



## AMELIORATING ROLE OF CURCUMIN ON OXIDATIVE STRESS INDUCED BY ENDOTOXEMIA IN RATS

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### ABSTRACT

The objective of this study is to investigate the neuroprotective and antioxidant effects of subsequent pretreatment with curcumin as natural antioxidant on biomarkers of oxidative stress and inflammatory response brain tissue induced by endotoxemia. This study was carried on 42 male rats. The rats were divided into three groups. Group I (control) 12 rats not receive drugs, Group II 15 rats injected intraperitoneally (i.p) with a single dose of Lipopolysaccharide (LPS) at a dose 200 mg/kg b.wt, Group III 15 rats administered Curcumin orally at dose 100 mg/kg/b.w/day for 3 weeks and then injected with a single dose of LPS at dose 200 mg/kg/b.w. Blood samples for serum separation and brain tissue were collected from all animal groups twice at one and three hours from the onset of injection with Endotoxin. All sera were subjected directly for determination of Tumor Necrosis Factor (TNF), Interleukin -6 (IL-6), Sialic acid (SA) and Nitric oxide (NO). In addition determination of brain tissues of Glutathione peroxidase (GPx) Superoxide Dismutase (SOD), Catalase (CAT) and L-MDA. The obtained result revealed that Endotoxemia could be potentially significant increase serum TNF, IL-6 and brain L-MDA. Also induced a significant decrease in SA, NO and GPX, CAT and SOD in brain tissues. Treatment with curcumin significantly decreased in serum TNF, IL-6, and L-MDA in brain tissue. Also induced significantly increased in serum NO, SA and GPX, CAT and SOD in brain tissue. From the obtained results it could be concluded that curcumin protects against lipid peroxidation, oxidative stress and decrease the inflammatory response to endotoxin injection.

**KEYWORDS:** Endotoxin, Curcumin, Oxidative stress, inflammatory markers, Antioxidant status.

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### 1. INTRODUCTION

Lipopolysaccharide (LPS) is one component of gram negative bacterial cell wall, which released by destruction of cell wall and acts as a potent bacterial product in the induction host inflammatory responses and tissue injury. (Kheir el Din., 2001). Endotoxemia –induced toxicity is characterized by disturbed intracellular redox balance and excessive reactive oxygen species (ROS) accumulation leading to DNA proteins and membrane lipid damages. (Mallis et al., 2000). Oxidative stress occurs when oxygen free radicals are generated in excess through a reduction oxygen or when neural are

under oxidative stress, excessive oxygen species (ROS) are produced that may inducer neural death (Slatter et al., 2000). Reactive oxygen species are thought to play a vital role in the pathogenesis of Hepatic Encephalopathy. Oxidative stress has also been implicated in liver and in neurodegenerative diseases like AIDS-Dementia, Huntington Chorea, Schizophrenia, Parkinson's disease, etc (Smythies, 1999). In recent years, there has been renewed interest in the treatment against different diseases using herbal drugs as they are generally non-toxic and world health organization has also recommended

the evaluation of the effectiveness of plants in condition where we lack safe modern drugs (Ayyanar et al., 2008).

Curcumin, commonly called diferuloyl methane is a hydrophobic polyphenol derived from the rhizome (tumeric) of the herb *Curcuma longa*. Tumeric has been used traditionally for many ailments because of its wide spectrum of pharmacological activities. Curcumin has been identified as the active principle of tumeric, chemically, it is a bis  $\alpha$ ,  $\beta$ -unsaturated  $\beta$ -unsaturated  $\beta$ -diketone that exhibits Keto-enol tautomerism. Curcumin has been shown to exhibit antioxidant, anti-inflammatory, antimicrobial and anticarcinogenic activities. It also has hepatoprotective and neuroprotective activities, suppresses thrombosis, protects against myocardial infarction and has hypoglycemic and anti-heumatic properties. (Agarwal et al., 2007).

## 2. MATERIALS AND METHODS

Twenty four white male albino rats of 8-10 week old and weighing 150 - 180 g were housed in separated metal cages and kept at constant environmental and nutritional throughout the period of experiment. The animals were fed on standard lab animal diet and water was supplied ad-libitum.

### 2.1. Induction of endotoxemia

Endotoxemia was induced by injecting the rats intraperitoneally with a single dose of LPS from *Escherichia coli* (serotype 055:B5) at a dose 200 mg/kg/b.w. (Harbuz et al., 1993).

LPS is manufactured, through the Egyptian International Center for Import Cairo, Egypt.

### 2.2. Preparation and dosage of curcumin

Curcumin was administered orally at a dose of 100mg/kg/b.w daily for 3 weeks (Kalpana and Menon., 2004). Curcumin was dissolved in ethanol, diethyl sulfoxide (DMSO), and purchased it from El-goumhoria CO. for trading chemicals medicines and medical appliances, Egypt.

### 2.3. Experimental design

Animals were randomly divided into three main groups placed in individual cages and classified as follow: Group I: (Control group): 12 rats administered standard lab diet and water was supplied ad-libitum for 3 weeks. Group II: (Endotoxin group): 15 rats injected intraperitoneally (i.p) with a single dose of LPS at dose 200 mg /kg/b.w. Group III: (Endotoxin pre-treated curcumin group): Rats received curcumin orally in a daily dose of 100 mg/kg/b.w for 3 weeks before endotoxin injection. One hour after the last dose of curcumin pretreatment rats were injected intraperitoneally (i.p) with a single dose of endotoxin (200 mg / kg/b.w).

### 2.4. Sampling

#### 2.4.1. Blood samples:

Blood samples for serum separation were collected by ocular vein puncture in dry, clean, and screw capped tubes after overnight fasting from all animals groups (control and experimental groups)

Twice along the duration of experiment at one and 3 hours from the onset of LPS injection. Serum was separated by automatic pipette and received in dry sterile samples tube, then kept in deepfreeze at 20°C until used for subsequent biochemical analysis of

Tumor Necrosis Factor (TNF) according to (Beyaert and Fiers, 1998), Interleukin-6 (IL-6) according to (Chan and Perlstein, 1987), Sialic acid (SA) according to (human sialic acid (SA) ELISA kit (cat No.CSB-Eo6o5h) and Nitric Oxide (NO) according to (Vodovotz, 1996).

#### 2.4.2. Tissue samples

Rats were dissected by decapitation the skull was opened carefully and the brain was removed gently and immediately transferred in to ice cold saline and stored at -20°C for subsequent biochemical analysis. Brain tissues were divided into appropriate portions and 0.5 g from each were homogenized in 5 ml -10% (W/v)- cold phosphate buffer saline (PBS) per gram

tissue, using tissue homogenizer centrifugated at 10,000 r.p.m for 20 minutes at 4 c the result supernatant was assayed for antioxidant enzymes GPx according to (Gross et al., 1967), Catalase activity according to (Luck, 1974) and SOD according to (Kakkar, 1984). Centrifugation at 4000 xg for 15 minutes for estimation MDA according to (Mesbah et al., 2004).

### 2.5. Statistical Analysis

The obtained data were statistically analyzed by one - way analysis of Variance (ANOVA) followed by the duncan multiple test. All analyses were performed using the statistical package for social science (Spss, B.o software, 2009) values  $p < 0.05$  were considered to be significant.

## 3. RESULTS

The obtained data in table (1) revealed that endotoxin treated rats showed significant increase in serum TNF- $\alpha$  and IL-6 and significant decrease in serum NO and SA concentrations at one and 3 hours in comparison with control group. Pretreatment with curcumin endotoxin group significant decreased in serum TNF- $\alpha$ , IL-6 and significant increased in serum NO and SA concentrations at one and 3 hours in comparison with endotoxin group only. The obtained data in table (2) revealed that endotoxin treated rats significantly increased in brain MDA and non-significantly decreased in SOD accompanied with significant increase in GPX and CAT concentrations at one and three hours in comparison with control group. Pretreatment with curcumin significant increase in brain MDA and increase in brain SOD, CAT and GPx concentrations at one and 3 hours in comparison with endotoxin group only.

## 4. DISCUSSION

The obtained data in table (1) revealed that the main value of serum Tumor Necrosis

Factor (NF- $\alpha$ ) and interleukin-6 (IL-6) concentration increased significantly in rat injected with LPS after one and three hours when compared with control group. These results are agreed with (Beulter et al., 1988) who investigated that inflammatory cytokines are released by macrophages and neutrophils in response to a variety of stimuli including endotoxin and gram positive bacteria. Activated kupffer cells where shown to increase proinflammatory cytokines such as (TNF- $\alpha$ ) and (IL-6) (Chiaie et al., 2005). The obtained data in table 1 showed that curcumin pretreatment endotoxin group significantly result in decreased serum (TNF- $\alpha$ ) and (IL-6) at one and three hours after endotoxin injection in rats when compared with endotoxin group. Curcumin can inhibit haemorrhage/resuscitation induced IL-6 production and TNF- $\alpha$  mediated activation of Nuclear Factor Kappa-B (NF-kB) cell proliferation and IL-8 release (Biswas et al., 2005). The obtained data in table (1) revealed that the main value of serum sialic acid (SA) concentration decreased significantly in rat injected with LPS after one and three hours when compared with control group. Sialic acid (SA) is the generic term given to a family of acetylated derivatives of neuraminic acid which occur mainly at terminal positions of glycoprotein and glycolipid oligosaccharide side-chains. Several biological functions have been suggested for SA, such as stabilizing the conformation of glycoproteins and cellular membranes, assisting in cell-cell recognition and interaction, contributing to membrane transport, providing binding sites for ligands for the membrane receptor functions, and affecting the function, stability and survival of glycoproteins in blood circulation (Sumangala et al., 1998). (Sophanpal et al., 2004) suggested that the levels of sialic acid were found to be reduced after injection of endotoxin the decrease due to sialic acid modify bacterial cell surfaces. Sialic acid is produced by the host in the course of inflammation, it is possible that a sialidase negative

Table (1): Effects of pretreatment with curcumin, on serum Tumor Necrosis Factor (TNF), Interleukin 6 (IL-6), Sialic acid and Nitric oxide (NO).

Animal groups		TNF- $\alpha$ pg/ml	IL-6 pg/ml	Sialic acid mg/ml	Nitric oxide (umol/ml)
Control group		83.34 $\pm$ 7.34 <sup>de</sup>	31.91 $\pm$ 5.14 <sup>d</sup>	3.75 $\pm$ 0.17 <sup>b</sup>	36.73 $\pm$ 1.92 <sup>a</sup>
Endotoxin group	1 hr.	232.3 $\pm$ 13.3 <sup>b</sup>	107.17 $\pm$ 5.25 <sup>a</sup>	1.84 $\pm$ 0.07 <sup>e</sup>	14.93 $\pm$ 2.29 <sup>c</sup>
	3 hr.	261.71 $\pm$ 9.41 <sup>a</sup>	96.04 $\pm$ 14.51 <sup>a</sup>	2.15 $\pm$ 0.24 <sup>de</sup>	13.68 $\pm$ 2.50 <sup>c</sup>
Curcumin + Endotoxin group	1 hr.	69.23 $\pm$ 5.19 <sup>e</sup>	56.87 $\pm$ 5.38 <sup>bc</sup>	3.02 $\pm$ 0.10 <sup>c</sup>	30.87 $\pm$ 2.48 <sup>ab</sup>
	3 hr.	102.47 $\pm$ 5.29 <sup>d</sup>	34.76 $\pm$ 7.24 <sup>d</sup>	2.35 $\pm$ 0.12 <sup>de</sup>	34.86 $\pm$ 4.66 <sup>ab</sup>

Mean values with different superscript letters in the same raw are significantly different at ( $P < 0.05$ ) Data are presented as means + S.E. S.E.: Standard Error

Table (2): Effects of pretreatment with Curcumin on Brain Super Oxide Dismutase (SOD), Glutathione peroxidase (GPX), Catalase and L-MDA.

Animal groups		(SOD ) u/g tissue	L-MDA umol/ml	(GPX) n/ g tissue	(CAT) mmol/min/g tissue
Control group		36.00 $\pm$ 0.31 <sup>ab</sup>	90.61 $\pm$ 1.61 <sup>b</sup>	23.57 $\pm$ 1.70 <sup>bc</sup>	42.00 $\pm$ 0.73 <sup>b</sup>
Endotoxin group	1 hr.	27.14 $\pm$ 0.76 <sup>bc</sup>	129.03 $\pm$ 18.61 <sup>a</sup>	10.67 $\pm$ 1.25 <sup>e</sup>	26.86 $\pm$ 1.71 <sup>e</sup>
	3 hr.	19.50 $\pm$ 2.69 <sup>c</sup>	132.15 $\pm$ 7.41 <sup>a</sup>	10.67 $\pm$ 2.11 <sup>e</sup>	30.88 $\pm$ 1.92 <sup>de</sup>
Curcumin + Endotoxin group	1 hr.	35.00 $\pm$ 7.41 <sup>ab</sup>	67.46 $\pm$ 3.30 <sup>bc</sup>	25.21 $\pm$ 1.04 <sup>ab</sup>	50.05 $\pm$ 1.91 <sup>a</sup>
	3 hr.	36.70 $\pm$ 1.54 <sup>a</sup>	63.09 $\pm$ 7.66 <sup>c</sup>	28.27 $\pm$ 0.95 <sup>a</sup>	51.40 $\pm$ 1.28 <sup>a</sup>

Mean values with different superscript letters in the same raw are significantly different at ( $P < 0.05$ ) Data are presented as means + S.E.

bacterium such as E.coli recognize free sialic acid as indicator of inflammation and down regulates expression and adhesions in response (Sopphanpal et al., 2004, 2007). Sialic acid in LPS could have a more indirect effect on cell surface properties of the bacterium it could influence either the general charge on the bacterial surface, especially other cell surface determinates such as fimbriae (Vanhan et al., 1995) or outer membrane (St Geme et al., 1993) contributing to host interaction the serum resistance. The obtained data in table 1 showed that curcumin pretreatment endotoxin group significantly increased sialic acid (SA) concentrations at one and three hours after endotoxin injection in rats when compared with endotoxin group only. (Eguchi, et al., 2005) indicated that the

glycosidic linkage of sialic acid is a potential target for superoxide and other related ROS. The free radicals activate NF- $\kappa$ B leading to an increase in production of TNF- $\alpha$  followed by eventually by tissue damage. (Dey and Cederbaum, 2006). The increase in the serum SA reflects increased sialylation of glycoproteins or glycolipids due to increased sialotransferase activity and or increased secretion of sialic acid from the cell membrane due to elevated of sialidase activity (Knuiman et al., 2004). In present study, the administration of curcumin cause increasing in serum sialic acid (Ou et al., 2013) reported that curcumin inhibits type A influenza virus (IVA) interfering with viral haemagglutination (HA) activity. Moreover stimulation curcumin with HA structure

revealed that curcumin binds to the region constituting sialic acid anchoring residues, supporting the results obtained by inhibition HA activity assay. The loss of the HA activity of curcumin treated influenza viruses suggests curcumin interrupts the link between the viral HA molecule and its cellular receptor by preoccupying the binding site on HA protein or by modification of the virus envelope. (Chen, XU and Johnson, 2006). The obtained results revealed that in table (1) revealed that the main value of serum and brain Nitric oxide (NO) concentration decreased significantly in rat injected with LPS after one and three hours when compared with control group. (Young and Yu., 2000) found that the release and activity of NO is reduced in LPS causing dysfunction of smooth muscle system and reduced vascular relaxation with consequent dysfunction. This function is attributable to high levels of free radicals that inactivate NO and or reduced its expression there for supplementation with antioxidants may have a positive effect in preventing the complications of rats injected LPS. (Young and Yu., 2000). The obtained data in table 1 showed that curcumin pretreatment endotoxin group significantly decreased serum NO concentrations at one and three hours after endotoxin injection in rats when compared with endotoxin group only. The present study suggests that increased NO is protected and treated period may be curcumin has been found attenuate various behaviour and biochemical alternation induced by chronic fatigue. Chronic fatigue represents one of core symptoms of depression. In one of the studies animals exposed to chronic fatigue demonstrated an increase in the immobility period in the forced swim test which was reversed by daily administration of curcumin (5-60 mg/kg) further curcumin attenuated chronic fatigue induced alternations in various oxidative stress parameters such as enhanced lipid peroxidation nitrite TNF- $\alpha$  and reduced glutathione levels thus

showing the antioxidant property (Gupta *et al.*, 2009). The obtained results revealed that in table (1) revealed that the main value of serum and brain L-MDA concentration increased significantly in rat injected with LPS after one and three hours when compared with control group. (Igabavboa *et al.*, 1989) reported that, formation of lipid peroxides in the crude homogenates resulted in response to administration of LPS. This may be due to an enhanced generation of O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> that accelerated peroxidation of native membrane lipids. peroxidation of mitochondrial membrane lead to loss of cell integrity increase in membrane permeability and alternation of Ca<sup>++</sup> homeostasis that contribute to cell death due to alternation in the inner membrane (Igabavboa *et al.*, 1989). The obtained data in table 1 showed that curcumin pretreatment endotoxin group significantly decreased serum and brain L-MDA concentration at one and three hours after endotoxin injection in rats when compared with endotoxin group only. These results are confirmed with (Sreejayan *et al.*, 1994) who claimed that curcumin inhibit iron-catalyzed lipid peroxidation in rat brain homogenates by chelation of iron an increasing number of studies have now established the ability of curcumin to mainly eliminate the hydroxyl radical, superoxide radical, singlet oxygen, nitrogen dioxide and nitric oxide. The obtained data in table (3) revealed that the main value of brain SOD (superoxide Dismutase, GPx (glutathione peroxidase) and CAT (catalase) concentration increased significantly in rat injected with LPS after one and three hours when compared with control group. These results are agreed with the recorded data of (Gilad., 1996) who indicated that, no changes was observed in the activity of glutathione peroxidase in the brain of rats exposed to LPS. Moreover, (Aydin *et al.*, 2010) found that no change in GSH-PX enzyme activity in LPS rats determined compared to control group. Decreased GSH-PX activities or its hidden may indicate that high amount of hydrogen

peroxide which may have accumulated in the cells increased hydrogen peroxide may be transformed to hydroxyl radicals and increase oxidative stress also (Gilad., 1996) reported that LPS exposure resulted significant reduction in superoxide dismutase in liver, kidney and brain this is due to superoxide dismutase or ubiquitous metalloproteins that are considered fundamental in the process eliminating superoxide by reducing it (adding electron to) form oxygen and H<sub>2</sub>O<sub>2</sub> (European bioinformatics Institute., 2009). SOD has a substantial role on the metabolism of O<sub>2</sub> anions (Faraei and Didion., 2004). (Gilad et al., 1996) reported that the activity of catalase was significantly depleted in liver, kidney, brain due to LPS exposure this significant decrease in the activity of catalase in LPS rats as ascribed as that catalase is the enzyme which protects the cells from the accumulation of hydrogen peroxide by dismutating it from water and oxygen or by using it as antioxidant in which it works as peroxide (Harishekar., 2012). The obtained data in table 1 showed that curcumin pretreatment endotoxin group significantly increased brain SOD, GPX and catalase concentrations at one and three hours after endotoxin injection in rats when compared with endotoxin group only. (Manikandan et al., 2011) recorded that curcumin treatment induced increase in LPO levels by significant increasing the activities of GSH, GPX, SOD, CAT and GST in the rat kidney tissue. Curcumin effect in restoring these antioxidant enzyme activities ( Reddy and Lokesh., 1994<sup>b</sup>) this observed antilipo effect of curcumin may be attributed to the redox metal binding activity (Yang et al., 2005) free radical scavenging properties (Mastuda et al., 2001) and antioxidative potential (Kelloff et al., 1996).

#### ***Conclusion & recommendations***

From the obtained results, it could be concluded that, the present study demonstrated that Curcumin provided an effective in protection against lipid

peroxidation, oxidative damage in brain and liver tissues and decrease inflammatory response to endotoxin injection in rats. We recommend that administration of diet rich in the antioxidants curcumin is very important for protection of different body tissue specially liver and brain tissues against oxidative stress or even inflammatory or erosion.

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## الدور الوقائي للكرمك على الاجهاد التأكسدي المحدث بالاندوتوكسين في الجرذان

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قسم الكيمياء الحيوية – كلية الطب البيطري – مشتهر-جامعه بنها

### الملخص العربي

في هذه الدراسة تم تقييم التأثير الوقائي لمادة الكرمك على التغيرات في مستوى أكسيد النيتريك وحمض السياليك، عامل نتخروم الفأ، انترلوكين-6 والأكسدة الفوقية للدهون (تركيز المالون داي الدهيد) والانزيمات المضادة للأكسدة في دم وانسجه المخ في الجرذان المستحدث فيها التسمم البكتيري بماده التوكسين. هذا وقد تم استخدام لإجراء هذه الدراسة على عدد 42 من ذكور الجرذان البيضاء اعمارهم تتراوح من 12-16 أسبوع واوزانها 200-250 جرام وقد قسمت الى ثلاث مجموعات وتم توزيعها كالاتي: -المجموعة الاولى (المجموعة الضابطة): اشتملت على 12 فأر لم تعطى اى ادويه واستخدمت كمجموعه ضابطه للمجموعات الأخرى. المجموعة الثانية (المجموعة المحدث بها التسمم البكتيري) تكونت من 15 فأر تم حقنهم بماده الاندروتوكسين في الغشاء البريتوني بجرعه واحده 200 ملجم لكل كيلوجرام من وزن الجسم. والمجموعة الثالثة (مجموعه الكرمك المحدث بها الاندروتوكسين) اشتملت على 15 فأر تم تجريعهم بماده الكرمك يوميا عن طريق الفم بجرعه مقدارها 100 ملجم لكل كيلو جرام من وزن الجسم لمدة 21 يوم ثم حقنهم بماده الاندروتوكسين في الغشاء البريتوني بجرعه مقدارها 200ملجم لكل كيلو جرام من وزن الجسم وقد تم تجميع عينات الدم والأنسجة في اليوم الثاني والعشرين من بداية التجربة بعد ساعه وثلاث بعد تجريعها بماده الاندروتوكسين وقد أسفرت نتائج التحليل البيوكيميائي عن وجود انخفاض في كلا من اكسيد النيتريك وحمض السياليك بالدم وايضا نقص في نشاط الجلوتاثيون بيروكسيداز، سوبر اكسيد ديسميوتيز ، انزيم الكتاليز في انسجه المخ في الفئران وزياده كلا من عامل نتخروم الفأ ، انترلوكين -6 بالدم والأكسدة الفوقية للدهون (تركيز المالون داي الدهيد) بأنسجة مخ الفئران بعد ساعه وثلاث ساعات عند مقارنتها بالمجموعة الضابطة. كما أوضحت النتائج ان مجموعه الجرذان المحدث بها التسمم البكتيري والتي تم وقايتها بماده الكرمك أظهرت زياده كلا من اكسيد النيتريك وحمض السياليك بالدم بالإضافة الى زياده سوبر اكسيد ديسميوتيز، انزيم الكتاليز وانزيم جلوتاثيون بيروكسيداز في انسجه مخ الفئران كما اسفرت النتائج عن نقص معامل تركيز عامل نتخروم الفأ، انترلوكين-6 والأكسدة الفوقية للدهون (تركيز المالون داي الدهيد) بعد ساعه وثلاث ساعات من تجريعها بماده التوكسين عند مقارنتها بالمجموعة المحدث بها الاندوتوكسين . اوضحت الدراسات ان استخدام الكرمك كماده واقية مضادة للأكسدة كان لها دور فعال في حماية الجرذان من التسمم البكتيري المحدث تجريبيا باستخدام ماده التوكسين وادي استخدامها للحفاظ على نسب القياسات البيوكيماوية في الدم والأنسجة لما يقارب النسب الطبيعية لذلك توصي الدراسة بضرورة استغلال المزايا الهائلة للكرمك كماده وقائية مضادة للأكسدة وادخاله كماده فعاله في صناعه العقاقير الطبية المستخدمة في وقاية وعلاج التسمم البكتيري.

(مجلة بنها للعلوم الطبية البيطرية: عدد 26(2):177-185, يونيو 2014)