# Protective effect of curcumin against injury induced stress i albino rats.

### A.F.El-Kirdasy<sup>a</sup>, Saad Shousha<sup>b</sup>, Osama A. Hassan<sup>c</sup>

#### **Abstract**

Curcumin is a phytopolylphenol pigment isolated from the pl Curcuma longa, commonly known as turmeric, with a variety pharmacologic properties. Curcumin blocks the formation of reacti oxygen species, possesses anti-inflammatory properties as a result inhibition of cyclooxygenases (COX) and other enzymes involved inflammation. These effects may play a role on the agent's hav antineoplastic properties, which include inhibition of tumor cell proliferat and suppression of chemically induced carcinogenesis and tumor growtl animal models of cancer. This study was designed to clarify the effect curcumin on induced stressing male albino rats. For this purpose about male albino rats weighting110-130gm were divided into four groups; the group considered as a healthy matched group (control) and the 2nd group exposed to intraperitoneal puncture (sham stress). While the 3rd group administered orally with curcumin powder in a dose of (80\mg/kg but weight). The 4th group exposed to Sham stress and administered v curcumin in the same previous dose.

Three different samples were taken; liver and kidney tissue were tal for measuring of malondialdehyde (MDA), Superoxide dismutase (SC reduced glutathione (GSH). Blood samples were taken, where the plas was used for estimation of Total protein, Albumin, Urea and Creating The whole blood was taken for erythrocytic count (RBCs), total leukoc count (WBCs), packed cell volume (PCV) and hemoglobin. Resrevealed that sham stress (2<sup>nd</sup> group) show significant increase in mean values of MDA, SOD and a significant decrease in the mean value of GSH, WBCs count, PCV and hemoglobin concentration.

The obtained results revealed non significant changes in total protalbumin, creatinine and urea. However, the results revealed that adcurrumin significantly alleviated or these changes caused due to strand returned these changes near to the normal values. Sham stress taken as a model of sever stress which the body can be exposed to These results suggest that curcumin may play an important role alleviating the changes caused by exposure to stress.

Key words: Curcumin; Antioxidants; Stress.

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#### Introduction

In the US, turmeric is generally recognized as safe (GRAS) as a food a the FDA. Serious adverse effects have not been reported in humans taking I of curcumin. Curcumin supplementation up to 8 g/day for 3 months was repr well-tolerated in patients with precancerous conditions or noninvasive canc et al., 2001). Each injury is accompanied with the oxidation stress with an i the level of free oxygen radicals and decrease in the body's antioxidant (Motycka et al., 2006). Reactive oxygen species (ROS) may be generated after physical exercise in working muscles, and in the tissues that undergo reperfusion (Sjödin et al., 1990; Ji, 1995). It is estimated that 2-5% of the tol flux leaks to form primary short-lived ROS such as superoxide radical ar hydrogen peroxide (H2O2), and hydroxyl radical (OH) (Boveris and Chan Sources of ROS during exercise include enhanced purine oxidation, dama containing proteins, disruption of Ca2+ homeostasis (Jackson, 2000), and flo endothelium ROS production (Shi et al., 2001). Neutrophils infiltrating the m injury by exercise may also produce ROS (Jones et al., 1986), which r oxidative tissue damage (Sen, 1995), e.g., DNA injury (Inoue et al., 1 peroxidation (Meydani et al., 1993) and protein oxidation (Saxton et al., 19 processes are associated with the appearance of an increase in the severi diseases (Ames et al., 1993; Gutteridge, 1993). If the production of free excessive as observed during strenuous aerobic exercise (Ji, 1995) antioxidant defense mechanisms are impaired, the balance between pro-ox antioxidants will be lost. Increased MDA levels and decreased GSH levels stressed group were reversed to control values after antioxidant treatment al., 2007). In the same aspect, Ohta et al., (2007) reported that increases in peroxidation), NOx (nitrite/nitrate) concentrations and myeloperoxidase a decreases in ascorbic acid, reduced glutathione concentrations and dismutase activity.

Findings of Yu et al., (2006) revealed that chronic oral treatmer galactosides and glucosides in a polysaccharide extract elevates enzymat of SOD and GPx (glutathione peroxidase) accompanied by a correspondin in malondialdehyde level. The ant- oxidative activities of these compour exercise-induced oxidative stress are correlated with various activities reducing the production of superoxide and hydroxyl radicals, inhi peroxidation, enhancing antioxidative defenses, and increasing the producti glutathione peroxidase activity and expression in different tissues. Sahin an (2007) found that the level of SOD, GSH and MDA in both kidney and live affected by stress. There is an apparent paradox between the benefits (moderate) and strenuous aerobic exercise or exposure to bad atmospheric such as torture. For these reasons the intrapretonial injures (Sham considered as sever physiological stressors to which animals exposed. The study was to investigate if curcumin which is a phytopolylphenol pigment is the plant Curcuma longa, can ameliorate or alleviate the changes due to stre

#### Materials and methods

#### 1- Animals and design:

This study was carried out on 80 male albino Wistar strain rats. Their body ranged from 110-130 g and their ages ranged from 60-70 days. All animals wer their plastic cages for two weeks for acclimatization in suitable well ventilated They were maintained under controlled temperature (23°C ± 2 °C) and light cor (lights on, 0700-1900 h) with adlibitum access to water and standard laborator that was purchased from the B.M. Company for International Commercial Development in Egypt. These animals were divided into 4 groups each one c 20 male albino rats. The 1st one considered as control group (G1) was not re anything except the used ration for 3 successive weeks and from the 4<sup>th</sup> week ε received corn oil (250µl/ kg body weight) which is the vehicle of the drug use other day by using stomach tube till the 11th weeks of experimental period. group called sham stressed group (G2) subjected to false intraperitoneal injection the third week till the 10<sup>th</sup> week and at the 11<sup>th</sup> week false intraperitoneal injecti stopped for the recovery. The 3rd group called curcumin group (G3) in wh animals received curcumin only in an oral dose of 80 mg/kg body weight (kalps Menon, 2004) dissolved in (250ml/ kg body weight) corn oil which is the ve curcumin from the third week until the end of the experiment. The animals of group (G4) were subjected to false intraperitoneal injection and the curcum administered in the same previously mentioned dose from the 3rd week till the the experiment. These experiments were performed in duplicate. All procedure performed in accordance with the Menoufia University Guide for the Care and Experimental Animals. All rats included in this study were certified as healt exhibited normal grooming and feeding behavior on the day of studies.

#### 2. Ration ingredients:

The ration was purchased from the BM. Company for International Comme Development in Egypt.

### 3. Sampling:

From the fifth week of starting experiment, 5 rats were killed by decapitation each group every two weeks. The liver and the kidneys were taken and dishomogenized with buffer (phosphate buffer pH 7.4) and stored at -20 °C until to the determination of antioxidant enzymes and malondialdehyde (MDA). From the fifth week of starting experiment, 10 rats were taken every two weel

blood samples were taken from the orbital sinus puncture; one in heparenized hematological examinations and the 2<sup>nd</sup> left to clot and serum was aspiral collected at -20 °C until used for biochemical studies.

### 4. Biochemical and hematological analysis:

The biochemical parameters as malondialdehyde (MDA) was measured at to the method described by Yashkochi and Masters (1979), while Surdismutase (SOD) was measured according to the method of Giannopolitis a (1977) and reduced glutathione (GSH) as showed by Patton and Chanarin (\*)

liver and kidney tissues. The tissue homogenate was prepared according method described by Combs et al., (1987).

Total protein, albumin and creatinine were estimated calorimetrically as desc Young (2001) using kits obtained from Elctroscient Company. Serum Ur measured according to the method described by the Patton and Chanarin (198 kits from Diamond diagnostic company. The RBCs and total leukocytes were as the method described by the Feldman et al., (2000). Packed cell volume (P hemoglobin were measured as described by Feldman et al., (2000).

#### 5. Statistical analysis:

Data were expressed as means + SEM (Standard error mean) and resuconsidered statistically significant at  $P \le 0.05$ . All data were subjected to an variance (ANOVA) test according to Snedecor and Cochran (1980).

#### Results

## 1. Effect of curcumin on malondialdehyde (MDA) level.

Our results revealed a significant ( $P \le 0.05$ ) increase in Malondialdehyde both liver and kidney tissue in the  $2^{nd}$  group (sham stress group) compared 1 control group as shown in both figure 1A and figure 2A, respectively. Ad curcumin to the stressed group ( $4^{th}$  group) significantly ( $P \le 0.05$ ) amelior increase of MDA and returned its values to normal in both liver and kidney. T of curcumin on this change due to induced stress was very clear and promine all the time of experiment (at the  $5^{th}$ ,  $7^{th}$ ,  $9^{th}$  and the  $11^{th}$  week) in case of kid liver.

# 2. Effect of curcumin on superoxide dismutase (SOD) activity.

A significant ( $P \le 0.05$ ) increase in superoxide dismutase (SOD) in both kidney tissue in the 2nd group (sham stress group) compared to the 1<sup>st</sup> control figure 1B and figure 2B, respectively). Adding the curcumin to the stressed g group) significantly ameliorated the increase of SOD and returned its values normal in both liver and kidney. The effect of curcumin on the induced stress clear and prominent during all the time of experiment (at the 5<sup>th</sup>, 7<sup>th</sup>, 9<sup>th</sup> and week) in both liver and kidney (figures 1B and 2B).

# 3. Effect of curcumin on reduced Glutathione (GSH) level.

MDA level and SOD activity increased significantly ( $P \le 0.05$ ) in both kidney due to the induced stress in the  $2^{nd}$  group, while the values of GSH d significantly ( $P \le 0.05$ ) in both liver and kidney at  $5^{th}$ ,  $7^{th}$ ,  $9^{th}$  and  $11^{th}$  week (fi and 2C, respectively). The effect of curcumin on this change due to induced s very clear and prominent during all the time of experiment (at the  $5^{th}$ ,  $7^{th}$ ,  $9^{th}$   $11^{th}$  week) in both kidney and liver (figure 1C and 2C).

# 4. Effect of curcumin on total protein, albumin, creatinine and urea levels

A non significant changes due to sham stress (2<sup>nd</sup> group) or due to cure group) on total protein, albumin, creatinine and urea was observed (3A, 3E 3D, respectively).

#### 5. Effect of curcumin on some hematological parameters

Sham stress (2<sup>nd</sup> group) significantly ( $P \le 0.05$ ) decreased the value leukocytic count , packed cell volume (PCV) and hemoglobin content thro experiment as shown in figures 4B, 4C and 4D, respectively. Oral adminis curcumin to the stressed group (4<sup>th</sup> group) significantly (P < 0.05) alleveranges caused due to stress and returned these changes to normal values B, C and D).

#### Discussion

In our experiment, we used group of rats administered orally with without induction stress (3<sup>rd</sup> group) to confirm that all the significant changes in the 2<sup>nd</sup> group were due to stress induced by intraperitoneal puncture and t any other cause.

Malondialdehyde (MDA) is the most abundant product of lipid pe (Chaudhary et al., 1994). The statistical analysis in the present study ship there was a significant increase in the level of (MDA) in liver and kidney tiss 2<sup>nd</sup> group as shown in figure 1A and figure 2A due to exposure of the animal stress. This increase in the level of malondialdehyde is due to ability of sharr generate (ROS) which leads to oxidative damage in several tissues by incre peroxidation. This result is in agreement with (Halliwell and Gutteridage Curcumin is a potent anti-oxidant was shown to protect experimental animal lipid peroxidation (Sreejayan and Rao, 1997; Dinkova-Kostova and Talalay, the present study, the curcumin significantly (P ≤ 0.05) decreased the mean the MDA in the liver and kidney tissues in sham stressed rats (the 4th grou results come in accordance with the results of Reddy and Lokesh (1994); r (2000) and Reyes-Gordillo et al., (2007). This decrease of MDA attrit explained by enhancing the activities of antioxidant enzymes (Reddy and 1994; Mohanty et al., 2004), and inhibiting the generation of ROS (Somasui al., 2002).

In the same manner, Soudamini et al., (1992); Unnikrishnan and Rao ( Sreejayan and Rao (1994) attributed the antioxidant mechanism of curcur neutralizing effect of free radicals and decreasing the  $\rm O_2$  and made it less at oxidation and also may attributed the potent inhibitant effect of curcumin to c lipo-oxygenase and cyclo-oxygenase in arachidonic acid during the metabolic acids (Huang et al., 1997) on opposite side Reyes-Gordillo et al., (2007) attractional activation and thus production of proinflammatory cytokines.

Superoxide dismutase (SOD) acts on Superoxide radical and conhydrogen peroxide radical. SOD activity produces  $H_2O_2$  which is a toxic performing reactive hydroxyl radical with iron and hydrochlorus acid (HOC (Lunec, 1990). Statistically, there was a significant (P  $\leq$  0.05) increase in SC in both liver and kidney tissues in sham stress group (2<sup>nd</sup> group) when co control group (1<sup>st</sup> group) ( Figures 1B and 2B, respectively). This increase

returned to its normal activity after oral administration of curcumin to stresse (4th group). These results may be due to an increase in lipid peroxidati consequently increase free radicals production. These results were in accordar that showed by Goel et al., (2005). They attributed this increase to the oxidative which leads to an increase in SOD activity to compensate this stress.

Reduced glutathione (GSH) is a pool for three important metabolic path enters as cofactor for glutathione-S-transferase (GST) during the prodetoxification, and it is considered as a substrate for the  $\gamma$ -glutamyl transferase, as it plays an important role as free radical scavenger like antioxidant  $\varepsilon$  (Meister, 1994; Anderson, 1997). In the present study, the statistical analysis  $\tau$  that sham stress significantly ( $P \le 0.05$ ) decreased the level of reduced glu (GSH) in both liver and kidney tissues in comparison to the control group (fig and 2C, respectively). After oral administration of curcumin to the sham stresse  $\tau$  group, we found that curcumin overcomed the effect of stress cal intraperitoneal puncture. These results may be due to lowering peroxidal maintaining the activities of antioxidant enzymes at higher levels. Our data accordance with that exhibited by Reddy and Lokesh, (1994) or by increasing of glutathione to prevent the decrease that takes place during the process of a (Jaruga et al., 1998; Yu et al., 2006).

In this study, there was a significant ( $P \le 0.05$ ) decrease in total leukocy and hemoglobin concentration in sham stress group ( $2^{nd}$  group) when compace control group ( $1^{st}$  group) (figures 4B, 4C and 4D, respectively. After oral admir of curcumin to the sham stressed rats in  $4^{th}$  group, the results revealed that covercomed the effect of stress caused by intraperitoneal puncture. These resu agreement with Littarru et al., (1994); Tesoriere et al., (2001); Jaja et al., (2005 results maybe attributed to the oxidative stress caused by intraperitoneal puncture. We can concluded that, curcumin, appears to play important roles an antioxidant, anti-inflammatory agent and has non toxic or harmful effects whe to the food in a recommended dose and therefore we recommend adding of to the diet of animals or peoples those exposed to different conditions of stress ameliorates and alleviates the changes caused due to stress. Further researc required to elucidate the exact actions of curcumin and any further role that have.

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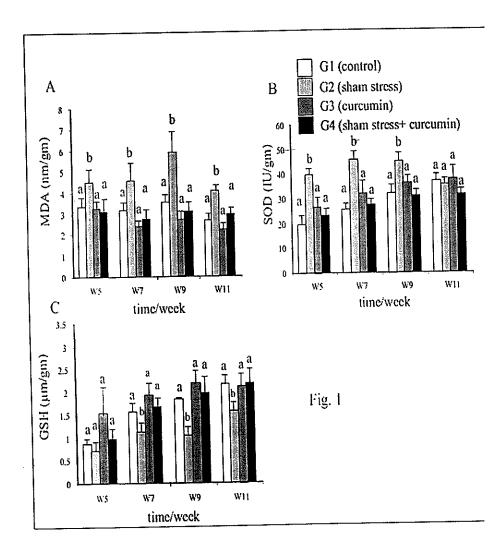


Figure 1. Effect of curcumin on the level of MDA, SOD and GSH in rat live G1 (1<sup>st</sup> group or control group), G2 (2<sup>nd</sup> group or sham stress group) group that received oral administration of curcumin in a dose of 80 mg weight from the 3<sup>rd</sup> week till the end of the experiment), G4 (4<sup>th</sup> group the rats subjected to false intraperitoneal puncture and the curcu administered orally with the same dose mentioned in the 3<sup>rd</sup> group). and vertical line represents the mean  $\pm$  SEM (n = 5). Bars with differ from control group (G1) are significantly different;  $P \le 0.05$ .

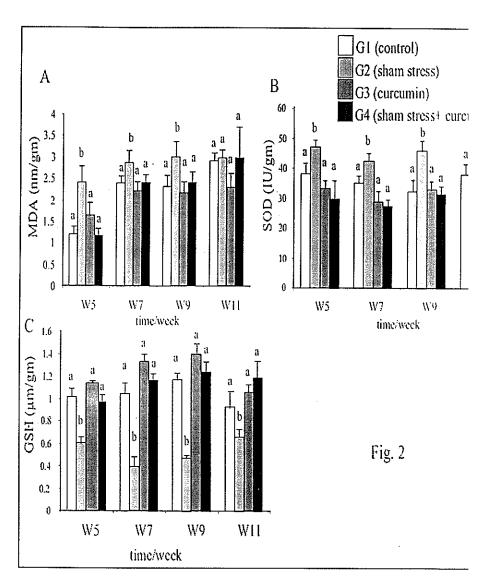


Figure 2. Effect of curcumin on the level of MDA, SOD and GSH in ratissue. G1 (1<sup>st</sup> group or control group), G2 (2<sup>nd</sup> group or sham stress gr (3<sup>rd</sup> group that received oral administration of curcumin in a dose of 8 body weight from the 3<sup>rd</sup> week till the end of the experiment), G4 (4<sup>th</sup> which the rats subjected to false intraperitoneal puncture and the curcu administered orally with the same dose mentioned in the 3<sup>rd</sup> group). I and vertical line represents the mean  $\pm$  SEM (n = 5). Bars with different from control group (G1) are significantly different;  $P \le 0.05$ .

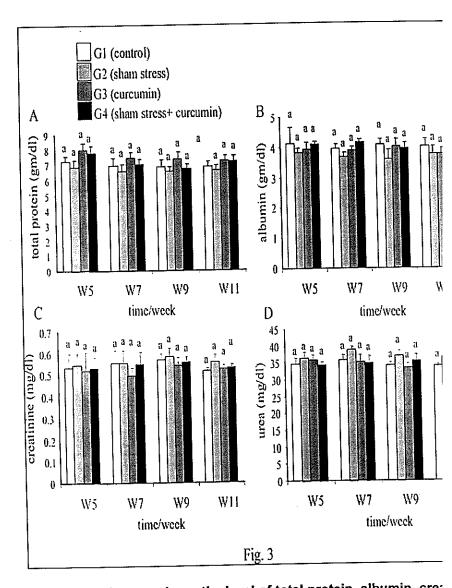


Figure 3. Effect of curcumin on the level of total protein, albumin, creaturea in rat sera. G1 (1st group or control group), G2 (2nd group or st group), G3 (3rd group that received oral administration of curcumin in 80 mg/kg body weight from the 3rd week till the end of the experime group in which the rats subjected to false intraperitoneal punctual curcumin was administered orally with the same dose mentioned group). Each bar and vertical line represents the mean  $\pm$  SEM (n = 5) different letter from control group (G1) are significantly different;  $P \le 0$ 

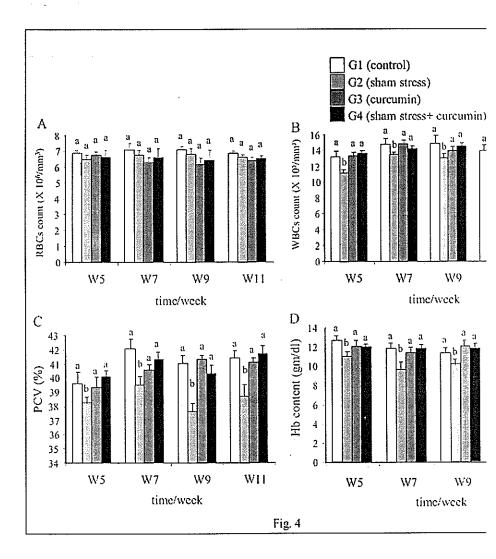


Figure 4. Effect of curcumin on RBCs count, total leukocytic count count), packed cell volume (PCV) and hemoglobin concentration in rat. group or control group), G2 ( $2^{nd}$  group or sham stress group), G3 ( $3^{rd}$  gro received oral administration of curcumin in a dose of 80 mg/kg body weig the  $3^{rd}$  week till the end of the experiment), G4 ( $4^{th}$  group in which t subjected to false intraperitoneal puncture and the curcumin was admin orally with the same dose mentioned in the  $3^{rd}$  group). Each bar and verti represents the mean  $\pm$  SEM (n = 5). Bars with different letter from contro (G1) are significantly different;  $P \le 0.05$ .

# ، العربي

# الوقائية للكوركومين ضد الاجهاد المستحث في فنران الالبينو.

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اء الحيوية وكيمياء التغذية كليه الطب البيطرى- جامعة المنوفيه بالسادات \*\* قسم الفسيولوجيا- كليه الطب معة بنها. \*\*\*قسم الطب الشرعي السموم كليه الطب جامعة المنيا.

سبرنبات الكركومين من النباتات التي تمتلك خصائص دوانيه كثيرة حيث يمنع تا ثير انزيم حينيز ولذلك فله خاصيه كمضاد للالتهاب. كما اثبتت الابحاث العلمية المختلف ان ، تاثيرات مضادة لنمو الخلايا السرطانية وقد اجريت هذه الدراسه لدراسة بعض التاثيرات رركومين في الفئران المعرضة للاجهاد المستحدث صناعيا . ولمعرفة هذه التاثيرات تم أر من النوع الالبينو يتراوح وزنها من ١١٠-الي١٣٠ جرام قسمت الى اربع مجموعات ة اشتملت على ٢٠ فار .الاولى G1) وهي المجموعة الضابطة والمجموعة الثَّانية ((G2) ع هذه المجموعة للوخذ البريتوني(Sham stress). وتم تجريع المجموعة الثالثة (G3) ن عن طريق القم (٨٠ ملجرام/كجم) من وزن الحيوان والمجمّوعة الرابعة (G4) تم رجهاد وكذلك تم تجريعها الكوركومين بالجرعة السابقة بعد ذلك تم تجميع عينات بعد اسبوع بالترتيب من الكبد والكلى لقياس مستوى المالوندالديهيد والجلوت اليون المختزل لم انزيم السوبر اوكسيد ديسميوتيز. كما تم تجميع عينات الدم وتم فصل البلازما لقياس ئى والالبيومين واليوريا والكرياتينين ايضا تم عد كل من كرات الدم الحمراء والبيضاء ات المضغوطة والهيموجلوبين. وقد اوضحت النتائج ان هناك زيادة معنوية في كل من وندالديهيد ونشاط السوبر اوكسيد ديسميوتيز ونقص معنوى في مستوى كل من الجلوت اثيون كرات الدم الحمراء وحجم الكريات المضغوطة والهيموجلوبين. كما اوضحت ايضا عدم فى مستوى البروتين الكلى والألبيومين والكرياتينين. ومن هذه النتائج يتضـح لنـا ان اضــافـة ادى الى علاج الاثار السلبيه الناشئة من الاجهاد عن طريق الوخذ البريتوني.