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Role of common fig and olive oil on the Nitropropane-induced submandibular gland changes: Role of IL1β, IL6 and TNFα

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Abstract
Objectives: 2-Nitropropane (2-NP) is a rat liver carcinogen. Animals chronically exposed to 2-nitropropane by inhalation, liver and pulmonary effects have been observed. The aim of this study was to verify if common fig and olive oil have a synergistic effect on the nitropropane alterations on the IL-1β, TNFα and IL 6 in submandibular gland of Mice.
Methods and materials: The present investigation was carried out on forty adult BALB/c male mice. The mice were divided randomly into 5 equal groups. Group I, a control group (n=8), which were injected with vehicle alone (canola oil, 5 mL/kg). The other four groups (n = 32) by intraperitoneal (i.p.) injection of 2- Nitropropane (100 mg/kg body weight dissolved in canola oil) 2 time/week for 4 weeks. At the same time of 2- NP injection, mice of the latter three groups were orally drinking water supplemented with fig extract, olive oil and mixture of fig and olive oil 50 (Mg/Ml)/ 4 weeks respectively. The gene expression levels of tumor necrosis factor-alpha (TNF-a), interleukin 1-β (IL-1β), and interleukin 6 (IL-6) were tested in submandibular gland of mice by RT-qPCR.
Results: the gene expression analysis showed that the 2-nitropropane caused statistically significant upregulation of TNF-α, IL-1β, and IL-6 compared to the untreated control group. Elevated expression of IL-1β and IL-6 were significantly downregulated by olive oil and fig treatment. Co-administration of fig with olive mixture markedly down-regulate the gene expression analysis TNF-α, IL-1β, and IL-6 compared to the 2-NP treated group.
Conclusions: The findings conclude that olive oil and fig extract may attenuate the alterations of 2-NP by down-regulating proinflammatory cytokines IL-1β, IL6 and TNF α.

Keywords: Olive oil, Fig, 2-Nitropropane, IL-1, IL-6, TNFα, submandibular salivary glands

1. Introduction

Interleukins are a group of cytokines that expressed by leukocytes [1]. IL1 α is participating in the regulation of immune responses, inflammatory reactions, and hematopoiesis [2]. IL-6 plays an essential role in the final differentiation of B cells into immunoglobulin-secreting cells [3]. Several genetic modifications or mutations associated with dysregulated IL-1 activity and autoinflammatory disorders were identified in mouse models and in patients [4]. Expression of IL-1 is up regulated in different tumor phenotypes and is implicated as an important factor in tumor progression via expression of metastatic, angiogenic genes and growth factors. Therefore, down regulation of expression of IL-1 may be able to inhibit cancer progression [5]. The miR146a and miR147b were associated with increased expression of genes related to the immune/inflammatory response. Overexpression of miR147b reduced the expression of the pro-inflammatory mediators IL-6 and COX-2 after IL-1β stimulation in both astrocyte and tuberous sclerosis complex cell cultures [6].

Various disorders affecting the salivary glands are known. Sialadenitis is inflammation of a salivary gland, usually caused by infections, although there are other less common causes of inflammation such as irradiation, allergic reactions or trauma [7].

Nitropropane (2-NP) is a colourless, oily liquid with a mild odour. It is used as a solvent, principally in blends, and has many industrial applications [8]. Previously, severe liver damage, as well as some kidney damage, has been observed in workers fatally poisoned from acute inhalation exposure to high concentrations of 2-nitropropane [9]. The results indicated that 2-NP inflicted DNA damage in the bone marrow cells and thus could be leukemogenic

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[10]. Studies were carried out in the rat indicated that 2-NP induced chromosome aberrations as well as DNA repair in vivo [11].

Olive oil is a liquid fat obtained from olives by pressing whole olives. It was evidenced that increased dietary extra virgin olive oil have beneficial synergistic effects on lipid metabolism and oxidative stress in patients with metabolic syndrome [12]. Analysis of liver miRNAs showed a selective modulation of certain miRNAs by hybrid palm oil which has been proposed to be somehow equivalent to extra virgin olive oil [13]. Use of extra-virgin olive oil (EVOO) combination with donepezil up-regulated synaptic proteins enhanced blood-brain barrier tightness and reduced neuroinflammation in Alzheimer's disease patients [14]. The olive oil phenolic compounds induced cell maturation in vitro, increasing alkaline phosphatase synthesis and reducing the expression of antigens involved in immune functions of the osteoblast which would improve bone density [15].

Ficus carica (Fc) is an Asian species of flowering plant in the mulberry family, known as the common fig. It is the source of the fruit also called the fig [16]. It has been used for metabolic, cardiovascular, respiratory, gastrointestinal, and skin disorders. Several studies were performed showing its anti-inflammatory, anti-angiogenic, anticancerogenic, and tissue-protective effects. The gene expression analysis showed that the plant extract caused statistically significant downregulation of TNF-α and IL-1α compared to the untreated cells.

Also, topical Fc leaf extract may be beneficial for some inflammatory disorders and androgen-dependent disorders of the skin such as androgenetic alopecia [17]. It was reported that F. carica exhibited remarkable antidiabetic properties with various mechanisms of action. Moreover, Ficus species are versatile sources of bioactive metabolites such as flavonoids, phenolic acids, tannins, alkaloids, glycosides, coumarins, triterpenoids, sterols and vitamin E. These extracts and isolated compounds significantly have enhanced insulin secretion and subsequently reduced blood glucose level in various in vivo studies [18].

The aim of this study was to verify if common fig extract and olive oil have a synergistic effect on the nitropropane alterations on the IL-1β, TNFα and IL 6 in submandibular gland of mice.

2. Material and methods
2.1 Experimental design and doses
A total of 40 adult BALB/c male mice weighting 25–30 g/each were used in this experiment. Mice were purchased from the Institute of Theodor Bilharz (Cairo, Egypt).

2.1.1 Ethical considerations
All experimental animals maintained and monitored in a specific pathogen-free environment. All experimental animal protocols were performed according to regulations set by the Institutional Animal Care and Use Committee and were approved by Assiut University. All animal procedures were also performed according to the Declaration of Helsinki and the guidelines for the care and use of experimental animals established by the National Institutes of Health (NIH). All animals were allowed to acclimatize in plastic cages (five animals per cage) inside a well-ventilated room for one week prior to the experiment. The animals were maintained under standard laboratory conditions (temperature of 23 °C, relative humidity of 60– 70%, and a 12 h light/dark cycle) and were fed a diet of standard commercial pellets and water containing

libitum. We made every effort to minimize animal stress.

2.1.2. Grouping and experimental planning
After 1 week of acclimatization, mice were randomly categorized into five main groups (8 mice each).
a) Group I (control mice).
b) Group II (2- Nitropropane inject group mice).
c) Group III (2- Nitropropane inject group treated with Fig).
d) Group IV (2- Nitropropane inject group treated with Olive).
e) Group V (2- Nitropropane inject group treated with fig and olive mixture).

Oral toxicity was induced in mice in the latter four groups (n = 32) by intraperitoneal (i.p.) injection of 2- Nitroprobane (100 mg/kg body weight dissolved in canola oil) 2 tme/week for 4 weeks; mice in the control group were injected with vehicle alone (canola oil, 5 mL/kg). At the same time of 2-NP injection, mice in groups III, IV and V were orally drinking water supplemented with fig, olive and mix of fig and olive 50 (Mg/Ml)/ 4 weeks respectively.

2.2 Sample collection
All animals were sacrificed at day 31 post- 2- NP injection. The submandibular salivary gland was removed and cut into small pieces in sterile saline. The pieces were suspended in Trizol for RNA extraction and gene expression analysis.

2.3 Gene expression analyses
The effect of fig, olive and co-administration of both on the expression of a panel of selected genes involved in the inflammation and toxicity of oral mucosa were investigated using real-time quantitative PCR.

2.3.1 Primers used in RT-PCR
The primers used for quantitative real-time PCR analysis were designed using the Primer Express 1.5 software (Applied Biosystems). The mouse primers were designed as following:
a) TNF-α Forward: 5′- ATGAGCACAGAAAGCATGA-3′, Reverse 3′-AGTAGACAGAAGAGCGTGGT- 5′.
b) IL-6 Forward: 5′- ACCGAGCTCTGTTGACAAG-3′, Reverse 3′- TCCTCGCCACACTTCTCTTT- 5′.
c) IL-1β Forward: 5′-GCACTACAGGCTCCGAGATGAAC-3′, Reverse 3′-TTGTCGTTGCTTGGTTCTCCTTGT- 5′ and normalized using GAPDH Forward: 5′-GTTGTCTCCTGCGACTTCA-3′, Reverse 3′-GGTGGTCCAGGGTTTCTTA - 5′.

2.3.2 Titration of mRNA for IL-1, IL-6, and TNFα by RT-PCR in tissues
After performing the indicated treatments, RNA was extracted and then the corresponding cDNA was prepared and then real time PCR was applied for gene expression analysis as previously described [19]. In brief, all cDNA samples were processed in a 96-well plate using the following cycling conditions: 10 minutes at 95 ℃, and 40 cycles at 95 ℃ for 15 seconds ended by one min. at 60 ℃. These data were analyzed according to Livak and Schmittgen [20].

2.4 Statistical analysis
We used one way analysis of variance ANOVA for determination of the statistical significance of differences between mean values. A probability of ≤ 0.05 defined this significance.

3. Results
Real time- PCR and mouse primers specific for IL-1β, IL-6 and TGFα were used to detect the presence of mRNAs. IL-1, IL-6 and TGFα could be detected in mouse submandibular gland in all groups. The results shown in table [1] indicate a dramatic difference between different study groups.

Data in (Table 1) show the IL-1β, IL-6 and TGFα mRNA expression in BALB/c mice salivary gland in different study groups. The means of IL-1, IL-6 and TGFα levels demonstrated a significant increase in mice with 2-NP when compared to controls ($P<0.001$). In contrast, use of olive oil and common fig extract revealed that the relative mRNA levels of the IL-1β, IL-6 and TGFα decreased significantly compared with the 2-NP treated mice (Figs. 1-3).

Table 1: Comparison of the mRNA expression of IL-1β, IL-6 and TNFα in BALB/c mouse submandibular salivary glands using real time PCR

	Group I (Control group)	Group II (2-NP-treated group)	Group III (Olive treated group)	Group IV (Fig treated group)	Group V (Fig and Olive treated group)
IL-1β	1	5.94592	2.511622	2.683212	1.616579
IL-6	1	8.56671	6.959865	5.273673	4.819247
TNFα	1	7.00216	4.953422	3.266623	1.876182

Figure (1) shows that expression of mRNA for IL-1β genes steadily increased with the administration of 2-NP ($P<0.001$). Significantly lower levels were observed with olive and fig treatment ($P<0.05$).

As shown in Fig. 2, the expression of IL-6 in tissue samples was significantly lower after olive and fig. the differences in mean IL-6 expression appeared statistically significant, with a P value of 0.01. However, tissue expressions of IL-6 in 2-NP treated group had risen. Similar trends were observed in tissue samples, as shown in Fig. 3. After olive and fig treatment, expressions of TNFα were significantly lower ($P<0.05$).
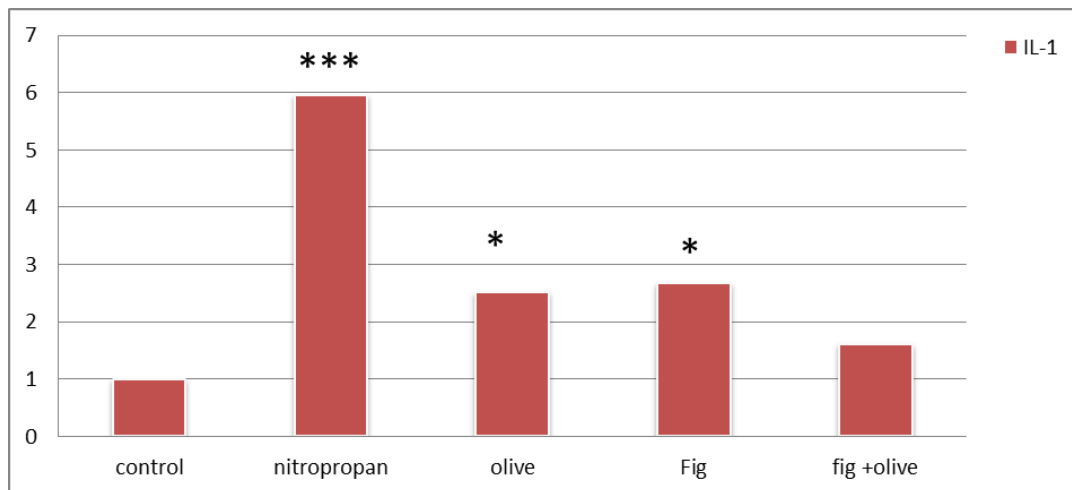

Fig 1: Effect of Fig, Olive and combination on the IL-1 mRNA expression in BALB/c Mouse Salivary gland after exposure to 2- Nitropropane as shown in materials and methods and the IL-1 mRNA expression was measured using real time PCR.
*$P<0.05$: mildly significant,**$P<0.01$: significant, ***$P<0.001$: highly significant, compared to normal control.
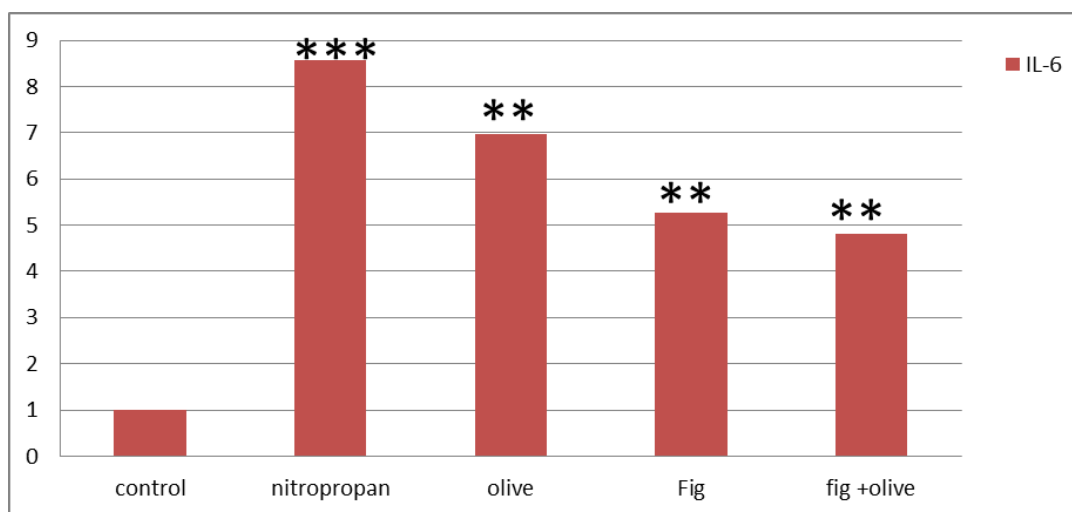

Fig 2: Effect of Fig, Olive and combination on the IL-6 mRNA expression in BALB/c Mouse Salivary gland after exposure to 2- Nitropropane as shown in materials and methods and the IL-1 mRNA expression was measured using real time PCR.
*$P<0.05$: mildly significant,**$P<0.01$: significant, ***$P<0.001$: highly significant, compared to normal control.
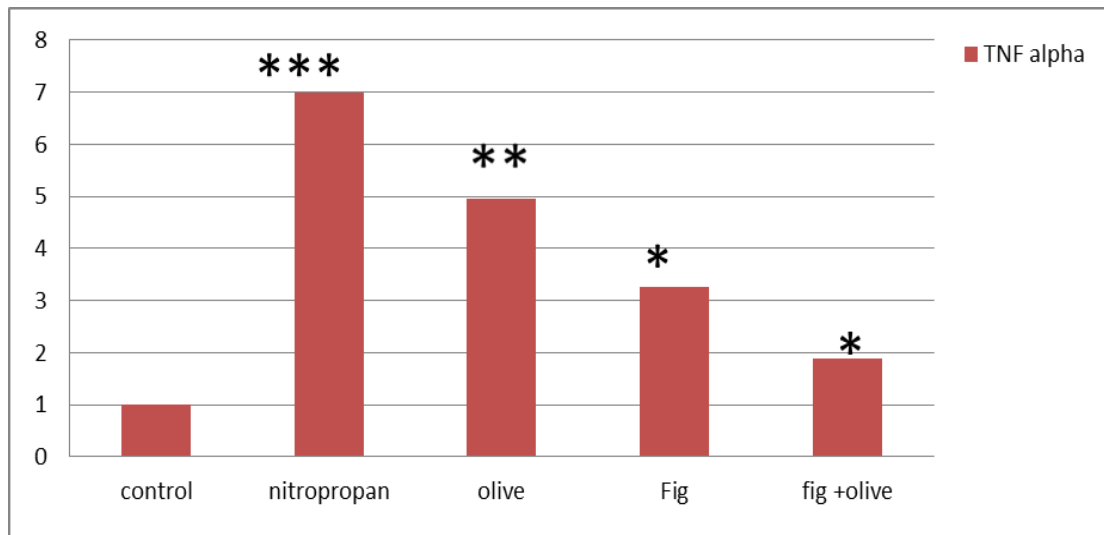
Fig 3: Effect of Fig, Olive and combination on the TNFα mRNA expression in BALB/c Mouse Salivary gland after exposure to 2-Nitropropan as shown in materials and methods and the IL-1 mRNA expression was measured using real time PCR.
*$P<0.05$: mildly significant,**$P<0.01$: significant, ***$P<0.001$: highly significant, compared to normal control.

4. Discussion

In the present work it was investigated that the expression levels of IL-1β, IL-6 and TNF-α in the submandibular glands of BALB/c mice were significantly higher in the 2-NP treated animals compared to the control group. These changes were comparable to those demonstrated in other studies [7, 21]. It was suggested that the liver damage induced by 2-NP is related to oxidative damage, lipid peroxidation [22]. Previous results indicated that 2-NP inflicted DNA damage in the bone marrow cells [10] and induced chromosome aberrations as well as DNA repair [11].

In contrast, the findings of this study suggested that the expression levels of IL-1β, IL-6 and TNF-α in the submandibular glands from mice treated with olive oil and fig extract were significantly decreased. Use of fig and olive mix greatly decreased the gene expression analysis of TNF-α, IL-1β, and IL-6 compared to the 2-NP treated group.

These results may be correlated with several studies which evidenced possible health benefits of olive oil. It was evidenced that increased dietary extra virgin olive oil have beneficial synergistic effects on lipid metabolism and oxidative stress in patients with metabolic syndrome [23]. On the other hand, analysis of liver miRNAs showed a selective modulation of certain miRNAs by hybrid palm oil [24]. The olive oil phenolic compounds induced cell maturation in vitro, increasing alkaline phosphatase synthesis and reducing the expression of antigens involved in immune functions of the osteoblast which would improve bone density [25].

In respect to the role of cytokines, expression of IL-1 is up regulated in different tumor phenotypes and is implicated as an important factor in tumor progression via expression of metastatic, angiogenic genes and growth factors. Therefore, down regulation of expression of IL-1 may be able to inhibit cancer progression [5].

Similarly, Interleukin 6 (IL6) and TNF α are involved in a wide variety of biological functions. Tumor necrosis factor receptor-1 (TNFR1) is involved in apoptosis through extrinsic pathway initiation. The level of soluble TNFR1 is reported increased in primary Sjögren's syndrome patients [26].

Furthermore, it was found that stimulation with IL-1β and TNF-α increased submucosal gland secretion in a concentration-dependent manner. The cytokine effect was dependent on cAMP. It was suggested that during bacterial infections and resulting release of proinflammatory cytokines, the glands are stimulated to secrete fluid [27].

Additionally, higher expression of IL-6 was found in salivary gland cancer (SGC) (70.7%) than in normal tissue (20%). There was a high association of cytomegalovirus CMV antigen presence with the presence of IL-6, and with the IL-6 expression intensity. Positive expression of CMV antigens in a high percentage of SGC cells suggested that it might play an important role in carcinogenesis by increasing IL-6 production and leading to inhibition of apoptosis and tumor development [29]. It was demonstrated that IL6 pretreatment prevented both senescence and salivary gland hypofunction via a mechanism involving enhanced DNA damage repair. Collectively, previous results indicated that cellular senescence is a fundamental mechanism driving radiation-induced damage in the salivary gland and suggested that IL6 pretreatment may represent a promising therapeutic strategy to preserve salivary gland function in head and neck cancer patients undergoing radiotherapy [28].

Similarly, protein expression levels of IL-17 and IL-6 were detected in parotids and submandibular glands by ELISA [30]. In this study there is a strong association between administration of fig and the expression levels of IL-1β, IL-6 and TNF-α which can be interpreted by many authors. It was suggested that the ethanolic extract of the fruit of F. carica may have potential antidiabetic and antiobesogenic agents [31]. It could be concluded that a herbal mixture composed of black berry, artichoke, and fig could afford an excellent natural candidate to combat oxidative stress and counteract hepatic toxins owing to its phenolic compounds [32]. F. carica leaves exerted significant effect on carbohydrate metabolism enzymes with promising hypoglycemic and hypolipidemic activities in type 2 diabetic rats [33]. Other study demonstrated that aqueous methanol extract of F. carica fruit exerted hypotensive and antihypertensive effects in glucose-induced hypertensive rats [34]. It was indicated that the dried fig could be as important as diet-derived antioxidants and antihepatotoxicity in preventing oxidative damage in the tissues by inhibiting the production of ethanol-induced free radicals and hepatotoxicity in rats [35]. Finally, we concluded that olive oil and fig extract may attenuate the alterations of 2-NP by down-regulating proinflammatory cytokines IL-1β, IL6 and TNF α.

5. References

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