



## PROTECTIVE EFFECT OF RUTIN ON SOME BIOCHEMICAL PARAMETERS IN A RAT MODEL OF ENDOTOXEMIA.

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### ABSTRACT

The objective of this study is to investigate the neuroprotective and antioxidant effects of subsequent pretreatment with Rutin as natural antioxidant on biomarkers of oxidative stress and inflammatory response in brain tissue induced by endotoxemia. This study was carried out 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. bwt, group III rats administered rutin orally at a dose 200 mg/kg b.wt/ day for three weeks and then injected (i.p) with a single dose of LPS at a dose 200 mg/kg/B.w. Blood samples for serum separation and brain tissues were collected from all animal groups two times at one and three hours from the onset 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 results revealed that, endotoxemia could potentially significantly increased serum TNF, IL-6 and brain L-MDA. Also induced a significant decrease in serum (SA), NO, and GPX, CAT, and SOD in brain tissues. Treatment with Rutin significant decrease in serum TNF, IL-6, and brain L-MDA. Also induced significant increase in serum (SA), NO, and GPX, SOD, and CAT in brain tissues from the obtained results it could be concluded Rutin may be protects against lipid peroxidation, oxidative stress and decrease the inflammatory response to endotoxin.

**KEYWORDS:** Endotoxin, Rutin, Oxidative stress, Inflammatory response, Antioxidant status.

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### 1. INTRODUCTION

Lipopolysaccharide (LPS) is a glycolipid component in the cell wall of gram-negative bacteria including deleterious effects on several organs including the liver and eventually leading to septic shock and death. Endotoxemia. Induced hepatotoxicity is characterized by disturbed intracellular redox balance, excessive Reactive oxygen species (ROS) and accumulation including DNA, proteins and membrane lipid damages. (Sebai et al., 2010). Moreover, lipopolysaccharide is a potent inducer of the host immune system, including the overproduction of numerous pro and anti-inflammatory cytokines, an increase in oxidative stress, and the

induction of nitric oxide synthase (iNOS) and hemeoxygenase -1 (HO-1) together, these events result in severer tissue injury. Furthermore, LPS causes endotoxemia, which is associated with multiple organ failure and is often lethal (Takamiya et al., 2009). In recent years, there are renewed interest in the treatment against different diseases using herbal drugs 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). Rutin quercetin-3-rhamnosylglucoside is a kind of flavonoid glycoside found in buckwheat, many

vegetables, fruits, and plant. Derived beverages such as tea and wine (Manach et al., 2003). Rutin is also known as vitamin P and has antiplatelet, antiviral and antihypertensive properties as well as strengthen the capillaries, which is the result of its high radical scavenging activity and antioxidant activity (Guo et al., 2007).

## 2. MATERIALS AND METHODS

Forty-two white male albino rats of 8-10 week old and weighting 150 -180 gm were housed in separated metal cages and kept at constant environmental and nutritional throughout the period of experiment. The animals were fed on constant ration 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 200mg/kg/b.w (Harbuz et al., 1993).

LPS is manufactured in Sigma Chemical CO. (St. Louis, MO, USA) and purchased from Schneldorf, Germany through the Egyptian international center for import Cairo, Egypt.

### 2.2. Preparation and dosage of rutin

Rutin was administered orally at a dose of 200mg/kg/b.w daily for 3 weeks (Tongjaroenbuanyam et al., 2011). Rutin was dissolved in propylene glycol solution and administered to rats at a dose level of (200 mg/Kg b.wt/P.o) once daily for 21 days (Abdel-Raheem, I.T. 2010). Rutin was manufactured by Epico (Egyptian International pharmaceutical Industries Company).

### 2.3. Experimental design

Animals were randomly divided into three main groups placed in individual cages and classified as follows: Group I: (Control group): 12 rats administered constant ration and water was supplied ad-libitum for 3 weeks. Group II: (Endotoxigen group): 15 rats injected intraperitoneally (i.p) with a single

dose of LPS at dose 200 mg /kg/b.w. Group III: (Endotoxin pretreated rutin group): 15 rats received rutin orally in a daily dose of 200 mg/kg/b.w for 3 weeks. Before endotoxin injection. Two hours after the last dose of rutin 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 animal 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 sample tube, then kept in deep freeze at -20°C until used for subsequent biochemical analysis for Tumor Necrosis Factor (TNF) according to (Beyaert and Fiers, 1998), Interleukin – 6 (IL-6) according to (Chan, and Perlstein, 1987), Sialic acid according to human sialic acid (SA) ELISA kit (cat NO.CSB-Eo6o5h) and Nitric oxide according to (Vodovotz, 1996).

#### 2.4.2. Tissue samples (Brain and liver).

Rats were dissected by decapitation, the skull was opened carefully, quickly removed, cleaned by rinsing with cold saline 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 gm from each were homogenized in 5 ml- 10 % (W/v) –cold phosphate buffer saline (PBS) per gram tissue using tissue homogenizer centrifuged at 10,000 r.p.m. for -20 minutes at 4°C. The resulting supernatant was assayed for antioxidant enzyme GPX according to (Gross, et al., 1997), Catalase activity (Luck, 1974) and SOD according to (Kakkar, 1984). Centrifuge at 4000 r.p.m. for 15 minutes for estimation of L-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.

Table (1): Effects of pretreatment with rutin 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>
Rutin + Endotoxin group	1 hr.	176.31 $\pm$ 6.80 <sup>c</sup>	31.94 $\pm$ 6.00 <sup>d</sup>	1.83 $\pm$ 0.07 <sup>e</sup>	25.04 $\pm$ 1.18 <sup>b</sup>
	3 hr.	220.88 $\pm$ 9.53 <sup>b</sup>	38.19 $\pm$ 6.12 <sup>cd</sup>	2.45 $\pm$ 0.19	34.50 $\pm$ 2.15 <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.

Table (2): Effects of pretreatment with rutin on Brain Super Oxide Dismutase (SOD), Glutathione Peroxidase (GPX) and Catalase. (CAT).

Animal groups		(SOD) u/g tissue	(GPX) n/g tissue	(CAT) mmol/min/ g tissue	L-MDA umol/ml
Control group		36.00 $\pm$ 0.31 <sup>ab</sup>	23.57 $\pm$ 1.70 <sup>bc</sup>	42.00 $\pm$ 0.73 <sup>b</sup>	90.61 $\pm$ 1.61 <sup>b</sup>
Endotoxin group	1 hr.	27.14 $\pm$ 0.76 <sup>bc</sup>	10.67 $\pm$ 1.25 <sup>e</sup>	26.86 $\pm$ 1.71 <sup>e</sup>	129.03 $\pm$ 18.61 <sup>a</sup>
	3 hr.	19.50 $\pm$ 2.69 <sup>c</sup>	10.67 $\pm$ 2.11 <sup>e</sup>	30.88 $\pm$ 1.92 <sup>de</sup>	132.15 $\pm$ 7.41 <sup>a</sup>
Rutin + Endotoxin group	1 hr.	34.00 $\pm$ 0.65 <sup>ab</sup>	17.22 $\pm$ 0.06 <sup>d</sup>	34.67 $\pm$ 1.96 <sup>cd</sup>	76.85 $\pm$ 5.46 <sup>bc</sup>
	3 hr.	32.00 $\pm$ 1.73 <sup>ab</sup>	18.12 $\pm$ 1.56 <sup>d</sup>	38.38 $\pm$ 0.92 <sup>bc</sup>	71.30 $\pm$ 10.23 <sup>bc</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.

### 3. RESULTS

The obtained data in table (1) revealed that endotoxin treated rats had significantly increased in serum TNF- $\alpha$ , IL-6 and significantly decreased in serum NO and SA concentrations at one and three hours in comparison with control group. Pretreatment with rutin 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 L-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 rutin significant decrease in brain L-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 (TNF- $\alpha$ ), and (IL-6) concentrations increased significantly in rat injected with LPS after one and three hours when compared with control group. LPS can trigger groups of different toll – like receptors (TLR) especially TLR4 expressed on the cell surface of immune cells ( Sweet et al., 1996). The binding of LPS to TLR4 triggers downstream signaling cascades, including mitogen activated protein kinases (MAPKS) (Doyle et al., 2006). the nuclear transcription kappa –B (NFkB) pathway which lead to the production of inflammatory mediators from macrophages such as (TNF- $\alpha$ ), (IL-1), (IL-6) and Nitric oxide (NO) (Takeda et al., 2008). The obtained data in table 1 showed that Rutin pretreatment endotoxin group significantly decreased serum (TNF- $\alpha$ ) and (IL-6) at one and three hours after endotoxin injection in rats when compared with endotoxin group. (Kwon et al., 2005) reported that rutin has anti-inflammatory effect and influence on the cytokine patterns reducing IL-1B, IL-6, IL-10, IL-13 and TNF- $\alpha$  in mice. (Bashkar et al., 2011) showed that cells of BWE (flavonoid compound including rutin and quercetin) may interrupt the interaction in LPS to toll – like receptor ( TLR4) activation to produce various inflammatory mediators like NO, COX-2 and inflammatory cytokines like IL-6, TNF- $\alpha$  and IL-1B and chemokines CCL2 / MCP1. The obtained data in table (1) revealed that the main value of serum (SA) concentration decreased significantly in rat injected with LPS after one and three hours when compared with control group. (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) 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 bacterium such as E.coli recognize free sialic acid as indicator of inflammation adhesions in response (Sophanpal 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 Rutin pretreatment endotoxin group significantly increased serum and sialic acid (SA) concentrations at one and three hours after endotoxin injection in rats when compared with endotoxin group only. However, most sialic acid is abundant as the terminal sugar of sialoglycoprotein and sialoglycolipids in vivo. Further (Egushi et al., 2005) indicated that, the glucosidic linkage of sialic acid is a potential target of superoxide and other related ROS. The free radicals activate NFkB leading to increase in production of TNF- $\alpha$  followed eventually by tissue damage (Dey and Cederbaum, 2006). One of the hallmark cellular responses to influenza virus infection is the activation of transcription factor NFkB signaling by the action of double stranded viral RNA and viral protein. (Aggarwal & Schishodia 2004). Sialidase (neurominidase) catalyzes the hydrolysis of sialic acid residues from sialoglycoconjugates and may have an effect on biological functions such as antigen presentation and receptor function. Mouse liver sialidase was non competitively inhibited isoscutellurein-8-o-glucuronide (Ic50, v influenza virus

sialidase was only weakly inhibited (Nagi *et al.*, 1990). The obtained data in table (1) revealed that the main value of serum 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 rutin pretreatment endotoxin group significantly decreased serum NO concentrations at one and three hours after endotoxin injection in rats when compared with endotoxin group only. In present study suggests that increased NO is protected and treated period may be Rutin has been found attenuate various behaviour and bio chemical 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 (5-60mg/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 data in table (1) revealed that the main value of brain L-MDA concentration increased significantly in rat injected with LPS after one and three hours when compared with control group. (Kheir el-din *et al.*, 2001) discussed that, stress elicited by LPS administration alters the oxidative status of liver and the most prominent among this evidence was the increase in free radical generation, as

reflected by an increase in brain MDA. Even though (Liu *et al.*, 2000) proposed that, MDA an end product of lipid peroxidation was shown to increase in the mitochondria from rat liver in response to exhaustive tread mill running in rats.

The obtained data in table 1 showed that Rutin pretreatment endotoxin group significantly decreased brain L-MDA concentration at one and three hours after endotoxin injection in rats when compared with endotoxin group only. (Russo *et al.*, 2000) Rutin treated groups showed dose dependent significant decrease in MDA levels this may be due to the acute antioxidant effects of the bioflavonoid rutin that showed maximum benefit, higher scavenger efficiency more antioxidant activity, which seems to be correlated to the catechol structure of the ring B, the 2, 3 double bond in conjugation with a 4-oxo function and the presence of both 7- and 5-hydroxyl groups. The obtained data in table (3) revealed that the endotoxin treated rats non significantly decreased brain SOD and significantly decreased brain GPX and, CAT concentrations after one and three hours in comparison with control group. These results are agreed with the recorded data of (Gilad., 1996) who indicated that, no changes were 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 hydroxyl radicals 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 on the metabolism of O<sub>2</sub> anions (Faraei and Didio., 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 Rutin 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. (Wei et al., 2011) Rutin possesses an antioxidant activity that can lead to increased antioxidant enzymes SOD, CAT and GPX activities. (Arjumand et al., 2011) these three cytoprotective enzymes enhancement, that can prevent damage by detoxifying ROS, may be attributed to rutin protective action on LPO, and to its enhancing effect of the cellular antioxidant defense contributing to the protection against oxidative damage. (Mahmoud, 2011).

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

In conclusion, the present study demonstrated that rutin 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 recommended 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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## التأثير الوقائي للروتن على بعض المكونات الكيميائية الحيوية لنموذج التسمم البكتيري في الجرذان

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

### الملخص العربي

في هذه الدراسة تم تقييم التأثير الوقائي لماده الروتن على التغيرات في مستوى أكسيد النيتريك وحمض السيليك، عامل نتخرورم الفا، انترلوكين-6 والأكسدة الفوقية للدهون (تركيز المالون داى 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 أي ادويه واستخدمت كمجموعه ضابطه للمجموعات الاخرى. المجموعة الثانية (المجموعة المحدث بها التسمم البكتيري) تكونت من 15 فأر تم حقنهم بماده الاندروتوكسين في الغشاء البريتوني بجرعه واحده 200 ملجم لكل كيلو جرام من وزن الجسم. والمجموعة الثالثة (مجموعه الروتن المحدث بها الاندروتوكسين) اشتملت على 15 فأر تم تجريعهم بماده الروتن يوميا عن طريق الفم بجرعه مقدارها 200 ملجم لكل كيلو جرام من وزن الجسم لمدة 21 يوم ثم حقنهم بماده الاندروتوكسين في الغشاء البريتوني بجرعه مقدارها 200 ملجم 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 السيليك بالدم وايضا نقص في نشاط الجلوتاثيون بيروكسيديز، سوبر اكسيد ديسميوتيز، انزيم الكتاليز في انسجه المخ في الفئران وزياده كلا من عامل نتخرورم الفا، انترلوكين-6 بالدم والأكسدة الفوقيه للدهون (تركيز المالون داى 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 الروتن أظهرت زياده كلا من اكسيد النيتريك وحمض السيليك بالدم بالإضافة الى زياده سوبر اكسيد ديسميوتيز، انزيم الكتاليز وانزيم جلوتاثيون بيروكسيديز في انسجه مخ الفئران كما اسفرت النتائج عن نقص معامل تركيز عامل نتخرورم الفا، انترلوكين-6 والأكسدة الفوقيه للدهون (تركيز المالون داى الدهيد) بعد ساعه وثلاث ساعات من تجريعها بماده التوكسين عند مقارنتها بالمجموعة المحدث بها الاندوتوكسين. اوضحت الدراسات ان استخدام الروتن كماده واقية مضادة للأكسدة كان لها دور فعال في حماية الجرذان من التسمم البكتيري المحدث تجريبيا باستخدام ماده التوكسين وأدى استخدامها للحفاظ على نسب القياسات البيوكيماوية في الدم والأنسجة لما يقارب النسب الطبيعية لذلك توصى الدراسة بضرورة استغلال المزايا الهائلة للروتن 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):191-199, يونيو 2014)