.*, 1993). The potential anticancer activity of turmeric was first estimated by Kuttan *et al.* (1985) who reported that ethanolic extract of turmeric and curcumin inhibited cell growth of Chinese hamster ovary cells, and were cytotoxic to Dalton's lymphoma cells. In the same study, IP administration of curcumin inhibited tumor formation in mice injected Dalton's lymphoma. In other studies, dietary administration of 2% turmeric in the diet, inhibited the tumors induced by DMBA, BP, and azoxymethane (AOM) in skin, forestomach, small and large intestine in mice, respectively (Azuine and Bhide, 1992 ; Huang *et al.*, 1993 and 1994). Furthermore, Rao *et al.* (1995) observed that dietary administration of pure curcumin had the same inhibitory effect of turmeric on AOM-induced colon

carcinogenesis. Moreover, Ghanem and El-Mofty (1998) reported that dietary administration of 20% turmeric powder exerted a strong chemopreventive effect against BP-induced tumors in the oesophagus of mice. The aim of this study is to investigate the influence of dietary supplementation of turmeric powder on the reduced glutathione level, glutathione S-transferase activity, and lipid peroxidation in the various body organs of rats, as possible mechanisms underlying the previously reported antioxidant and anticarcinogenic actions of this plant.

MATERIALS and METHODS

Animals:

This study was carried out on 75 Albino rats of 70 ± 2 days old and weighing 120 to 150 g. The animals were purchased from the Medical Research Institute of Alexandria University. Rats were housed in metal cages (4–6 rat/cage) with soft-wood chips for bedding. They were given a commercial basal diet and water *ad libitum*. Rats were housed under the mentioned environmental conditions and the basal diet for a minimum of two weeks before the experiment for acclimatization and to ensure normal growth and behaviour.

Plants:

Powdered turmeric was purchased and identified in the Herbarium of Faculty of Science, Alexandria University, Egypt.

Chemicals:

5,5-dithiobis-(2-nitrobenzoic acid: DTNB, 1-chloro-2, 4-dinitrobenzene (CDNB), disodium salt of ethylene diamine tetra-acetic acid (EDTA), were obtained from Sigma Che mical Co. (St. Louis, MO, USA). Thiobarbituric acid (TBA), trichloroacetic acid (TCA), and reduced glutathione (GSH) were obtained from Fluka Co. Kits for determination of liver aminotransferases (AST and ALT) activities, and urea, and creatinine content were supplied by Isotech Co. and Biocon Co., respectively. All other chemicals and reagents were of the highest purity grade and commercially available.

Experimental design: Rats were divided into 3 groups:

Group I: 25 rats were supplemented with the basal diet mixed with 10% powdered turmeric. Animals were housed in groups each of 5 rats, and kept under the same conditions of light, climate, diet and water for 4 weeks.

Group II: 25 rats were supplemented with the basal diet mixed with 20% powdered turmeric and housed as the above group for 4 weeks.

Group III: 25 rats kept on the basal diet as a control group.

At the end of experiment all animal were killed by decapitation. Blood samples were collected for determination of serum AST and ALT activities, urea and creatinine. Tissue homogenates and supernatants were prepared from liver, kidneys, and brain for the assessment of glutathione level, glutathione S-transferase and lipid peroxidation content.

Methods:

Reduced glutathione (GSH) was assayed by spectrophotometric technique according to the method described by Sedlack and Lindsay (1968) using Ellman's reagent. Glutathione S-transferase (GST) activity in tissues homogenate supernatants, was measured spectrophotometrically at room temperature as the rate of GSH conjugation of CDNB according to the method described by Habig *et al.* (1974). Lipid peroxides as malondialdehyde (MDA), was measured spectrophotometrically after the reaction with thiobarbituric acid (TBA) according to the method described by Placer *et al.* (1966). Serum urea and creatinine levels were determined spectrophotometrically after the method described by Fawcett and Scott (1960), and Henry (1974) respectively. Serum AST and ALT activities were determined photometrically according to the method described by Reitman and Frankel (1957)

Statistical analysis: Obtained data was statistically analysed using the GLM procedure of the Statistical Analysis System computer package (SAS, 1987). Results were presented as means ± S.D, $P<0.05$ was considered statistically significant.

RESULTS

Clinical manifestation of treated rats:

Along the experimental duration, no symptoms were observed on the treated rats and they did not show any changes in the general behaviour or their physiological activities.

Biochemical changes of GSH level, GST activity, and LP level in tissue homogenates of rats: The results presented in Table 1 and Fig. 1 show a significant decrease in the level of reduced glutathione in liver, kidnyes and brain homogenates in all treated groups when compared with their respective control values. On the other hand, the activity of glutathione S-transferase was significantly increased, such increase was greater in rat received diets supplemented with 20% turmeric powder

than those given 10% turmeric, where the values were 1.9, 1.7, and 1.8 times as their respective control in liver, kidneys, and brain, respectively (Table 2 and Fig. 2). Also, the lipid peroxidation was significantly decreased in all treated groups especially those received 20% turmeric, and in all tissues homogenates where the reduction percentage was 53, 25, and 35% in liver, kidneys and brain, respectively, when compared with their respective control values (Table 3 and Fig. 3). Regarding the serum biochemical parameters presented in Table 4, revealed that there is no significant changes in the values of serum ALT and AST activities, also, serum urea and creatinine were not significantly changed when compared with their respective controls.

Table 1: Effect of dietary supplementation of powdered turmeric on the concenteration of reduced glutathione (GSH) in rat tissue homogenates after 4 weeks.

Groups	*GSH (µmol/g wet tissue)*		
	Liver	Kidneys	Brain
Control	38.82 ± 0.87 a	24.72 ± 0.53 a	18.10 ± 1.33 a
Powdered Turmeric 10 %	29.43 ± 1.04 bc	21.59 ± 1.15 b	16.62 ± 0.23 b
Powdered Turmeric 20 %	28.81 ± 0.99 bc	20.58 ± 0.90 b	14.67 ± 0.52 c

Table 2: Effect of dietary supplementation of powdered turmeric on glutathione S-transferase (GST) activity in rat tissue homogenates after 4 weeks.

Groups	*GST (mol CDNB/min/g wet tissue)*		
	Liver	Kidneys	Brain
Control	1342.5 ± 56.56 c	435.51 ± 22.3 c	187.95 ± 4.70 c
Powdered Turmeric 10 %	2145.7 ± 115.73 b	595.80 ± 50.5 b	307.80 ± 19.60 b
Powdered Turmeric 20 %	2579.4 ± 114.14 a	755.95 ± 73.4 a	338.45 ± 22.30 a

Table 3: Effect of dietary supplementation of powdered turmeric on lipid peroxidation (LP) level in rat tissue homogenates after 4 weeks.

Groups	*LP (nmol MDA/g wet tissue)*		
	Liver	Kidneys	Brain
Control	172.66 ± 4.88 a	116.07 ± 1.68 a	41.88 ± 2.00 a
Powdered Turmeric 10%	85.82 ± 11.88 cb	80.36 ± 5.00 d	31.10 ± 1.52 cb
Powdered Turmeric 20%	82.93 ± 6.08 cb	88.80 ± 5.92 c	27.45 ± 2.85 c

Table 4: Liver and kidneys functions after 4 weeks of dietary supplementation of powdered turmeric in rats.

Groups	Liver and kidneys functions			
	ALT (U/l)	AST (U/l)	Urea (mg/dl)	Creatinine (mg/dl)
Control	54.00 ± 2.45 a	18.80 ± 0.97 a	25.72 ± 0.82 a	0.58 ± 0.04 a
Powdered Turmeric 10 %	46.67 ± 2.89 a	15.00 ± 2.31 ab	24.53 ± 0.40 a	0.57 ± 0.03 a
Powdered Turmeric 20 %	55.00 ± 2.89 a	16.33 ± 1.33 ab	22.00 ± 0.53 a	0.53 ± 0.03 a

Means in the same column with similar letter do not differ significantly at $P \leq 0.05$.

DISCUSSION

The aim of this study was to investigate the influence of dietary supplementation of turmeric powder on the level of GSH, GST activity, and lipid peroxidation in the various body organs of rats, as possible mechanisms underlying the previously reported antioxidant and anticarcinogenic actions of this plant (Sharma *et al.*, 1972; Aruna and Sivaramakrishnan, 1990; Azuine and Bhide, 1992 and Negriz and Otles, 1993). Apparently, no clinical symptoms of illness or poisoning could be observed in rats received turmeric powder in their diet along the whole period of experiment. Such results are in harmony with those obtained by Wahlstrom and Blennow (1978) who found no apparent toxic effects in rats upon feeding curcumin. The antioxidant and anticarcinogenic studies of turmeric powder proved that the highest concentration of reduced GSH was detected in the liver followed by kidneys then brain, (Fig. 1). These results are consistent with those obtained by Dencke and Fanburg (1989) who attributed such distribution in GSH levels to the fact that the liver is specialized to synthesize GSH and serves as its main storage, and exported GSH to other organs, where 60% of the exported GSH goes to the kidney; the second organ of detoxification following liver, and the remaining 40% of GSH goes to other organs such as lungs, brain and heart. However, the results present in table 1 and figure 1 showed that different doses of turmeric powder 10 and 20% resulting in a significant decrease in the level of GSH in all

studied tissues. These results are in substantial agreement with those obtained by Donatus *et al.* (1990), Susan and Rao (1992) and Rady *et al.* (1997) who attributed such depletion in GSH level to the spontaneous or enzymatic conjugation of □ - and β-unsaturated ketone groups present in these plants with GSH and/or protein thiol. On the other hand, these results contrdict those obtained by Saad *et al.* (1998), Singh *et al.* (1998) and Badary *et al.* (1999) who reported no significant changes in the hepatocytes GSH level upon oral administration of curcumin 2% in female mice. Also, from the present study, it was evident that the GST activity behaves the same distribution in the various body organs in control rats as that of GSH (Fig. 2). Also, such distribution is comparable with that reported by Kraws and Kloft (1980) ; Joseph and Louise (1986) and Prochaska and Fernandes (1993). Dietary supplementation of turmeric powder 10 or 20% significantly increased the GST activity in the liver, kidneys and brain, such induction in GST activity in tissues of rats was more sensitive to the higher dose of turmeric than the lower dose (Table 2 and Fig. 2). Such results are in agreement with those reported by Azuine and Bhide (1992), Susan and Rao (1992), Goud *et al.* (1993) and Singh *et al.* (1998) who studied the effect of oral administration of turmeric on the hepatic GST activity either in rats or mice. On the other side, the in-vitro studies using tissue culture and cell lines revealed that curcumin was a potent inhibitor of GST activity as observed by Oetari *et al.* (1996) and Van Iersel *et al.* (1996) who explained such differences between the in-vitro and in-vivo results of GST activity on the basis of differential metabolism of curcumin between in-vitro and in-vivo systems. Concerning lipid peroxidation, our results revealed that the highest level of lipid peroxidation was observed in the liver and kidneys (Fig. 3). These results may be explained by the higher metabolic activities in the liver and kidneys putting them at an increased risk for exposure to the oxidative stress resulting from reactive oxygen species formation. Turmeric powder produced a significant reduction in the lipid peroxidation in the liver, kidneys and brain tissue homogenates. These results are consistent with those obtained by Donatus *et al.* (1990), Soundamini *et al.* (1998), Sreejayan and Rao (1993), Ruby *et al.* (1995), Cohly *et al.* (1998) and Makamura *et al.* (1998) who reported that the turmeric and/or curcumin are potent inhibitors of lipid peroxidation in the liver, kidney and brain of rats, mice and cell lines. Although the exact mechanism by which turmeric or its products inhibit the lipid peroxidation process, was not fully revealed, several possibilities may be

considered rendering them potent antioxidants. One possible is their content of polyphenolic groups which induce protective effects against reactive oxygen species as reported by Sharma (1976), Negriz and Otles (1993) and Okuda *et al.* (1993). A second possible mechanism of lipid peroxidation inhibition is inhibition of eicosanoid generation, and cyclo-oxygenase and lipoxygenase pathways of arachidonic acid metabolism which are potent modulators of lipid peroxidation and tumor promotion (Fisher *et al.*, 1988 and Bond *et al.*, 1993). Furthermore, it was proved that the active principles of turmeric (curcumin) and its products were potent inhibitors of eicosanoid generation and lipid peroxidation via inhibition of lipoxygenase and cyclo-oxygenase pathways of arachidonate metabolism as reported by Houghton *et al.* (1995) and Huang *et al.* (1991 and 1997). Another possible mechanism, the antioxidant effect of such plant and its products may be due to scavenging of peroxides and other radical oxygen species as did by natural protectors against lipid peroxidation such as retinol, ascorbic acid, □-tocopherol, glutathione and antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase which have the capacity to scavenge reactive oxygen species and lipid-free radicals (Fujii *et al.*, 1984; Fukuzawa *et al.*, 1985; Wald *et al.*, 1987; Srinivas *et al.*, 1992; Makamura *et al.*, 1998, Burits and Bucar, 2000 and Kruck *et al.*, 2000). All of these may explained how this plant and its products produced their reductive effects on the lipid peroxidation in the different tissues of rats, and therefore play an important role as protective agents against cancer. Serum urea, creatinine, AST and ALT activities shown in table 4 revealed that the turmeric powder either 10 or 20% had no significant effect on these parameters. Similar observation was reported by Sambaiah *et al.* (1982). Furthermore, Srimal and Dhawan (1973), Deshpande *et al.* (1998) and Park *et al.* (2000) reported that the curcumin and turmeric returned the elevated levels of AST and ALT activities to normal levels in rats treated with either formaldehyde or CCl_4.

It can be concluded that the dietary supplementation of turmeric powder 10 or 20% for 4 successive weeks had no toxic effects and without any apparent abnormal clinical signs especially at higher dose level as evidenced by their effects on the liver and kidney functions. Also, turmeric powder imparted a chemoprotective effect against oxidative stress , and against cancer as evidenced by its inductive effects on the GST and activity and reduction of lipid peroxidation in various body organs of rats.

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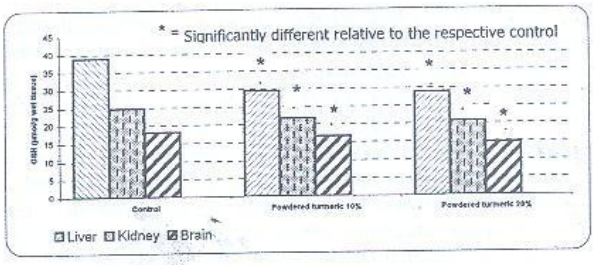

Fig. 1: Effect of powdered turmeric on GSH level in different body organs of rats after 4 weeks.

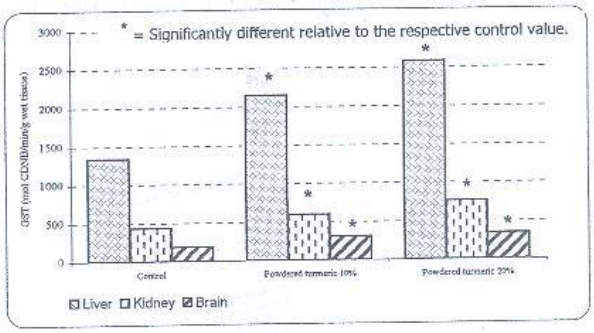

Fig. 2 : Effect of powdered turmeric on GST activity in different body organs of rats after 4 weeks.

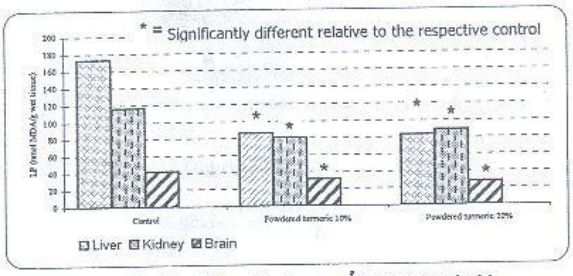

Fig. 3 : LP level in different body organs of rats treated with powdered turmeric after 4 weeks.