Peppermint (*Mentha piperita* L.) and Thyme (*Thymus vulgaris*) attenuate the Immune and Inflammatory Disorders in Rats Consumed Repeatedly Heated Palm oil

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ABSTRACT

The re-heating of palm oil leads to the breakdown and release of trans fatty acids (TFA) absorbed by fried food, which enters the body and can cause damage to the immune system and lead to inflammation. The aim of this study was to evaluate the protective role of Mentha piperita L. and Thymus vulgaris in alleviating the immune and inflammatory disorders in rats after chronic oxidized palm oil intake. Fifty male Wistar rats weighing between (150-200 g) were isolated into five groups: Group1, the control group, received a basal diet; Group 2 received basal diet sustained with 15%(w/w) palm oil heated for 10 minutes; inGroups 3-5, the rats were fed oxidized palm oil (OPO), the same as group 2, and received water extract of menthe (290mg/kg BW/day), thyme (500mg/kg BW/day) and their combination by gavage, respectively for 6 weeks. The results showed that the rats fed with OPO had a significant decrease in the immunoglobulins (IgG, IgM, and IgA), and significant increases in the inflammatory markers (TNF-α, CRP, IL-1□, IL-6, and MCP-1). The results obtained revealed that feeding rats on OPO induced notable increases in the thiobarbituric acid reactive substance (TBARS) and protein carbonyl contents (PCC) associated with a marked depletion in reduced glutathione (GSH) and superoxide dismutase (SOD). However, the treatment of the oxidized palm oil group with menthe and/or thyme extracts led to the improvement of the mentioned parameters compared to the OPO group. In conclusion, our findings showed that men and thyme plants have beneficial health properties through immunomodulatory, anti-inflammatory, and antioxidant effects.

Key Words: *Menthapiperita L., Thymus vulgaris, oxidized palm oil, immunoglubulins, inflammatory markers, antioxidant enzymes.*

eIJPPR 2020; 10(2):59-66

HOW TO CITE THIS ARTICLE: Nadia Nour Osman, Mashael Arif Alsharari, Hadeil Muhanna Alsufiani (2020). "Peppermint (*Mentha piperita* L.) and Thyme (*Thymus vulgaris*) attenuate the Immune and Inflammatory Disorders in Rats Consumed Repeatedly heated Palm oil", International Journal of Pharmaceutical and Phytopharmacological Research, 10(2), pp.59-66.

INTRODUCTION

Deep frying is the world's most popular way of preparing food [1]. When heated repeatedly, the physical and chemical properties of the oil change considerably because of oxidation, hydrolysis, and polymerization, which may change the composition of fatty acids of the oil. During this process, the fried food absorbs many oxidizing products such as aldehydes and hydroperoxide [2].

Fried foods, for example, potato chips and fried pastries are rich sources of trans fatty acids (TFA), which can increase body weight [3]. Also, its effect turns out to be more hazardous at higher doses since it can instigate oxidative stress by the formation of free radicals [4]. Free radicals that are formed by the oxidation reaction are termed as either reactive oxygens species (ROS) orreative chlorideespecies ROS mainly led to oxidative damage to

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Received: 24 November 2019; **Revised:** 26 March 2020; **Accepted:** 02 April 2020

biological molecules such as proteins, carbohydrates, lipids, and nucleic acids [5–7]. Increased oxidative stress plays a role in the pathophysiology of chronic inflammatory diseases. The utilization of oxidized heated oils can also cause chronic inflammatory diseases and increases the risk of obesity [7,8].

Plants in the Lamiaceae family such as Menthapiperita L. [9] and Thymusvulgar is [10] are rich in polyphenols which act as antioxidants protecting normal cells against free radicals [11].

Mentha is an important genus of Lamiaceae, which is recognized for vital oils, medicinal uses, and the antimicrobial activity of different species [12]. Mentha spp. has been used as a medicinal and aromatic plant since prehistoric times [13, 14].

Menthapiperita L. Containes phenoliccompounds [15] and recentresearch hasreportedon thepotential effect of the peppermint (in the form of leafextract) as antioxidants, antiallergics, and anti-inflammatory [16–18]. *Thymus vulgaris* is a flowering plant in the mint family *Lamiaceae.* It contains high concentrations of phenols. Thyme is used as anti-inflammatory, antibacterial, antioxidant and antiviral [19–21].

The antioxidant effects are primarily due to phenolic substances as phenolic diterpenes. Apart from polyphenols, among the important components involved in the cell defense system against free radicals are carotenoids and flavonoids [22]. The Aim of our investigation was the determination of the protective role of *Mentha piperita L.* and *Thymus vulgaris* alone or in a mixture in alleviating the immune and inflammatory disorders in rats after chronic oxidized palm oil intake.

MATERIALS AND METHODS

Peppermint Extract Preparation

The leaves of *Mentha piperita L.* were purchased from the local traditional market in Jeddah, Saudi Arabia. Ten grams of dried peppermint was dissolved in 100 ml of distilled water. Then, the water extract was filtered into an amber bottle and kept in -80º conditions until further use. The extract was thawed and was let stand at room temperature for about two hours before use [16].

Thyme Extract Preparation

The leaves of *Thymus vulgaris* were purchased from the local traditional market in Jeddah, Saudi Arabia. Thirty grams of dried leaves of *Thymus vulgaris* were dissolved in distilled water (60 ml). Then, the sample was filtered using filter paper and these filtrates were stored at 20ºC for 3 days only (i.e., freshly prepared every 3 days) [22, 23].

Preparation of oxidized palm oil

The palm oil was purchased from the local traditional market in Jeddah, Saudi Arabia. Palm oil was used as anoxidized palm oil (OPO). The frying process was involved 1Kg of potatoes that were fried in the 2.5L of palm oil for 10 min at 180ºC. The cooling process for five hours took place between intervals of the heating process. The whole frying process was repeated with fresh potatoes without changing the palm oil for the compensated loss of oil [24].

Experimental animals and their management

Fifty adults male Wistar rats (150-200g) from the central Animal House, King Fahad Medical Research Center, King Abdulaziz University, Saudi Arabia used as experimental animals. The rats were kept in plastic cages and kept in a 12h light-dark cycle at 25±2ºC of room temperature two weeks before the rats started to be placed under observation. The animal were treatmented according to the Ethics Committee approval number 581-17 of the King Fahad Medical Research Center, and recommendations were made regarding the proper care and handing of the animals. The rats were divided into five groups, as follows:

Group 1, normal group: Animals received a basal diet.

Group 2: Animals received a basal diet sustained with 15% (w/w) of palm oil heated for 10 times [25].

Groups3-5: Animals were fed oxidized palm oil as in group 2 and received water extract of menthe (290 mg/kg BW/day) [15], thyme (500mg/kg BW/day) [26] and their combination by gavage, respectively for six weeks.

Blood collection and serum separation

After completing the investigation time (6 weeks) and overnight fasting under ether anesthesia, the rats were sacrificed, and blood samples were collected immediately into Serum Separator Tubes (SST) tubes. the serum was separated by centrifugation at 3000g for 10 min and stored at -20ºC until biochemical analysis.

Biochemical Analysis

Serum immunoglobulins (IgG, IgA and IgM) and inflammatory markers (tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), monocy techemotactic protein-1 (MCP-1) and C-reactive proteins) were determined by using ELISA kits. Levels of serum glutathione reduced (GSH), superoxide dismutase (SOD), protein carbonyl content (PCC), and thiobarbituric acid reactive substances (TBARS) were analyzed using assay kits that were purchased from Abcam (Cambridge, UK).

Statistical Analysis

The data were analyzed usingMegaStat (Add-in for Excel) version 10.0. one-way ANOVA and the LSD test Post Hoc test for multiple comparisons were used. P<0.05was considered as statistically significant.

Feeding rats with OPO led to reducing of the serum IgG, IgM, and IgA, compared with the normal control rats P<0.05 (Table 1). The rats who weref ed with OPO and treated with menthe and/or thyme showed more improvement in their immune function.

RESULTS

Table 1: Effects of *Mentha piperita L* and/or *Thymus vulgaris* extracts on Immunoglobulins in the serum of rats fed diets containing 15% oxidized palm oil for 6weeks.

Parameters / Groups	IgG (µg/ml)	IgM (µg/ml)	IgA (µg/ml)
Control	6076.580±780.915	739.843±122.861	6332.064±604.895
OPO	4173.448±643.533 ***	465.651±112.629 ***	4491.502±515.207 ***
OPO+AEMP	5213.550±605.112 *^^&	618.066±81.578 *^^&	5635.068±462.800 *^^^&
OPO+AETV	5254.123±335.785 *^^&	620.995±78.461 *##^^&	5679.770±462.980 *^^^&
OPO+ MIX	5983.045±590.317 ^^^	718.716±85.317 ^^^	6307.981±753.336 ^^^

Each value represents the mean of 8 rat's ± SD
P < 0.05*, 0.001*** means significant difference when compared with control
P <0.01^^, 0.001^^^ means significant difference when compared with OPO
P <0.05$^{\&}$ means significant difference when compared with OPO+ MIX
Oxidized palm oil (OPO), aqueous extract of *Mentha piperita* (AEMP), aqueous extract of *thymus Vulgaris* (AETV).

The results in Table (2) show the effects of menthe and/or thyme on some serum inflammatory markers (TNF-α, CRP, IL-1β, IL-6, and MCP-1) in rats fed OPO. It was indicated that the OPO group showed highly significant increases in the serum inflammatory markers compared with the control group. Supplementation of menthe and/or thyme along with OPO significantly limited the elevation in these inflammatory parameters.

Table 2: Effects of *Mentha piperita L* and /or *Thymus vulgaris* extracts on inflammatory markers in the serum of rats fed diets containing 15% oxidized palm oil for 6weeks.

Parameters / Groups	TNF-α (pg/ml)	CRP (µg/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)	MCP1 (ng/ml)
Control	4.715±2.951	11953.287±2150.689	62.859±9.459	75.438±10.619	202.236±22.856
OPO	27.967±6.532 ***	16861.793±1466.338 ***	91.343±5.632 ***	122.218±28.984 ***	325.841±16.791 ***
OPO+AEMP	11.096±1.984 *^^^&	13974.451±1353.068 *^^&	74.708±9.089 *^^^&	95.376±7.561 *^^^&	247.134±23.316 *^^^&
OPO+AETV	10.956±1.477 *^^^&	13898.955±1252.780 *^^&	73.778±10.065 *^^^&	95.073±6.575 *^^^&	246.326±50.334 *^^^&
OPO+ MIX	6.119±1.562 ^^^	12042.870±1666.249 ^^^	63.499±9.740 ^^^	79.278±10.385 ^^^	209.826±33.135 ^^^

Each value represents the mean of 8 rat's ± SD
P<0.05*,0.001***means significant difference when compared with control
P <0.01$^{\wedge\wedge}$, 0.001$^{\wedge\wedge\wedge}$means significant difference when compared with OPO
P <0.05$^{\&}$ means significant difference when compared with OPO+ MIX
Oxidized palm oil (OPO), aqueous extract of *Mentha piperita* (AEMP), aqueous extract of *Thymus vulgaris* (AETV).

The results in Table 3 indicate a highly significant induction in the serum TBARS and PCC accompanied by a highly significant reduction in the serum GSH and SOD in the rats fed diets containing 15% OPO compared to the control rats. Administration of menthe and/or thyme in continuation with OPO for 45 days resulted in a significant induction in the activities for serum SOD and GSH with a significant reduction in TBARS and PCC as compared to rats fed OPO.

Table 3: Effects of *Mentha piperita L* and /or *Thymus vulgaris* extracts on oxidative and antioxidant markers in the serum of rats fed diets containing 15% oxidized palm oil for 6weeks.

Parameters / Groups	GSH (mg/g)	SOD Inhibition Rate %/mg of protein	TBARS (µM/mg)	PCC (nmol/mg)
Control	5.99±1.308	149.316±18.176	0.0057±0.0002	2598.112±384.145
OPO	3.01±1.018 ***	108.357±19.208 ***	0.0117±0.0034 ***	5592.248±1149.554 ***
OPO+AEMP	4.74±0.437 *^^^&	132.206±12.189 *^^^&	0.0083±0.0009 *^^&	3690.815±632.988 *^^^&
OPO+AETV	4.83±0.667 *^^^&	133.156±9.040 *^^^&	0.0082±0.0015 *^^&	3685.561±590.663 *^^^&
OPO+ MIX	5.80±0.912 ^^^	146.683±8.696 ^^^	0.0060±0.0011 ^^^	2748.664±585.507 ^^^

Each value represents the mean of 8 rat's ± SD
P<0.05*,0.001***means significant difference when compared with control
P <0.01^^, 0.001^^^means significant difference when compared with OPO
P <0.05& means significant difference when compared with OPO+ MIX
Oxidized palm oil (OPO), aqueous extract of *Mentha piperita* (AEMP), aqueous extract of *Thymus Vulgaris* (AETV).

DISCUSSION

The human body has been exposed to various types of factors that lead to the production of free radicals (ROS, RCS, RNS), causing oxidative damage to the body's biological molecules [5]. Recently, studies suggest that consumption of fried foods induced oxidative stress [27].
An immune system is an organism's system of biological structures that defend against infection by pathogens and tumor cells being detected and destroyed [28]. Antibodies such as IgG, IgM, IgA, IgE and IgD are part of the immune system and play important role of the immune response [29]. The OPO group had high reducting of serum IgG, IgM, and IgA compared to the control group. Our data was consistent with the previous study conducted by Egbung et al. [30] who observed a decreased immunity in Wistar albino rats following the consumption of trans fatty acids. Moreover, Harbige [31] showed that an increased intake of oxidized oil can affect lymphoid organs and affect immune responses in control rats and may be involved in allergic diseases. In contrast, the aqueous extract of menthe and/or thyme showed a significant increase in the serum immunoglobulins when compared with the OPO group. Peppermint extract has multiple advantages over the classical immune stimulants: it is easy to obtain, it is less expensive and it can be given orally, which is the most convenient method for immunostimulation [32]. The current results are in line with Abdel-Wahab [33], who reported an increase in IgG, IgM, and IgA production in Japanese quail on a diet of peppermint (3% and 1%). Nazarizadeh et al [10] showed that treatment broilers fed aflatoxin B1 and ochratoxin A contamination diets with chamomile flower and thyme-oil extract can ameliorate adverse effects in the immune response by increasing plasma IgG and IgM (p<.001), but could not have the effect of IgA. The improvement of the immunity system could be explaned the presence of antioxidants and the free radical scavenging capacity of polyphenols found in Mentha and Thyme [34-38].
Oxidative stress Caused by an imbalance between was developing the free radicals and antioxidant defenses that will lead to oxidative damage [7]. Inflammation is a complex biological response of vascular tissues to harmful stimuli such as irritants, pathogens, or damaged cells [39]. Both inflammation and oxidative stress can induce the pathogenesis of chronic diseases and metabolic disorders. The phenolics have anti-oxidative activity and anti-inflammatory effects that may reduce oxidative stress and inflammation biomarkers [40]. The intake of trans-fatty acids was also associated with inflammatory factors [41]. Therefore, unhealthy diets, such as fast foods, have resulted in chronic inflammation with higher pro-inflammatory cytokines levels as CRP, which are inflammatory behavior sensitive markers in the body [42]. The results of this study show that the OPO group indicated highly significant increases in the serum TNF-α, CRP, IL-1β, IL-6 and MCP-1 compared to with the control group. That outcome was consistent with Ng et al. [43] who observed that prolonged consumption of repeating heat

palm oil led to blood pressure and increasing VCAM-1, which may be attributed to inflammation. Furthermore, tissue damage may be caused by ROS produced by the thermal oxidation of repeated oil heating, resulting in endothelial cell injury and induced inflammatory parameters [44]. In contrast, the aqueous extracts of menthe and thyme showed to be decreasing in the serum inflammatory markers compared to the oxidized palm oil groups. These findings are consistent with the previous study by Modarresi, Farahpour and Baradaran [45], who reported *M. piperita* at 8% could reduce the expression of TNF-α, and conversing raise of TGF-β1 and IL-10. Elbahnasawy et al. [46] found that supplementation with *Thymus vulgaris L.* prevents inflammation and oxidative stress. Mentha and/or thyme Extracts showed a significantly improved inflammatory system through reduced TNF-α, CRP, IL-1β, IL-6, and MCP-1, which are rich in polyphenol compounds [47,48].

The findings of the present study showed that an OPO diet significantly increased the TBARS and PCC accompanied by significantly decreased SOD and GSH levels compared to the control group, which was in agreement with Zhang [49] who observed an increase of MDA (oxidative stress) in their blood, and increased PCC in breast meat in rats fed 5% oxidized oil for 4 weeks. In our study, supplementation of menthe and/or thyme along with OPO significantly reduced the levels of TBARS and PCC accompanied by a significantly induced level of serum SOD and GSH content. These results agree with Bellassoued et al. [50] who found a significant reduction in hepatic and kidney lipid peroxidation (TBARS) and an increase in antioxidant enzymes SOD, CAT, and GPx after treatment with *Mentha piperita* leaf essential oil (40 mg/kg) in the rats treated with CCl4. Khalifa and Alkhalf's [51] results showed that dietary supplementation with powdered thyme leaves (PTL) reduced malathion (MAL) toxicity through different antioxidant and anti-inflammatory mechanisms. Mentha and thyme are rich polyphenolic mixtures, and the protective effect can be related to the antioxidant activity of their polyphenolic mixtures [52, 53]. Therefore, the treatment with menthe and thyme extracts results in the amelioration of oxidative stress by their antioxidants

CONCLUSION

In conclusion, the data from this study indicates that *Mentha piperita L.* and/or *Thymus vulgaris* extracts can induce antioxidant activities and immunity and reduce TBARS and PCC; Therefore would protecting immunity and inflammatory system from oxidative damage that could affec the health negatively.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Abbreviations:

OPO, oxidized palm oil; **TNF-α**,tumor necrosis factor-alpha; **CRP**,C-reactive protein; **IL-1β**, interleukin-1beta; **IL-6**,interleukin-6; **MCP-1**,monocyte chemotactic protein-1; **TBARS**,thiobarbituric acid reactive substance; **PCC**, protein carbonyl contents; **GSH**, reduced glutathione; **SOD**, superoxide dismutase.

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