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Effects of *Rosmarinus Officinalis* Extract on Induced Nitric Oxide and Stimulated Proinflammatory Mediators Via- Bacterial Lipopolysaccharide (LPS)

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Abstract: The effect of Rosemary (*Rosmarinus officinalis*) ethanolic extract on inflammatory mediators stimulated by bacterial lipopolysaccharide (LPS) has been investigated *in vivo* and *in vitro* studies. When the administration of various Rosemary ethanolic extract significantly suppressed various proinflammatory mediators, the highly phytochemical mixtures specially phenolic compounds reflected an inhibitory effect on Cyclooxygenase-2 (Cox_2) activity and oxidative burst induced via LPS administration, since Rosemary extract significantly decrease Nitric oxide (NO), prostaglandin-E2 (PGE2) and malonaldehyde (MDA) contents, the crude extract also acted as a cytoprotective agent when reflected a Free radical – scavenging activity that elicited widespread damage to cell constituents such as membrane lipids and significantly increased the normal cells viability and the antioxidant enzymes activity superoxide dismutase (SOD) and Glutathione reductase (GR) combined with a significant increase in the reduced (glutathione GSH) contents throw attenuation of the induced inflammation and NO dependent signaling mediators via-lipopolysaccharide-activated macrophages. Supplementation with these natural extracts may prove valuable in limiting the pathophysiology of numerous disorders associated with oxidative damage and inflammation.

Key words: Rosemary extract; inducible nitric oxide synthase; proinflammatory mediators and antioxidant enzymes.

INTRODUCTION

Extracts from Rosemary (*Rosmarinus officinalis*) strengthens the nervous system, stabilizes emotions, and minimizes mood swings. It is an effective antiseptic. Usually found in hair and skin products. Today, the Rosemary extract is prescribed for treatment of disorders of some diseases and neuronal hypoxia, both of which have etiologies associated with oxidative stress (Kleijnen and Knipschild, 1992; Itil and Martorano, 1995; Oken et al., 1998 and Pitchumoni and Doraiswamy, 1998).

Rosemary is a potent free radical scavenger and inhibitor of NADPH-oxidase (Maitra *et al,*1995 and Pincemail *et al*., 1987). The extract inhibits nitric oxide (NO) production in lipopolysaccharide-activated macrophages by concomitantly scavenging NO accumulation.(Kobuchi and Packer, 1997 and Marcocci *et al.*, 1994).

Humans are continuously exposed to different kinds of chemicals such as food additives, industrial chemicals, pesticides and other under-irable contaminants in the air food and soil (Hasegawa *et al*., 1995). Most of these chemicals induce a free radical-mediated lipid peroxidation leading to disruption of biomembranes and dysfunction of cells and tissues (Cho *et al*., 2003). Therefore lipid peroxidation is a crucial step in the pathogenesis of free radical-related diseases including inflammatory injury hepatic dysfunctions. Free radical reactions are associated with a range of degenerative diseases and acute ischemic insults affecting liver and other tissues, several human diseases have been associated with the overproduction of reactive oxygen species (ROS) (Gőksel *et al.*, 2006).

During oxidative stress, reactive oxygen species, superoxide anion ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2) can elicit widespread damage to cell constituents such as membrane lipids. These free radical combines with the cellular lipid and proteins to produce lipid peroxidation, measured through its catabolite malonadyaldhyde (MDA), resulting in structural changes of endoplasmic reticulum and other biomembranes and loss of metabolic activity leading to cellular damage (Kadiiska *et al.*, 2000).

Lipid peroxidation is a free radical-mediated chain reaction, since it is self-perpetuating. The length of the propagation depends upon chain breaking antioxidant, such as the enzymes superoxide dismutase, catalase and glutathione peroxidase (Diniz *et al.*, 2003).

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On the contrary, at low concentrations, ROS, including nitric oxide (NO) and superoxide anion ($O_2^{\bullet -}$), play a crucial role as regulatory mediators in cellular signaling processes (Remacle *et al*., 1995) and in many physiological functions such as regulation of vascular tone, monitoring of oxygen tension in the control of ventilation, signal transduction from membrane receptors, and erythropoietin production (Droge, 2002). Another important physiological function of ROS is the phagocyte-dependent killing of pathogens during the respiratory burst, a process characterized by an increase in oxygen consumption, activation of a metabolic hexose monophosphate shunt, and the generation and release of chemically reactive oxygen metabolites (Ramarao *et al*., 2000). Additionally, ROS generated either extracellularly or intracellularly through legend–receptor interactions can function as signal transduction molecules to activate cytokine production and modulate genome expression by specific and precise mechanisms during cell activation (Roebuck, 1999).

Nitric oxide (NO) is a short-lived free radical with many well-known physiological functions gas and highly reactive free radical, is produced by monocytes and macrophages upon exposure to LPS; one these are its abilities to act as either a cytoprotective or a cytotoxic agent (Bogdan, 2001). It is accepted that the difference between the protective and the deleterious effects of this free radical is determined both by concentration and time of exposure and by cell type (Leong *et al*., 2002).

NO plays an important role in host defense. Overproduction of NO and its metabolites, however, has been implicated in the pathogenesis of conditions such as bacterial sepsis and chronic inflammation (Laskin and Pendino, 1995 and Evans *et al*., 1993). The resultant nitrotyrosine is used as a marker of peroxynitrite or at least nitration reactions in gastritis (Evans *et al*., 1993), inflammatory bowel disease (Yoshimi *et al*., 2001 and Ichiki *et al*., 1998) as well as general inflammation (Lowenstein *et al*., 1993). While we have demonstrated that peroxynitrite-induced apoptosis of macrophages and colon epithelial cells is reduced by ascorbic acid (Xie *et al*., 1994), it is not known if other dietary antioxidants exert similar benefits or if they are present in epithelial cells from the upper gastrointestinal tract. However, flavonoids and related phenolic compounds have been described.

Currently, there is increasing interest in therapeutic use of antioxidants to prevent tissue damage induced by overproduction of ROS, by reducing free radical formation or by scavenging or promoting the breakdown of these species (Young and Woodside, 2001; Collins, 1999 and Cuzzocrea *et al*., 2001). Experiments in different *in vitro* and *in vivo* systems have demonstrated the potent antioxidant action of plant polyphenols (Damianaki *et al*., 2000), and it has been suggested that they can prevent oxidative-stress-related diseases (Aucamp *et al*., 1997 and Chung *et al*., 1998).

Dietary antioxidants confer significant protection to normal cells from pro-apoptotic oxidant stress. The phytochemical mixtures found in the teas, cat's claw and green tea, appear to be more effective than vitamin C in some cell lines and at concentrations that suggest that they may be acting at levels distinct from the mere of the oxidant signal. Diet supplementation with these or related antioxidants may prove valuable in limiting disorders associated with inflammation.

In the present study, we have demonstrated that Rosemary extracts inhibit ROS production in rat peritoneal macrophages stimulated *in vitro* with LPS; however, little is known about the molecular bases of these inhibitory effects, or about the effects of Rosemary extracts on other important targets of oxidant stress.

We also investigated the molecular bases of the effects of Rosemary extracts, firstly *in vitro* studies to assess whether it scavenges $O_2^{\bullet -}$, and secondly in studies of effects on MDA formation and antioxidant defense enzymes. We also investigated its possible regulatory effect on inflammation processes.

MATERIAL AND METHODS

Plant Material:

Leaves samples of *Rosmarinus officinalis* (Rosemaryaceae) were collected in May (2008) from the Faculty of agriculture , Giza, Egypt. All leaves were washed then air dried in shade at room temperature for 5–7 days, grinded to fine powder and kept for phytochemical analysis.

Plant Extracts:

50 g of the air–dried powdered leaves of Rosemary were extracted separately by percolation in ethanol 70 % (Et), extracts were combined, filtered, and then freeze dried.

Quantitative Determination of the Phytochemicals in the Rosemary Extracts:

Total tannins, saponins, flavonoids, and phenolic compounds were determined according to the methods described by Balbaa, 1974; Ebrahimzadeh and Niknam, 1998; Zhuang *et al*., 1992 and Swain and Hillis, 1959 respectively.

Determination of Superoxide Radical Scavenging Activity:

Superoxide radical scavenging activity by various *Rosmarinus officinalis* extracts was determined according to Chung, *et al*.

Spleen Cell Suspension Preparation:

In order to prepare a single-cell suspension of spleen cells (splenocytes), spleens were collected aseptically and gently mashed between frosted ends of two sterile microscope slides. Unless otherwise indicated, splenocytes were resuspended in Roswell Park Memorial Institute (RPMI) 1640 media supplemented with 10% fetal bovine serum (FBS), 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 5% sodium bicarbonate, 50 mg/ml gentamicin, and 10 mM 2-mercaptoethanol (2-ME) (complete media) prior to adjusting cell concentrations as needed (Moldeus *et al.*, 1978)

Cell Culture:

Cells were cultured in phenol red free DMEM containing 50 units/ml penicillin, 50 mg/ml streptomycin, 44 mM sodium bicarbonate and 10% fetal bovine serum at 37°C in humidified air containing 5% CO_2, cells were plated in 1 ml of media in 24 well plates, cultured for 2 days then treated. In all cases, cells were washed and fresh complete media added before the indicated treatments. Stock solutions of Rosemary extracts were prepared in DMSO, the final concentration of DMSO did not exceed 0.5% (Moldeus *et al.*, 1978)

Cell Viability:

Cell viability was determined by MTT assay according to Heras *et al.*, 2001 after 24 h exposure to various concentrations of 10, 25, 50, or 100 mg/ml Rosemary leaves extracts. 5 mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide was added to 0.1 mL of cell suspension for 4 h, and the formazan formed was then dissolved in isopropanol. Optical density was measured using a plate reader at 570 nm .

Cell Culture and Treatments:

Cells were cultured in phenol red free DMEM containing 50 units/ml penicillin, 50 mg/ml streptomycin, 44 mM sodium bicarbonate and 10% fetal bovine serum at 37°C in humidified air containing 5% CO_2. cells were plated in 1 ml of media in 24 well plates, cultured for 2 days to 80% confluency (approximately 1×10^6 cells/well) then treated. with various LPS (0, 0.1, 1, 5 and 10 µg/mL) and Rosemary extracts 10, 25, 50, or 100 mg/ml Rosemary extract combinations In all cases, cells were washed and fresh complete media added before the indicated treatments. Stock solutions of Rosemary extracts were prepared in DMSO, the final concentration of DMSO did not exceed 0.5% (Ridnour *et al.*, 2000).

Protein Concentration:

The total protein concentration in the samples was measured by the method of Bradford (Bradford, 1976) with standard curves prepared using bovine serum albumin.

Biochemical Analysis:

The superoxide dismutase enzymes activity (SOD) was measured by the method of Santamar á *et al.,* 2003. The Glutathione reductase (GR) activity was measured according to Carlberg and Mannervik, 1975.

Determination of Superoxide Radical Scavenging Activity:

Superoxide radical scavenging activity of rutin standard expressed as % inhibition and different Rosemary extract concentrations using nitro blue tetrazolium reducing activity according to the method described by (Doke, 1983).

Determination of Total Glutathione:

The level of total acid-soluble SH compound (glutathione GSH) was determined with Ellman's reagent according to De Vos *et al*., 1992. The buffer were mixed with 630µl of 0.5 M K_2HPO_4 and 25 µl of mM 5, 5'–dithiobis (2-nitrobenzoic acid) (final pH 7). The absorbance at 412 nm was read after 2 min. GSH was used as a standard.

COX-2 Assays with Purified Enzymes:

For the determination of COX-2 activity, 900 µl of suspension was incubated at 37°C with 10 µl of acetylsalicylic acid (1 mg/ml in PBS), 2 µl of LPS 5 mg/ml in DMSO), and 100 µl PBS for 24 hr. Controls

were prepared by incubation of 900 µl of cell suspension with 10 µl of acetylsalicylic acid, 2 µl of DMSO, and 100 µl PBS. The reaction was terminated by quickly chilling on ice. Samples was separated by centrifuging, stored at -20°C, and PGE2 levels were determined (Nicolaas *et al.*, 2000).

Measurement of PGE_2 Production:

Cells were seeded in 96-well plates ($2x10^6$ cells/well) and stimulated with LPS (100 ng/ml) with or without rosemary extract for 16 h. The level of PGE_2 production was measured in cell culture supernatants of peritoneal macrophages by ELISA (Assay Designs, Ann Arbor, MI, USA).

Determination of Malonaldehyde (MDA):

Lipid peroxidation was measured according to the method of Burits and Bucar, 2000.

Measurement of Nitrite:

Six hours after LPS-treatment, the stable end products of L-arginine-dependent NO synthesis, nitrate and nitrite were measured cell culture medium using the Griess reaction as previously described Wang *et al.*, 2000.

Statistical Analysis:

Statistical analyses were done using SPSS (version 10) program. Mean and standard error were descriptive measures of quantitative data using the analysis of variance test (ANOVA) for independent samples. P-values <0.05 were considered significant.

RESULTS AND DISCUSSION

Data illustrated in Fig (1) represent that the percentages of total tannins, saponins, flavonoids and phenolic compounds contents in the Rosemary crude ethanolic extracts were 3.57, 2.12, 2.05 and 4.75% respectively based on the dry weight.

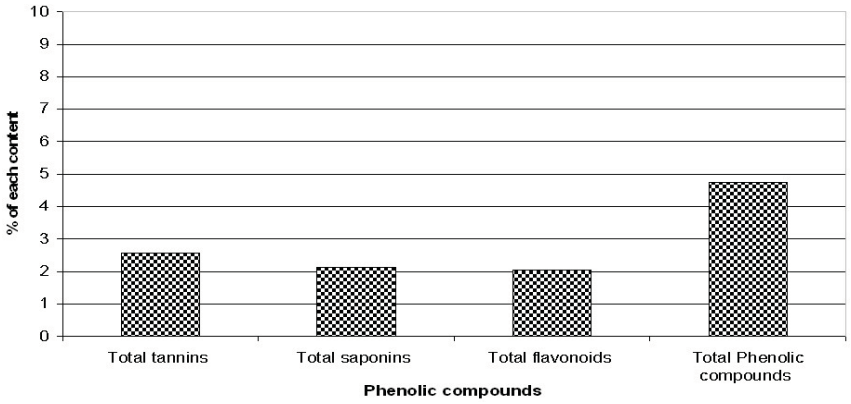

Fig. 1: Total tannins, saponins, flavonoids and phenolic compounds contents in the Rosemary leaves ethanol extract (70%)

These results are in agreement with Cimino *et al.*, 2007, who screened Rosemary crude extract showed a highly amounts of polyphenolic compounds that is optimum compounds helps reduce the risk of developing chronic diseases such as inflammation. Such ingredients of various phytochemicals are very large in number and structurally divers which have a health –promoting properties, especially in terms of inflammation as a chempreventive agents where phenolic compounds inhibited inflammation induced by LPS agents.

The obtained data from Table (1) showed that different Rosemary extract concentrations were more superoxide radical scavenging activity than the same concentrations of rutin. Also the highest superoxide radical scavenging activity was reported with 1000ppm crude extract concentration.

The antioxidant activity of the phenolic compounds against oxidative damage induced by LPS administration has been established by *in vitro* studies by (Chen *et al.*, 1999). Data revealed a significant activity of crude rosemary ethanolic extract against oxidative molecules as a highly scavenging agents against the highly produced amounts of reactive oxygen species (ROS) (Torras *et al.*, 2005).

Table 1:: Superoxide radical scavenging activity of rutin standard expressed as % inhibition and different Rosemary extract concentrations

Samples	Superoxide scavenging activity 100 %			
	100ppm	250ppm	500ppm	1000ppm
Rutin (Standard)	39.87	41.24	52.01	69.57
Ethanol 70%	51.54	56.54	59.87	78.49

* Different concentrations of rosemary extracts.
* All values mean of 3 replicates

The Effect of Various Concentrations of Rosemary Extract Administration on the Normal Cells Viability:

The illustrated data in Fig (2) showed a significant increase in the cell viability as a result of various concentrations of Rosemary extract administration and the highest observed increased viability was with 100 ppm concentration of rosemary ethanolic extract.

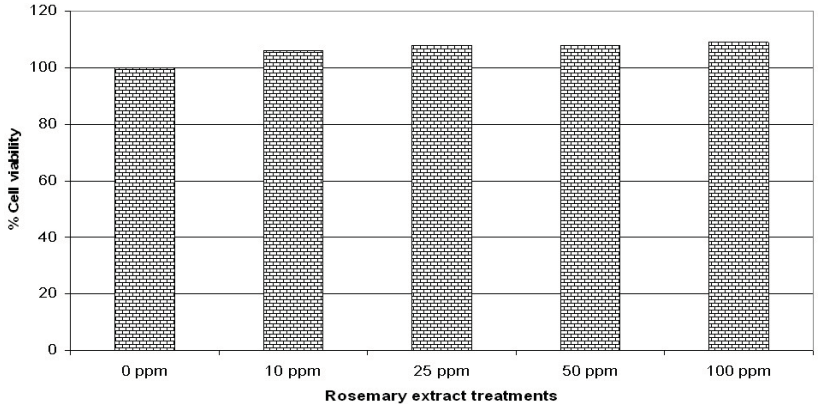

Fig. 2: The effect of various concentrations of Rosemary extract administration on the macrophage viability using MTT assay method.

Many studies of plant-derived anti-inflammatory compounds have investigated the potential inhibitory effects of natural products *in vivo* and *in vitro* systems (Chan and Riches, 1998 and Kang *et al*., 2007). Many herbs have a strong scent typical of an aromatic medicinal plant, and its roots are used for the treatment of rheumatism, toothache, lumbago, and fever remedy (Perry, 1980) and shown to inhibit cytotoxicity and COX_2 activity (Lee *et al.,* 2006).

Protein Contents:

Data in Table (2) revealed that there was a significant increase in the protein contents in response to various LPS treatments. On the other hand, the administration with Rosemary extracts significantly reduced the protein contents, the lowest responses have been observed with the highest Rosemary treatments 100 ppm compared with the corresponded control.

Table 2: The activity of Rosemary extract against proinflammatory included response

Treatment	*In vivo study*	
	Protein concentration mg/mL	PGE_2 content ng/mL
Control	7.67_f	3.21^f
LPS	73.26_a	39.62^a
10 ppm Ros. ext.	41.32_b	21.23^b
25 ppm Ros. ext.	32.61_c	17.34^c
50 ppm Ros. ext.	23.26_d	13.26^d
100 ppm Ros. ext.	19.36_e	9.18^e
L.S.D	1.942	2.494

PGE_2 Content:

The presented data in Table (2) revealed that the highest increase of PGE_2 contents observed with LPS administrations as an inflammatory mediator. On the other hand, the highest anti-inflammatory effect has been observed with the highest administrations of Rosemary extracts 100 ppm compared with the corresponded control. In light of the above , it is unclear whether phenolic phytochemicals inhibit inflammation using just

their antioxidant alone or their antioxidant defense enzymes activity promotion that inhibit iNOX, COX_2, various ROS formation and various inflammatory mediators that inhibit inflammation under LPS stimulation conditions.

The Suppression of Proinflammatory Oxidative Burst Induced Inflammation:
The Antioxidant Activities of Rosemary Extract as a Super Oxide Anion Scavenging Agents:

Data in Table (3) indicate that the highest inhibition agents of NBT has been observed with the highest administrations of Rosemary extracts 1000 ppm which has been associated with the lowest contents of $O_2^{\bullet-}$ that correlated positively with various levels of LPS treatments.

Table 3: The activity of Rosemary extract against proinflammatory included response

Treatment	*In vitro study* ($O_2^{\bullet-}$ scavenging)
	NBT %
Control	100
100ppm Ros. ext.	62.16
250ppm Ros. ext.	45.23
500ppm Ros. ext.	32.16
1000ppm Ros. ext.	20.68

Accordingly, modulating the concentration of various ROS molecules induced by LPS treatments (O_2, H_2O_2 and NO) in inflamated cells by either antioxidant enzymes activity or natural antioxidant products could be a means to control their inflammation process. Reactive free radicals, such as superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$), and peroxyl radical (ROO^{-}), are particularly reactive and are known to be a biological product in reducing molecular oxygen (Williams and Jeffrey, 2000). The cumulative production of superoxide anion, hydrogen peroxide and hydroxyl radicals which can be formed from H_2O_2 in the presence of metal ions to be all involved in the oxidative damage to macromolecules including, disruption of membrane fluidity, protein denaturation, lipid peroxidation, oxidative DNA and alteration of platelet functions (Kinsella *et al.*, 1993), which have generally been considered to be linked with many chronic health problems such as cancers, inflammation, aging and atherosclerosis (Babior, 1978).

The Antioxidant Activity of Rosemary Extract Against MDA Formation:

Data shown in Table (4) represent the subsequent increase in MDA contents as a response to various LPS administrations that responded to antioxidant activity of Rosemary extracts, also the administration of Rosemary extracts significantly decrease the MDA contents compared to the positive control.

Table 4: The antioxidant activity of Rosemary extract against MDA formation

LPS Concentration (µg/mL)	MDA nmol/ mg protein		
	Control	1000 ppm Ros. extract	% Reduction
0.00	1.36_d	1.032^b	75.74
0.10	6.12^c	3.26^{ab}	53.27
1.00	7.64^c	3.89^{ab}	50.92
5.00	9.32_b	4.98^a	53.43
10.00	11.21_a	5.78^a	51.56
L.S.D	1.648	2.66	-

The Effect of Various Concentrations of Rosemary Extract Administration on the NO Contents:

Data in Table (5) indicated that, there was a significant increase in the NO content as a result of various LPS administrations. On the other hand, the highest increase has been observed with 10 μg/ml LPS and the highest suppression positively correlated with the highest Rosemary extract administration 1000 ppm rosemary crude extract compared with its corresponded control.

Table 5: The effect of various concentrations of Rosemary extracts administration on the NO contents (nmol/10^6cells)

LPS concentration	Control	Rosemary extract treatments			
		100 ppm	250 ppm	500 ppm	1000 ppm
0.00 μg/ml	23.74^e	17.23^e	14.32^e	11.36^d	5.26^e
0.10 μg/ml	28.16^d	23.61^d	19.21^d	14.21^c	9.12^d
1.00 μg/ml	32.36^c	27.72^c	22.40^c	16.61^c	11.16^c
5.00 μg/ml	38.61^b	31.61^b	27.61^b	22.26^b	13.27^b
10.00 μg/ml	41.61^a	36.76^a	31.41^a	27.15^a	16.36^a
L.S.D	2.423	2.58	2.36	2.50	1.22

Nitric oxide (NO), identified in 1987 as a vasodilator of blood vessels, is a potent intercellular mediator that regulates several physiological and pathophysiological processes (i.e., blood pressure, hormone release, nerve transmission and the immune response) of higher organisms (Christopherson and Brent, 1997; Garthwaite and Boulton, 1995; Lipton, 2001 and Nathan,1997). Peroxynitrite (ONOO) which produced under activation of iNOS to produce NO that react with O_2 to form the highly toxic (ONOO) which intermediate alone or with H_2O_2 signal transduction leading eventually to the transcriptional activation of cyclooxigenase-2 (COX_2) as recorded in our study which induced by various LPS treatments and inhibited by various rosemary extracts administrations when compared to the corresponded control (Hinz and Brune, 2002).

The Effect of Various Concentrations of Rosemary Extract Administration on COX2 Enzyme Activity:

The enzymes relative activity of COX_2 had been mentioned in Table (6) which showed a significant increase in the COX_2 in a response to various LPS administrations; on the other hand, the anti-inflammatory activity of Rosemary extracts administration significantly decreased the relatively COX_2 activities compared to the positive control.

Table 6: The effect of LPS administrations on COX_2 activity in the response of Rosemary extract (Relative of the control %)

Treatment	Relative activity % (COX_2)
0.00 μg/ml LPS	12.76
10.00 μg/ml LPS	100
10 μg/ml LPS+100 ppm Ros. extract	55.47
10 μg/ml LPS+250 ppm Ros. extract	45.30
10 μg/ml LPS+1000 ppm Ros. extract	39.41

Cyclooxygenase-2 (COX_2) is a key enzyme that specifically converts arachidonic acid to prostaglandin H_2 (PGH_2), which is then converted to PGE_2 by PGE synthase (PGES). PGE_2, which is produced by various cells, plays an important role in various pathologic processes (Narumiya *et al.*, 1999). The activation of COX_2 is associated with a highly formation of prostaglandin E_2 an eicosanoid that was recently shown to induce growth factor receptors phosphorylation and mitogenic signaling under inflammation conditions (Shao *et al.*, 2004).

Phagocytic cells constitute the first line of immune defense against external agents, which known to play an important role in the host defense against noxious substances and are involved in a variety of disease processes, including autoimmune diseases, infections, and inflammatory disorders (Pierce, 1990). An inflammatory stimulus such as lipopolysaccharide can activate macrophages to produce a variety of pro-inflammatory cytokines, such as TNF-α, IL-6 and IL-8. Also, the formation of other inflammatory mediators, including prostaglandins and NO, is catalyzed by COX_2 and iNOS. These enzymes are detectable in only certain types of tissues and are induced transiently by growth factors, tumor promoters, and pro-inflammatory cytokines (Prescott and Fitzpatrick, 2000).

The Effect of Various Concentrations of Rosemary Extract Administration on Antioxidant Defense Enzymes:

The data represent in Table (7) revealed that, these was a significant increase in the antioxidant defense enzyme SOD activity as a response to the elevated administration of LPS *in vitro*, on the contrary the administration of various concentrations of Rosemary extract significantly reduced the SOD activity due to its antioxidant activity against induced oxidative stress induced by various LPS treatments.

Table 7: The response of SOD activity to various administrations with Rosemary extract (IU/mL)

LPS	Control	Rosemary extract treatments			
		10 ppm	25 ppm	50 ppm	100 ppm
0.00 μg/ml	7.68^{d}	6.96^{d}	7.03^{d}	6.86^{d}	6.84^{d}
1.00 μg/ml	10.36^{c}	9.06^{c}	8.64^{c}	8.31^{c}	7.94^{c}
5.00 μg/ml	15.62^{b}	14.94^{b}	13.64^{b}	12.76^{b}	11.63^{b}
10.00 μg/ml	16.82^{a}	16.81^{a}	15.94^{a}	14.66^{a}	13.93^{a}
L.S.D	0.526	0.636	0.76	0.886	0.738

The obtained data from Table (8) indicate that, there is a significant decrease in GR activity as a response to various LPS administrations, on the other hand, the data reflected a significant decrease in the GR activity compared to its corresponded control under various concentrations of rosemary extracts.

Table 8: The effect of various concentrations of Rosemary extract administration on Glutathione reductase (GR) activity (IU/mL)

LPS	Control	Rosemary extract treatments			
		10 ppm	25 ppm	50 ppm	100 ppm
0.00 μg/ml	9.26[a]	7.62[a]	6.36[a]	5.18[b]	4.98[b]
1.00 μg/ml	7.23[b]	6.21[b]	6.10[a]	5.98[a]	5.39[a]
5.00 μg/ml	6.61[b]	5.32[c]	5.21[b]	5.01[a]	5.32[a]
10.00 μg/ml	3.47[c]	4.61[d]	4.21[c]	4.02[c]	3.98[c]
L.S.D	0.636	0.346	0.352	0.517	0.298

A large amounts of H_2O_2 constitutively generated under inducing LPS increase protein tyrosine kinase (PTK) mediated phosphorylation that is with oxidation of GSH by H_2O_2 in the induced cells (Hildeman *et al.,* 1999) , the lower concentration of GSH allow activation of the initial signaling events leading to inflammations (Kang *et al.*, 2008). Since antioxidant defense enzymes (SOD and glutathione reductase) and natural antioxidant crude rosemary ethanolic extract prevented these effects and inflammation mediators which over activates transcription factors including factor – kappa B (NF-kB) and activator protein 1 (AP-1) which are most ubiquitous transcription factors and regulates the expression of genes involved in inflammatory responses) associated with inflammation (Dinarello, 2000) .Anti-inflammatory natural products have been shown to suppress the expression of these genes by inhibiting the NF-kB activation pathway (Gilroy *et al.*, 2004).

The Effect of Various Concentrations of Rosemary Extract Administration on Antioxidant Defense Natural Compounds from Rosemary Ethanolic Extract:

Data in Table (9) showed that, there was a significant increase in the GSH contents as a results of the elevated increase in Rosemary extract concentrations.

Table 9: The effect of various concentrations of Rosemary extract administration on GSH content (nmol/mL)

LPS	Control	Rosemary extract treatments			
		10 ppm	25 ppm	50 ppm	100 ppm
0.00 μg/ml	11.62[c]	12.01[c]	12.61[c]	13.01[c]	13.62[a]
1.00 μg/ml	22.68[b]	22.81[b]	23.01[b]	23.61[b]	23.94[b]
5.00 μg/ml	24.46[b]	24.32[b]	24.62[b]	25.82[b]	26.92[b]
10.00 μg/ml	30.61[a]	30.21[a]	32.62[a]	33.78[a]	35.47[a]
L.S.D	3.646	2.491	1.883	2.491	3.145

There is a growing interest in substances exhibiting antioxidant properties that are supplied to human and animal organisms as food components or as specific preventive pharmaceuticals. The plant kingdom offers a wide range of natural antioxidants. Antioxidant phenolics have been shown to provide a defense against oxidative stress from oxidizing agents and free radicals (Matkowski, 2006). Many herbal infusions, frequently used as home medicines have antioxidative and pharmacological properties related to the presence of phenolic compounds, especially phenolic acids and flavonoids. Polyphenols are also known for their ability to prevent fatty acids from oxidative decay (Chlopcikova *et al.*, 2004 and Fecka *et al.*, 2007).

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