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Neuroprotective effect of *Myrtus communis and Zingbar officinale* in LPS induced neurotoxicity in brain rats' model

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ABSTRACT

The current study was undertaken to elucidate a possible neuroprotective role of myrtle and ginger against lipopolysaccharides(LPS) induced neurotoxicity in experimental rat model. Ginger (Zingbar officinale)and myrtle((Myrtus communis)was administered orally (1 gand 300 mg /kg b.wt respectively for one month then neurotoxicity was produced in male rats by intraperitoneal injection of 200ug/g b.wt. After such treatment(4 hr after LPS injection), the animals were sacrificed and analyzed for oxidative stress biomarkers, such as, nitric oxide (NO) and malondialdehyde,(MDA), interleukine-1β (IL-1β),Tumour necrosis factor α (TNF-α) ,estrogen , 5LOX .15LOX ,lipoxin A4(LA4), Asymmetric dimethyl arginine (ADMA) and Willebrand factor(VWF)were determined in serum and brain tissue of challenged rats. The results revealed significant increase in all investigate stress parameters associated with significant decrease in the estrogen level inLPS-intoxicated rats. Marked amelioration was detected in all biomarkers under investigation upon treatment of LPS induced neurotoxic rats with myrtle or ginger with more or less similar ameliorative effects .

INTRODUCTION

The brain inflammation is accompanied by glial cell activation due to various signals such as LPS to imitate several harmful factor responsible for cytotoxicity including cytokines or free as superoxide [1] , and necrosis factor -α (TNF-α) [2] . While, activation of microglial is important as well as stringent for defense of host, overmicroglia stimulation is associated with brain toxicity [3] .

Microglial cells could be activated by lipopolysaccharide (LPS) [4] . There are a number of evidences indicating that neurodegenerative or affective disorders, such as Parkinson's disease (PD), Alzheimer's disease (AD) and schizophrenia[5] are associated with oxidative stress. The increased lipid peroxidation, protein and DNA oxidation were also demonstrated [4]. All these data indicated development of inflammatory reaction which may cause progressive degeneration of nigrostriatal neurons in PD.

The most necessary biomarkers associated with the process of pro-inflammatory related to LPS activation are TNF-α , interleukin (IL)-1β,reactive oxygen species (ROS), H_2O_2, reactive nitrogen species (RNS) ,that is induced by inducible nitric oxide synthase (iNOS), and prostaglandin E2 that is stimulated by cyclooxygenase (COX)-2 [6]. Neuronal death in the brain is the leading cause of the over production of the pro-inflammatory biomarkers .LPS has been the widely effective applied activator induced inflammatory dopaminergic neuro-degeneration of glial cells

[3]. It was found that, death of dopaminergic (DA)and non-dopaminergic neuronal cells was found as a direct effect of LPS .Hence, the destructive effect not restricted to LPS stimulated dopaminergic brain cells is supported by Qin et al., (2007) who found that LPS stimulated Aβ1–42 intracellular aggregation in the neurons of hippocampal [4]. However, Ling et al.(2004) declared that single injection of LPS stimulates vigorous reaction of microglial, creating a dopaminergic selective death while not neurons of GABAergic [7].The potential illustration for the incident of LPS selective or non-selective damage to dopaminergic neurons is related to the various zones, ambits, grade and time effectiveness by LPS in the cells of brain and the various themes noticed *in vitro*. Thus, not only can microglia stimulate destruction of neuron but also, they can become continually stimulated to output persistently ultimate brain cells toxicity that unsuccessful to establish after the exciting inducement has dissolute.

On the other hand, myrtle *(Myrtus communis* L. Myrtaceae) is an important medicinal plant grown in Mediterranean climates, due to its high leaf, flower and fruit essential oil [8].The medicinal function involved antimicrobial [9] antioxidant and anti-mutagenic [10], astringent, antiseptic, anti-hyperglycemic (Djenane et al., 2011)anti-nociceptive and anti-inflammatory [12] .

Considering, ginger, (*Zingibe rofficinale)* is used as dietary supplements [13] ,it considered to be safe and used to treat various disorders[14. Beside, ginger demonstrates anticancer activity through anti-inflammatory and anti-oxidative activities [13]. Further, ginger showed principle efficacy in the suppression of NFkB, COX2, and LOX, stimulates apoptosis, tumor suppressor gene activation as well as attenuates different biological functions. Hence, ginger and their ingredients perform hopefulness and confidence towards the new curative design. Outlook study should converge on clinical tests to demonstrate its efficiency, and their accurate effect in attenuation of genes [13]. Thus the current study is undertaken to determine how LPS influenced brain inflammation, transfer of peripheral inflammation to the brain through measuring TNFα; ,IL-1beta ,NO.LPO 5LOX .15LOX ,LA4,ADMA ,Estrogen, WVF levels as well as the effect of both myrtle and ginger extracts in attenuation of such neuro-inflammation.

MATERIALS AND METHODS

Chemical

All chemicals were purchased from Sigma Co (USA) and Laboratory Supplies, Poole (UK).I.Chemical

Rivastigmine and all chemicals were purchased from Sigma Co (USA) and aluminum chloride from BDH Laboratory Supplies, Poole (UK).TRIzol reagent was bought from Invitrogen (Germany). The reverse transcription and PCR kits were obtained from Fermentas (USA). SYBR Green Mix was purchased from Stratagene (USA).

Ginger extract preparation

The rhizome of ginger was purchased from the International Company (Cairo-Egypt). The plant was authenticated and a specimen voucher was deposited (NRC-0234) at the Cultivation and Production of Medicinal and Aromatic Plants Department, National Research Centre, Dokki, Giza, Egypt. In order to prepare the ethanolic extract ginger was ground into a fine powder using a pestle and mortar. The powder (30 g) was refluxed in ethanol (600 ml) in a Sechelt apparatus for two days. Ethanol in the extract was evaporated under reduced pressure to give a brown extract (yield: 11%). The material was subsequently reconstituted in a known volume of sunflower oil [15] .

Myrtel ethanol extract preparation One kilogram of fresh
Myrtus Communis L. leaves was obtained from the local market. The dried leaves of *Myrtus Communis* L. were grinded into powder. The powder was then extracted by 1 L of hydro-ethanol mixture (80/20, v/v) for 8 hours. This step was repeated for our times. Afterwards, the filtrate was pooled and concentrated under the vacuum at a temperature not exceeding 60°C. The obtained *Myrtus Communis L.* alcoholic extract was stored at -20°C before being used [16] .

Animals

Adult male Sprague-Dawley rats (180–220 g) were obtained from the National Research Centre, Cairo, Egypt. They were housed at 24 ± 1 _C, with a relative humidity of 45–55 % and 12:12 h dark / light cycle . The animals had free access to standard pellet chow and filtered water ad libitum throughout the experimental protocol. All experiments were carried out between 09:00 and 17:00 h. The protocol was approved by the National Research Centre Ethics Committee Guidelines for the use and care of animals, that animals are not suffered at any stage of experiment.

Drug and treatment schedule

A purified, lyophilized Escherichia coli endotoxin (Serotype055:B5; Sigma) was used; it was dissolved in sterile physiological saline, aliquoted, and frozen at - 20_C. The dose of LPS (200 ug/kg) was obtained according to [17]. Ginger was received orally with dose 1 gm/kg body weight for one month [15]. Also, myrtle was administered orally for one month at the dose 300 mg /kg b.wt [18] then at the last day the rats were received LPS intraperitoneally injection and after 4 hrs they were killed for biochemical analysis.

Animals were randomized into five groups (fifty adult Male Sprague-Dawley rats) based on their body weight. Each group having minimum ten numbers of animals. The groups were as follows:

Group one: Normal control rats
Group two: Normal control rats treated orally with *myrtle extract*
Group three : Normal control rats treated orally with *Ginger extract*
Group three : Serving as LPS -intoxicated rats
Group Four : Prophylactic treated for one month with myrte then ip injected with LPS .
Group Five : Prophylactic treated with ginger for one month then ip injected with LPS .

Brain tissue sampling and preparation.

At the end of the experiment, the rats were fasted overnight, subjected to anesthesia with diethyl ether and sacrificed. The whole brain of each rat was rapidly dissected, washed with isotonic saline and dried on filter paper. Each brain was weighed and homogenized in ice-cold medium containing 50 mM Tris/HCl and 300mM sucrose at pH 7.4 to give a 10 % (w/v) homogenate [19]. This homogenate was centrifuged at 1 400 × g for 10 min at 4 °C. The supernatant was stored at –80 °C and used for biochemical analyses that included MDA ,15LOX, 5LOX , VWF , ADMA and LA4 while , ,TNF-α and IL-1β were estimated in serum . The ethical conditions were applied such that the animals suffered no pain at any stage of the experiment and the study was approved by the Ethics Committee of the National Research Center. Animals were disposed of in bags provided by the Committee of Safety and Environmental Health, National Research Center. Estrogen was determined in serum by the quantitative determination fluorometric method .

Biochemical analyses

Serum TNF-α , IL-1α and estrogen were detected by ELISA technique .15 LOX was determined in brain tissue by HPLC method [20]. 5LOX was determined in brain tissue by colorimetric method according to [21].VWF was measured by a quantitative direct enzyme immunoassay according to [22] in brain tissue . ADMA was determined in serum by ELIZA method according to [23]. L A4 Invasion assay was performed using a method described previously by [24]. Lipid peroxide(MDA) was determined according to the method of [25].

Statistical analysis

Data were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program , version combined with co- state computer program and least significant difference (LSD) to compare significance between groups, where unshared letters are significant at $P \leq 0.05$

RESULTS

Significant increase in TNFα,MDA 15LOX, 5LOX, ADMA and LA4 in LPS neurotoxic rats with percentages increase reached to 65.56,566.66,32.00,57.68,806.00 and 38.46% respectively, as compared to control rats .While ,significant decrease was detected in VWF , estrogen and IL-1ß in LPS injected rats with percentages reduction 83.46 , 33.11 and 28.35%, respectively . Prophylactic treatments of inflammatory brain rats with ginger with myrtle exhibited marked amelioration and an insignificant difference in all biomarkers under investigation (Tables 1-4)

Table (1): Prophylactic effectiveness of ginger and myrtle on ADMA and MDA levels in LPS induced neurotoxicity in brain rats model

	CON	C+ Mrt	G+C	LPS	MYR+LPS	LPS+GIN
ADMA(Umol/L)	1.00±0.45 a	1.07±0.68a	1.06±0.67 a	9.06±0.01 b	4.8±0.05 c	5.44±0.4 c
%change	-	7.00	6.00	806.00	380.00	444.00
MDA(nmol/L)	0.12±0.01 a	0.12±0.003 a	0.11±0.004 a	0.80±0.002 b	0.10±0.004 a	0.11±0.005 a
%change	-	0	8.33	566.66	16.66	8.33

- *Data are expressed as mean ±SD of 10 rats / group*
- *Statistical analysis is carried out using SPSS computer program combined with Co-state computer program, where varied letters are significant at P≤0.05.*

Table (2): Prophylactic effectiveness **of ginger and myrtle on TNF-α and IL1-β levels in LPS induced neurotoxicity in brain rats model**

	CON	C+ Mrt	G+C	LPS	MYR+LPS	LPS+GIN
TNF-α(ng/L)	23.03±2.12a	22.56±1.22 a	23.43±6.45 a	38.13±4.56 b	30.78±3.23 c	24.34±2.12 a
%change	-	2.04%	1.74	65.56	33.65	5.69
IL1B(Pg/L)	3900.76±55.10 a	3689.00±5.45 a	3666.00±87.78 a	2794.66±56.67 b	3511.33±100.78 a	3501.00±57.89 a
%change	-	5.43	6.01	28.35	9.98	10.23

- *Data are expressed as mean ±SD of 10 rats / group*
- *Statistical analysis is carried out using SPSS computer program combined with Co-state computer program, where varied letters are significant at P≤0.05.*

Table (3): Prophylactic effectivenessof ginger and myrtle on VWF and LA4 levels in LPS induced neurotoxicity in brain rats model

	CON	C+ Mrt	G+C	LPS	MYR+LPS	LPS+GIN
VWF(ng/L)	1360.34±2.56a	1440.66±5.78 a	1331.00±45.54 a	225.00±13.50 b	1489.5±59.671 a	1555.00±40.23 c
%change	-	5.88	2.15	83.46	9.49	14.31
LA4(Pg/ml)	59.56±12.78 a	66.00±1.50 a	60.34±2.56 a	82.66±4.78 b	60.90±9.56 a	61.00±3.45 a
%change	-	10.81	1.30	38.78	1.38	2.41
ESTROGEN (Pg/ml)	22.77±2.00 a -	23.56±0.49 a 3.47	23.68±0.67 a 3.99	15.23±1.00 b 33.11	25.39±3.55 a 11.51	26.44±1.45 b 16.11

Table (4):Prophylactic effectiveness of ginger and myrtle on 15 LOX and 5LOX levels in LPS induced neurotoxicity in brain rats model

	CON	C+ Mrt	G+C	LPS	MYR+LPS	LPS+GIN
15LOX(U/L)	8.56±0.68 a	8.10±0.89 a	8.03±0.98 a	11.3±1.23 b	8.95±0.89 a	8.60±0.87 a
%change	-	5.37	6.19	32.00	4.55	0.46
5LOX(U/L)	12.50±1.00a	13.37±0.98 a	13.58±0.89 a	19.71±1.21 c	13.90±0.54 a	13.24±1.23 a
%change	-	6.96	8.64	57.68	11.20	5.92

- *Data are expressed as mean ±SD of 10 rats / group*
- *Statistical analysis is carried out using SPSS computer program combined with Co-state computer program, where varied letters are significant at P≤0.05.*

DISCUSSION

It is well previously known that, NO is implicated in neuronal damage *in vitro*[26]. NO perform highly reactive peroxynitrite species through its interaction with superoxide free radicals which is able to planting further neuronal destruction [26]. The addition of NO synthase inhibitors to cultures of neuron–glia suppress the nitrite and decreased neuronal cell loss induced by LPS [27]. Beside NO and TNFα have been speculated to be principal mediators of LPS motivated inflammation [26]. However, TNFα individually is not qualified for initiating neurotoxicity in vitro .While, markedly neurotoxicity can be determined upon the incorporation of TNFα with IL-1 and interferon-g [28]. It was found that ,LPS is contingent on dose which motivated the activation of microglia to output NO and TNFα, resulting in injury of dopaminergic as well as other neurons .

Neutrophils are considered as the main origin of pro-inflammatory mediators comprising iNOS, and cyclooxygenase-2which have principle effects in neuronal damage in LPS-induced rat's model. It was declared that LPS single injection elevated expression of brain TNF-α mRNA and protein for more than 10 months [4] . However , did not indicated the inflammatory mediators levels at that delayed timing, because of , cerebrospinal fluid is regenerate around 11 times /day in adult rats and preserves brain medium [29]. Post LPS injection, brain neutrophils die 1-5 days, considering soon reaction to injury [3]. Meanwhile, long-lasting inflammation is prevented through the suppression effect of cytokine signaling family proteins and antioxidant enzymes that disband

inflammatory mechanisms are activated [30]. Hence, acute inflammation of brainmay not continue for months in the loss of a recent stimulator .

It was found that dehydroepiandrosterone (DHEA) and prostaglandins are anti-inflammatory agents excreted by astrocytes [3]. (DHEA suppress nuclear factor-kappa Bactivation(NF-κB) stimulated by TNF-α [31], whereas prostaglandin E2 diminished Akt and the nuclear translocation of NF-κ Bactivation [32]. Astrocytes continue, normally in LPS iv injected in animals brain and probably, excreted anti-inflammatory agents inhibiting microglial expression of inflammatory mediators. In addition, neurotrophic factors such as transforming growth factor-β1, neurotrophin-3, and brain-derived neurotrophic factor are produced from activated microglia. So, not all inflammatory mediators created in the brain are neurotoxic. Such as, the neuroprotective influence of IL-1β [3].
Neutrophils have principle effect in the progress and inflammation aspects as well as they are the main origin of free radicals at inflammation area. The occurrence of lipid peroxidation as a result of free radicals might produce huge number of reactive aldehydes and lipid peroxides which are the reasons implicated in physiological alterations linked with cells and tissues oxidative injury .

The inducible COX-2isoenzymeperforms a necessary, function as ant-inflammatory mediator. COX-2 expression was found to be suppressed by antioxidants in human alveolar macrophages. COX-2 expression is regulated by one of control element which is NF- B activity. The suppression of the COX pathway ,elevates ROS production through peroxidative cleavage of 5-hydroxyeicosatetraenoic acid (5-HPETE), hence 5-LOX inhibitors would decline this effect[Roth et al., 2004] .Furthermore, apoptotic neutrophils were found in a higher number in COX-2 deficient mice rather than in COX-1 deficient mice ,indicating an anti-apoptotic role for COX-2.

During LPS induced inflammation, certain endogenous lipooxy-metabolites as lipoxin-A4 (LXA4) and prostaglandin-E2 (PGE2) are accelerated macrophage phagocytosis of apoptotic cells. Also, COX-2 inducement may be principal factor in inflammatory brain PGE2 output .SO, PGE2 output may occur at the area of inflammation in the brain parenchyma[33].

Moreover, lipoxins have important function in the resolution of inflammatory process, suppressing neutrophil mobilization and enhancing macrophage induction and phagocytosis of neutrophils by macrophages. Lipoxin A4 has been indicated to suppress LPS expression of granulocyte-macrophage inducing factor (GM-CSF)-activated inflammatory cytokines in peripheral blood leukocytes [34] and pulmonary microvascular endothelial cells [35] .

The starting point for synthesis of LXs is arachidonic acid (AA), the only two truly endogenous L Xs known, LXA4 and LXB4, are typically formed by transcellular metabolism of AA involving sequential LOX activity [36]. In one of these pathways, AA is oxygenated by15-LOX to generate 15S-HETE, which is then modified by5-LOX to originate both LXs. Another 2-step pathway forLXA4 and LXB4 formation involves the conversion of AA into leukotriene A4 by LOX-5, followed by its metabolism by LOX-12 [37]. Interestingly, the acetylation of COX-2 by aspirin, while inhibiting the synthesis of prostaglandins and thromboxane, favors the generation of 15R-HETE, which can then be converted by LOX-5 to generate the aspirin-trigger dLXs (ATLs) 15-epi-lipoxin A4 and 15-epi-lipoxin B4 [38]. The LXs are subjected to rapid enzymatic breakdown, but ATLs are more resistant to degradation and thus can exert longer lasting effects. In fact, their formation and functions are directly linked to a change in the phenotype of neutrophils present at the site of inflammation [36]. Once formed at the site of injury, LXs suppress neutrophil recruitment, enhance phagocytosis of apoptotic neutrophils by macrophages, and stimulate the accumulation of nonphlogistic type of monocytes/macrophages which do not produce pro-inflammatory mediators [36]. The study found that LXA4 levels in serum of LPS induce brain toxicity were lower than those of control rats and that this decrease was correlated with the degree of cognitive deficit and tissue accumulation of tau protein [36].
17b-estradiol (Estrogen) was found to prevent microglia activation: It was declared that estrogen suppresses the morphological transformation on behalf of reacting phenotype and prevent the LPS-stimulated output of mediators implicated in the inflammation , as NO and iNOS, PGE2, and MMP-9 [39]. Our results are in accordance with [39] who demonstrated that estrogen blocks iNOS expression stimulated by inflammatory signals, in different typeof cells .The activity of nitric oxide has been connected with the advanced destruction of neural cells and with the enhancement of microglia emigration, at the area of injury[40].

Valuable actions of estrogen on neuro-, degradation have been linked with different machineries stimulated by the direct action of hormone on neurons: (1) elevation in synaptic connections and neurotransmission; (2) blocking of apoptosis in brain cells ; (3) controlling the activity of mitochondria and (4) specific proteases inhibitory activity

that would cause to pathogenic peptides formation, as b-amyloid [39]. Estrogen might maintain, the brain integrity(functional and structural) by scavenging the inflammatory response linked with neuro-degradation. The estrogenanti-inflammatory function might also be implicated in the other inflammatory disorders , as osteoporosis, in which over output or disturbed action of the inflammatory elements and a useful function for estrogen have been renowned [39].

Regarding to estrogen mechanism of action on the inflammatory mediators, (1) estrogen performs its function at certain levels suitable for receptor stimulation (2) the activity of estrogen is prevented by the receptor antagonist ICI 182,780, and at 60 min between hormone and LPS injection (3) ER-a and ER-b are expressed in microglia and macrophages (4) estrogen regulate MMP-9 mRNA levels in microglia and macrophages (5) estrogen-stimulated ER-a prevents MMP-9 developer inducement [39].

Asymmetric dimethyl arginine (ADMA) is competitive inhibitor of NO synthase and by decreasing NO output it may elicit endothelial impairment and atherosclerosis [41]. ADMA is partially discarded, by the kidneys, hence, renal fail, elevates its level [41]. ADMA levels were detected to be elevated in end-stage renal disease (ESRD)subjects related to normal one [41]. Further, increased ADMA levels are proposed to be connected with the growing hesitancy of cardiovascular proceedings noticed in ESRD patients [42]. It is indicated that, ADMA might be a modern both on peritoneal dialysis and hemodialysis (HD) related to healthy subjects [43]. In our study, serum ADMA levels of LPS induced neuronal toxicity of rats were found to be markedly increased than normal controls. Direct relationship is ascertained between serum ADMA levels and CRP levels. So, a linkage exists between endothelial impairment and in LPS induced neuronal inflammation in rats [43].

In LPS -mediated inflammation in humans, ADMA was increased by the end of infection period, suggesting that the type of inflammatory stimuli plays a crucial role in ADMA regulation. ADMA levels are increased in the presence of chronic inflammation (atherosclerosis or rheumatoid arthritis), and ADMA is closely associated with IL-6 levels. In addition, acute inflammation induces a parallel elevation of serum ADMA and impairment of endothelial function in healthy individuals, implying that ADMA may mediate the development of inflammation related endothelial dysfunction in these subjects [44].

Considering, Willebrand factor (VWF) is present in the platelet granules and Weibel-Palade bodies of endothelial cells. VWF performs primary platelets adhesion, which is the first inflammatory process. VWF is concerning with acute and chronic inflammation [45]. The high plasma VWF levels have been demonstrated in different inflammatory diseases as rheumatoid arthritis [45]. However ,[46] declared that the inflammatory process is connected with a reduction in a disintegrin-like and metalloprotease with thrombos pond in type I repeats – 13 (ADAMTS13) activity, suggested that ULVWF that is probably liberated and found beneath, these state over elicits inflammatory process , as ADAMTS13 insufficiency leads to elevate neutrophils extra vasation in thioglycollate-induced peritonitis and wound healing. While, the reduction in neutrophils extravasation in VWF deficient mice, may be related to loss in the storage of P-selectin and delayed formation of atherosclerotic lesions in mice [45].

Our data clearly indicated that the antioxidants ability in decreasing reaction of inflammation, COX-2 expression, iNOS levels as well as oxidative damage by stimulating apoptosis in the inflammatory cells [47].

Myrtle oil and leaves extract induced the antioxidant enzyme activities, reduced lipid peroxidation and, scavenge free radicals .There is an evidence that oil is rich with polyunsaturated fatty acids (PUFA) in relieving cardiovascular diseases, inflammation , atherosclerosis and other diseases [48]. The fatty acid ingredient beside the elevating contents of PUFA could be causing seed lipids of myrtle significant for nutritional supplement as well as processing usage. Phenolic rich ingredients as flavonoids, phenolic acids, and tannins are greatly spread in plants [48], which have increased great concern , because of their antioxidant properties and quenching free radical or metal chelating capability [49], which probably have useful participation for health care of human . Oil of myrtle seed is described by the lack of flavonoids and proanthocyanidins, however, it hold low quantity of total phenols (0.25 mg GAE/g DW) and tannins (0.20 mg GAE/g DW). Methanol extract of myrtle leaves had a high level of phenolic compounds (25.25 mg GAE/g DW) with the prevalence of tannin fraction (20.33 mg GAE/g DW). While, flavonoids and proanthocyanidins were low in the extract of methanolic seed. *In vitro* antioxidant activity showed that myrtle seed methanolic extract exhibited greater antioxidant ability than oil [49].

Therapeutic use of extract markedly reduced MDA level, which might be related to extract free radical scavenging properties [50]. On the other, inflammatory process performed statistically increase in the level of NO , however, remediation with extract demonstrated obvious reduced level of NO. It has been declared that LPS injection showed ROS elevated levels which lead to stimulation of NF-k B, a transcription factor which maintains several genes of cytokine, such as TNF-a, IL-1b, IL-6 and IL-8 [50]. So, our results indicated, handling with extract may inhibit the LPS stimulated NF-kB activation in the brain and reduced pro-inflammatory output as well as mRNA levels of fibrogenic cytokine, as TNF-a, IL-1b, IL-6 and IL-8. Treatment by myrtle extract showed significant down-regulation in pro-inflammatory cytokines which may be due to inhibition of ROS. GA of myrtle seed was shown to ameliorate inflammation by enhancing antioxidant enzyme activity or suppressing reactive oxygen species production in LPS injected rats.

Essential oils of myrtle can also act as anti-inflammatory agents, because one of the inflammatory responses is the oxidative burst that occurs in diverse cells(monocytes, neutrophils, eosinophils, and macrophages).ROS and RNS overproduction may be responsible for damage at inflammatory sites by being trigger elements or by being signaling messenger molecules [51].

In prevalent, cytokines involved in inflammatory reaction are not continuously provide or are happened in low degree. However, the existence of suitable inducement, as LPS, stimulated the output of interleukin-1β (IL-1β), interleukin-6 (IL-6), and TNF-α pro-inflammatory cytokines , resulting in the prompting of an inflammatory response [52] Established on these data, the evolution of novel medication for inflammatory disorders has concentrated on the interception of inflammatory cascade cytokines members, [53]. Hence, in this study, we have indicated that extracts of myrtle and ginger were capable of suppress LPS-stimulated NO output , TNF-α and this could be attributed to the NO synthesis is adjusted by TNF-α cytokine signals . It was found that , LPS endotoxins and inflammatory cytokines, TNF-α and IL-1β, have been involved in the inducible nitric oxide synthase (iNOS) expression which gives high amounts of NO (Justo et al., 2015). It was ascertained the anti-inflammatory effects of ginger extracts [54]. Shimoda et al.(2010) found that extracts of ginger or their constituents influenced nitric oxide output by LPS-stimulated cells in a dose-dependent relationship for RAW264 cells [55]. Jiang et al. (2006), declared that , ginger bioactivity extracts may not be readily prophesied [56]. The authors confirmed that suppression of LPS stimulated output of prostaglandin E in histiocytes of human in vitro is probably with ginger crude organic extracts, whilst, in spite of their ingredients may do at different location , the extracts were not almost as efficient at prohibiting TNF-α. In dissimilarity, Surh (1999) declared that gingerol of rhizome of ginger can inhibit output mice TNF-α and has possibly for the remediation of TNF-α linked disorders [57] . Justo et al.(2015), showed that ginger extracts from supercritical fluid extraction exhibited quenching free radical activity , reducing and chelating properties in dose-dependent relationship [34]. Aly et al. (2013) demonstrated that, ginger attenuated inflammation caused by *Schistosoma mansoni* [15]. Thus, it could be concluded that myrtle and ginger ethanolic extract can able to modulated LPS-induced brain inflammation in rats. Extensive further studies are need for explore the exact mechanism and⁄or (s) of anti-inflammatory properties of both extracts.

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