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Oxidative stress status, Nitric oxide and Peroxynitrite levels in sera and saliva of Iraqi smokers.

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ABSTRACT

The aim of this study was to estimate the oxidative stress status in sera and saliva of smokers, and point on to the role of nitric oxide and peroxynitrite in this status. The study samples was divided into four groups,three of them were smokers groups:cigarette (CS), cigarette&narghile (CS&N), narghile (N).In addition to a non smokers group to be used as control (C).The measured parameters included serum and salivary Total Oxidant Status (TOS),Total Antioxidant Status(TAS),Nitric Oxide(NO)concentration and of Peroxynitrite ($ONOO^-$).The results showed a significant increase in serum(TOS)of(CS&N)group compared to that of (C)group,whereas a non-significant difference was found in(CS)as well as(N)groups compared to that of (C)group, with a non-significant change in saliva of all smokers groups compared with that of(C)group.Meanwhile the serum (TAS)showed that there was a non-significant difference in all smokers groups in comparison to that of(C) group, while in saliva this parameter showed a significant decrease in both(CS)and(CS&N)groups but non-significant change in(N)group compared with that of(C)group.A high significant increase was observed in nitric oxide level in serum of(CS&N)group with no significance difference in sera of(CS)group and(N)group compared with that of(C)group, while in saliva there was a significance increase in both(CS)and(CS&N)groups compared with(C),but no significance difference in saliva of(N) group compared with(C)group.Meanwhile no significant difference in peroxynitrite level was found in both serum and saliva of different smokers groups compared with that of(C)group.

Keywords: Saliva, Total oxidant status, Total antioxidant status, Nitric Oxide, Peroxynitrite, Cigarette &Narghile.

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INTRODUCTION

The introduction of contaminants into a natural environment, leading to instability, harm discomfort, disorder, and stress to the ecosystem, the physical systems or living organisms ,can be defined as Pollution. Pollutants included chemicals such as petroleum hydrocarbons, heavy metals, pesticides etc. that can lead to system stress[1]. Environment pollution is reported to be a worldwide problem and its potential to influence the health of human populations is great [2].The manufacture of cigarettes and cigars creates large quantities of waste in the form of tobacco slurries, solvents, paper , oils, wood, plastics, packaging materials and airborne pollutants[3].

For four centuries the most potent and prevalent addictive habits, influencing behavior of human beings is the using of tobacco smoking [4]. Throughout the developing world smoking is now increasing rapidly and it regards as one of the biggest threats to current and future world health[5]. According to World Health Organization (WHO) approximately 47% of men and 12% of women smoke worldwide, the most popular form of smoking is tobacco smoking and it practiced by over one billion people in majority of all human societies [6]. Smoking in accordance to the classification of (WHO) classified as a chronic, and progressive disease as well as contagious .Meanwhile, it can damage each part of the body [7].

The most popular ways of smoking are smoking cigarettes, cigars and pipes. In addition to these in the Subcontinent region, tobacco is smoked in the forms of “Beedi” (tobacco rolled in dry leaves) and “Huqqa” [8].Water pipe smoke (WPS; also called hubble bubble shisha, hookah, or narghile) is a traditional method of tobacco smoking commonly practiced especially in China , Turkey , India and the Arabian Peninsula and is now increasing globally [9].Many of the harmful chemicals and gases present in cigarette smoke are existing in equal, or even greater amounts in water pipe smoke, including nicotine , heavy metals and carbon monoxide [10].

The role of oxidant /antioxidant balance in physiological state and disease pathogenesis has been reported previously by many studies cited in reference (11)[11]. The chemicals which directly, or indirectly lead to the formation of free radical such as aldehydes, phenols, hydrocarbons, nitric oxide (NO), and quinone and semiquinone radical are reported to be present in the cigarette tobacco smoke[12].Therefore Tobacco smoke is considered as an abundant source of oxidants, the increased production of reactive oxygen species associated with smoking may surpass the capacity of oxidant defense system, which lead to oxidative damage [13].

NO is an important molecule involved in physiological and pathological processes in mammals. It can be protective or hazardous for organs or tissues in where it is present [14]. Its source into the body is from both metabolic and dietary substances followed by its transport to the salivary glands via blood[15]. Its injurious effects in the body is due to formation of ugly peroxynitrite through its reaction with superoxide [16]. The generation of a moderate flux of peroxynitrite over long periods of time cause substantial oxidation and potential destruction of host cellular constituents, leading to the disruption of cell signaling pathways, dysfunction of critical cellular processes, and the induction of cell death through both apoptosis and necrosis [17].

Several studies were done to show the effect of smoking on antioxidants and oxidative stress among the smokers, such as Ozbay & Dulger،s study: which showed that in cases of smoking and acute exercise there was a significant increase in lipid peroxidation activity with a decrease in activity of antioxidant enzyme.[18]. Also Morrow *et al*., showed there was an increase in lipid peroxidationin of the smokers [19] .In contrast to the study of Charalabopoulos et *al*., which measured a non-significant change in salivary antioxidant defense in young smokers group compared with that of non-smokers group while they exhibited a significantly reduced ability of blood lymphocytes to resist H_2O_2-induced DNA damage, in spite of their higher plasma antioxidant capacity, [20].

The suggestion that saliva could be used in diagnosis of disease, begun in the second half of the20thcentury. Today saliva is often used to diagnose systemic and local diseases [21-23].The main advantage of this medium is the easy, noninvasive sampling method compared with that for blood [21].

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Antioxidants are present in all body fluids including saliva. Saliva may comprise a first line of defense against oxidative stress and has protective effects against microorganisms, oxidants and toxins [24]. Several studies showed reduced levels of salivary antioxidants in smokers compared to nonsmokers, such as a study by Kanehira *et al*., who compared salivary antioxidant enzymes of elderly smokers with that of non-smokers[25], also Reddy and colleagues who studied the effect of cigarette smoking on salivary and blood super oxide dismutase levels between smokers and non-smokers [26].The results of these studies disagreed with those of Buduneli *et al*., who studied the influence of smoking and gingival inflammation on salivary antioxidant capacity [27],as well as Zappacosta & colleagues[28] who studied the effect of smoking one cigarette on salivary antioxidant metabolites of healthy smokers, in addition to Charalabopoulos *et al.*, who compared salivary antioxidant capacity between smokers and non-smokers[20].

Limited reports that utilized or appreciated using of saliva to detect the oxidative stress alteration in comparsion to the alteration in serum of smokers of (cigarette), (cigarette &narghile) & (narghile) compared to nonsmokers has been found in the literature.

Therefore, the aim of this project is to estimate the oxidative stress status in sera and saliva of Irqi individuals using different types of smoking, and point on to the role of nitric oxide and peroxynitrite in this status.

MATERIALS AND METHODS

Materials

All chemicals used in this study were of analytical grade.

Participants Criteria

The study was carried out on a total number of 95 person .They were Iraqi male with age ranged from (20 - 28 years).The study participants were divided into four groups, three of them were smoker groups .In addition to a fourth group who were healthy ,age matched nonsmoker male to be used as control for the purpose of comparison. The detailed information of the participants is illustrated in **(Table 1)**: The local Ethics committee of the College of Science approved the study protocol.

(Table 1): Details of participants groups

Characteristic	Cigarette(CS) smokers n=30	Cigarette and narghile(CS&N) smokers n=25	Narghile Smokers(N) n=18	Control(C) n=22
Gender	Male	Male	Male	Male
Age (year)	25± 5	25± 5	25± 5	25± 5
Method of Smoking	Cigarette	Cigarette& Narghile	Narghile	_
Period of Smoking (year)	3-5	3-5	1-3	
Number of Smokings (times per day)	10-40	10 – 40 2 - 4 (Puff of narghile per week)	2 – 4 (Puff narghile per week)	_
Medical History	None	None	None	None

Samples

Serum and saliva collection

Fasting whole blood was collected (5.0 ml) from the healthy Iraqi smokers and nonsmokers healthy individuals, kept in tube without any anticoagulant at room temperature for one hour. Then the tube was

centrifuged (2000×g) for 10 minutes, the clear serum was pipetted into clear dry test tube and then stored at (-20) C° for subsequent analysis.

Unstimulated saliva was collected in the overnight fasting state, after thoroughly rinsing the mouth with saline solution. It was centrifuged (2000×g) for 10 minutes and the supernatant was stored at (-20) C° until being used for different investigations.

Methods

Total Oxidant Status (TOS)

Total oxidant status value was determined using Erel (2005) [29].Which is based on the following:

Oxidants present in the sample oxidize the ferrous ion–*o*-dianisidine complex to ferric ion. The oxidation reaction is reported to be enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion with xylenol orange in an acidic medium makes a colored complex. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample.

Standard curve of total oxidant

The assay was calibrated with hydrogen peroxide(10mM)using different concentrations (0,25,50,100,150,200 µmol/L) of standard hydrogen peroxide (10 mM) and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (µmol H_2O_2 Equiv./L).Then from the equation that derived from the standard curve the (TOS) concentrations were calculated .

Total Antioxidant Status (TAS)

The total antioxidant status value was determined using Erel's developed method (2004) [30].The principle of the assay can be summarized as follows: A standardized solution of Fe^{2+}–*o*-dianisidine complex reacts with a standardized solution of hydrogen peroxide by a Fenton-type reaction, producing $OH^{\cdot}$. These potent ROS oxidize the reduced colorless o-dianisidine molecules to yellow-brown colored dianisidyl radicals at low pH. The oxidation reactions progress among dianisidyl radicals and further oxidation reactions develop. The color formation is increased with further oxidation reactions. Antioxidants in the sample suppress the oxidation reactions and color formation. This reaction can be monitored spectrophotometrically.

Standard curve of total antioxidant

The assay was calibrated using different concentrations (0, 0.2 , 0.4, 0.6, 0.8, 1.0 mmol/L) of standard Uric acid (Uric acid 1.0 mM which dissolved in 10mM NaOH solution). The results were expressed as in terms of millimolar uric acid equivalent per liter. Then the (TAS) concentrations were calculated from the equation that derived from the standard curve .Which constructed from the plot of different concentrations of standard against the absorbance.

Determination of nitric oxide concentration

Nitric oxide concentration was determined using (Jose *et al*, 1998) [31] method. In which nitric oxide concentration in the samples of the studied groups was determined based on the reduction of nitrate to nitrite by cadmium and the produced nitrite was determined by Griess reaction. The Formation of the azo dye was detected via its absorbance at λ=540 nm.

Standard Curve of NO

Standard NO curve was prepared using different concentrations (0, 50,100, 150,200, and 250µM) of stock sodium nitrite ($NaNO_2$).Then the nitric oxide concentration was calculated using the equation derived from the standard curve.

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Determination of peroxynitrite concentration

Peroxynitrite concentration was determined according to (Vanuffelenet al, 1998) [32] procedure .In which peroxynitrite mediated nitration of phenol, resulting in nitrophenol formation, which it's absorbance was measured at a wave length (λ=412 nm).

Calculation

$$\text{peroxynitrite concentration (mM)} = \frac{A\ test - A\ blank}{\text{Molar extinction coefficient}} * 10^3$$

Where:

A_{test}= absorbance of the test, A_{blank} =absorbance of blank

Molar Extinction Coefficient (ε) is equal to $4400M^{-1\ cm-1.}$

Statistical analysis

All statistical analyses were performed using SPSS program, version16.0 under Windows (Statistical Package for Social Science, Inc.,Chicago, IL, USA). Descriptive analysis was used to show the mean and standard deviation of variables. The significance of difference between mean values was estimated by Student independent T-test.and P-value < 0.05 was considered significant and P-value < 0.01 was considered as a high significant.

RESULTS

Total Oxidant Status (TOS) {which can be defined as the *in vivo* marker of a shift developing in an oxidative/anti oxidative ratio in favor of the oxidative side [33]}, was measured in serum and saliva of the different studied smokers groups as aforementioned in materials & methods part.

In serum of (CS&N) group a significant increase is obvious in (TOS) ($p<0.05$) compared with (C) group, but no significance difference ($p>0.05$) is found in (TOS) of (CS) as well as of (N) groups compared to that of the (C) group. While in saliva there is a non-significance change in this status ($p<0.05$) in the three studied groups compared to that of nonsmokers control group. **(Table 2)**

(Table 2): Total oxidant status (TOS) level in the three smokers groups [cigarette (CS), cigarette & narghile (CS&N), narghile (N)] in comparison to the control group.

Parameters	*group*	*Mean value±SD*	*group*	*Mean value±SD*	*P-value*
TOS serum µmol H_2O_2/L	**Control (C) n=22**	**16.5±3.9**	**(CS) n=30**	**17.1±4.40**	**0.608**
			(CS&N) n=25	**19.04±4.03**	**0.038***
			(N) n=18	**16.04±2.58**	**0.653**
TOS saliva µmol H_2O_2/L	**Control (C) n=22**	**7.07±2.91**	**(CS) n=30**	**8.7±3.70**	**0.064**
			(CS&N) n=25	**6.9±4.32**	**0.927**
			(N) n=18	**8.3±5.01**	**0.443**

*** Differences are statistically significant (P<0.05).**

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Total Antioxidant Status (TAS) {which is a biomarker for measuring the antioxidant potential of body fluids can be defined as the moles of oxidants neutralized by one liter of solution [34] }, was measured in the studied groups as mentioned in methods & materials section.

The results presented in **(Table 3)** and it is clear that there is no significant difference in this parameter in serum of all smokers (CS), (CS&N) &(N) groups in comparison to the control group. While this parameter in saliva show a significant decrease (P<0.05) in both (CS) and (CS&N) group with no significant change (P>0.05) in (N) group in comparison to that of the control group.

(Table 3): Total antioxidant status (TAS) level in the three smokers groups [cigarette (CS), cigarette &narghile (CS&N), narghile (N)] in comparison to the control (C) group.

Parameters		*Mean value±SD*		*Mean value±SD*	*P-value*
TAS serum mmole Uric acid/L	**Control (C) n=22**	**0.58±0.38**	**(CS) n=30**	**0.42±0.23**	**0.077**
			(CS&N) n=25	**0.53±0.28**	**0.606**
			(N) n=18	**0.45±0.28**	**0.288**
TAS saliva mmole Uric acid/L	**Control (C) n=22**	**0.64±0.43**	**(CS) n=30**	**0.41±0.27**	**0.017***
			(CS&N) n=25	**0.41±0.31**	**0.049***
			(N) n=18	**0.44±0.26**	**0.093**

***Differences are statistically significant (P<0.05).**

Nitric Oxide concentration was measured as mentioned in materials & methods section in serum & saliva of all studied groups and the results as described above in **(Table 4)** show that there is a high significant increase in nitric oxide level (p<0.01) in serum of (CS&N) group compared with (C) group, and no significance difference (p>0.05) in (CS) group and (N) group compared with that of (C) group. While in saliva there is a significance increase (p<0.05) in both (CS) and (CS &N) groups compared with (C),and no significance difference (p>0.05) in saliva of (N) group compared with (C).

(Table 4): Nitric Oxide concentration in the three smokers groups [cigarette (CS), cigarette &narghile (CS&N) , narghile (N)] in comparison to the control (C)group.

Parameters		*Mean value±SD*		*Mean value±SD*	*P-value*
NO serum µM	**Control (C) n=22**	**14.9±3.69**	**(CS) n=30**	**15.5±7.48**	**0.699**
			(CS&N) n=25	**18.6±4.24**	**0.004****
			(N) n=18	**17.2±3.62**	**0.107**
NO saliva µM	**Control (C) n=22**	**15.3±4.71**	**(CS) n=30**	**17.9±4.44**	**0.046***
			(CS&N) n=25	**18.8±4.21**	**0.014***
			(N) n=18	**18.4±4.21**	**0.069**

***Differences are statistically significant (P<0.05)**
****differences are statistically high significant (P<0.01).**

When the level of peroxynitrite was measured in serum and saliva of all the studied groups as mentioned in materials & methods section.

When serum & salivary peroxynitrite concentration were compared between the different smokers groups and that of control group, the results in **(Table 5)** illustrate a non-significant difference (p>0.05) in both serum and salivary peroxynitrite level in the different studied groups compared with that of control group.

(Table 5): Peroxynitrite concentration in the three smokers groups [cigarette (CS), cigarette &narghile (CS&N), narghile (N)] in comparison to the control (C) group.

Parameters		*Mean value±SD*		*Mean value±SD*	*P-value*
Peroxynitrite Serum µM	**Control (C) n=22**	**54.5±14.08**	**(CS) n=30**	**56.1±16.82**	**0.591**
			(CS&N) n=25	**53.6±12.16**	**0.827**
			(N) n=18	**56.6±15.26**	**0.701**
Peroxynitritesaliva µM	**Control (C) n=22**	**2.83±1.08**	**(CS) n=30**	**2.53±0.95**	**0.314**
			(CS&N) n=25	**2.98±1.07**	**0.650**
			(N) n=18	**2.66±1.02**	**0.604**

***Differences are statistically significant (P<0.05).**

DISCUSSION

During smoking, free radicals are formed that can activate inflammatory cells which generate high levels of reactive oxygen metabolites .The presence of high amount of free radicals in tobacco smoke may play a major role in depletion of antioxidant level, lipid peroxidation, protein modification , the product of such process in turn may react with tobacco smoke constituent resulting in additional toxic products .Therefore smokers are subjected to an increased oxidative stress situation, which can result in an imbalance between oxidants and antioxidants [35,36].

The total oxidant status (TOS) was measured in the present study ,and the results in **(Table 2)** show that in serum of (CS&N) group there is a significant increase (p<0.05) compared to that of nonsmokers control group .Whereas a non significant difference (p>0.05) was found in this status in serum of (CS) as well as of (N) groups compared with that of (C) group .The result of (CS&N) group is in line with the result of Shermatov *et al*., who reported that in children exposed to secondhand cigarette smoke the (TOS) level was significantly higher than that group who did not exposed to cigarette smoke [37] .While no significant difference was found in serum (TOS) level of (CS) as well as of (N) groups compared to that of (C) group.This result agrees with the result obtained by of Aslan *et al*., who studied the total antioxidant status as well as the total oxidant status in sera of 100 Turkish smokers compared with the same number of nonsmokers [38].

It is reported that smoking is an important factor influencing the salivary levels parameters of oxidative stress. It influences the oxidative balance in the whole body through stimulation the oxidative burst of neutrophils [39].Meantime several investigations, have suggested that saliva contains oxidation biomarkers akin to those in blood. Therefore, saliva can be considered as one of the chief defense systems against aqueous soluble components of tobacco[28].

The results of salivary (TOS) showed a non-significant difference (p>0.05) in all three studied smokers groups compared to that of the control group **(Table 2).**

Free radical formation is naturally controlled by antioxidants. Antioxidants are capable of deactivating or stabilizing free radicals before they attack the different components of the **cells [40].**

Since the measurement of all known antioxidants separately is time consuming and many antioxidants may remain undiscovered, in addition to that the total activity may be greater than the sum of the individual antioxidants because of their cooperative interactions, therefore it was recommended that measurement of total antioxidant activity better than measuring the individual antioxidant, as performed in the present study [41].

In serum and saliva the total antioxidant status of (CS, CS&N, N) smokers and nonsmokers were measured, the results in **(Table 3)** showed that in spite of the presence of difference but this difference was not significant .The measured total antioxidant status(TAS) in serum of nonsmokers was higher than that of all smokers groups. Moreover the salivary (TAS) of nonsmokers group was higher than that of (CS) and (CS&N) groups. This increase was statistically significant ($P < 0.05$). These study agree with that of Ziborro & Bartosz who compared the total antioxidant status of various human fluids and found a lower TAS values in saliva of the smoker than in that of non-smokers [42]. Also in line with Abdolsamadi *et al.,* results who studied some salivary antioxidant enzymes of the smokers with a daily consumption of 20 cigarettes for at least 10 years in comparison to that of non-smokers [43].

Such observed decrease in the total antioxidant status level may be explained as that due to the presence of a high amount of free radicals in tobacco smoke results in an oxidative stress in the smoker bodies, this leads to exhaustion antioxidants there.

Non-significant difference in serum and saliva of (TAS) was observed in the saliva of (N) group compared to that of control group. This result agrees with the results of Charalabopoulos *et al.,* who evaluated the antioxidant compensatory mechanisms, by estimating the antioxidant capacity of extracellular defence fluid (saliva and plasma) and the intracellular resistance of peripheral lymphocytes to oxidative stress in young healthy smokers [20]. As well as agrees with results of Buduneli *et al*., in their study of the influence of gingival inflammation and smoking on salivary antioxidant status and found that neither gingival inflammation nor smoking compromised the antioxidant capacity of saliva in systemically health gingivitis patients[27]. Such results can explained by that when the host antioxidant systems are generally activated in response to an oxidant attack, a balance exists as a protective mechanism between the oxidants and antioxidants system [38].

A highly significance increase in nitric oxide level ($p<0.05$) was detected in serum of (CS&N) group **(Table 4)** compared with control group. A result in line with that of Ghasemi *et al* 2010, who studied the influence of cigarette and narghile smoking on serum nitric oxide metabolite concentration, and found serum NO metabolites was significantly higher in the active smokers men compared to nonsmokers[44]. Also this parameter in saliva showed a significance increase ($p<0.05$) in both (CS) and (CS &N) groups compared with that of (C) group **(Table 4)**. These results are in line with the study of Preethi *et al.,* Who showed an elevated level of salivary nitric oxide in patients with oral lichenoid reactions and smokers, tobacco chewers when compared to normal controls[45].The results of the present study contradict the data of Bodis & Haregewoin, who studied nitric oxide level in freshly secreted saliva, and found reduced salivary nitric oxide levels in smokers compared to control subjects [46].

The measured increased nitric oxide concentration in serum of (CS&N) , as well as in saliva of (CS) and (CS&N) groups, may be due to that tobacco smoke contains very high concentrations of nitric oxide in fresh smoke which was reported to be as high as 500ppm [47]. In addition carbon monoxide, which is a major component of cigarette smoke, binds to the hemoglobin and therein prevents oxygen from doing so. This causes a lower amount of oxygen to be transported through the blood and carried to the cells of the body a situation refer to as a hypoxia[48] .A hypoxia is a synergistic inducer of iNOS expression and hence favours the reaction between nitric oxide and oxygen resulting in nitrosative modifications[49]. Therefore, the observed increase in nitric oxide level in smokers **(Table 4)** may be due to the release of cytokines by inflammatory mediator as a result of the existence of tobacco smoke, or may be due to a hypoxia which lead to over expression of iNOS synthesis [45].

A non-significant difference ($p>0.05$) in serum nitric oxide of (CS) group and (N) group compared with that of (C) group was observed **(Table 4)**. Meanwhile a non-significant difference ($p>0.05$) was found in this

parameter in saliva of (N) group compared with that of the control group. This result was in line with a study of Kurku *et al.,* who didn't find significant difference between the NO level of smokers and non smokers[11].

In this study non-significant difference was found in peroxynitrite concentration in both serum and saliva of different kinds of smokers compared to the control group **(Table 5)**.Superoxide and nitric oxide can react together to produce peroxynitrite, each can modulate the effects of the other. This also may imply that the rate of production of either nitric oxide , or superoxide, and the ratio of their concentrations, may be important in systems in which both radicals are produced[50].Superoxide is usually scavenged by superoxide dismutase (SOD), which is a metalo proteins present in all oxygen metabolizing cell. The reaction of NO with O^-_2 competes the reaction of scavenging O^-_2. And the presence of SOD in various compartments of our body enables it to dismutate O^-_2 radicals immediately which protects the cells from its oxidative damage [50]. It is worth to mention that several studies reported a significantly higher salivary SOD activity in smokers compared to nonsmokers [25,51,52]. Also a significant increase in serum SOD activity was observed in smokers upon comparison of its activity compared with that of the control group [53].Therefore it can be concluded that the reason of the observed non significant difference in peroxynitrite concentration in this study may be due to the reported high activity of SOD ,which scavenged the O^-_2 radical and prevent it's reaction with nitric oxide to produce peroxynitrite.

Finally, the reported unaltered salivary total oxidant status in the present study may be explained based on that even though the observed reduction in salivary total antioxidant status of (CS&N) group, the increase in nitric oxide concentration which is not accompanied with increase in peroxynitrite concentration as described above, are enough to cope with the increased free radicals induced by smoking. Therefore, here nitric oxide NO can be a very effective antioxidant to the reactive oxygen species (ROS). Such antioxidant mechanisms was suggested to be through the versatile chemistry of NO with ligand- metal and radical- radical [54].

The present study clarify the single effect of (CS) & (N) smoking and the binary effect of both (CS&N) .The design emphasizes the differences between narghile and cigarette smoke resulting from differing quality rather than quantity of smoke delivered. The usage of charcoal in the former as a heating source is considered an important characteristic distinguishing the narghile from the cigarette. Thus narghile includes smoke from charcoal burning products as well as those originating from the sweetened tobacco mixture. Therefore, it is predictable that several of the chemical compounds present in smoke of cigarette would also be present in narghile smoke [55]. The tar produced by a narghile may vary from that produced by a cigarette, because tobacco in a narghile is heated to about half the temperature (that is, 450˚C) of tobacco in a cigarette[10].In addition to that the volume of smoke inhaled during a typical 5-minute episode of cigarette smoking use is dramatically less than that inhaled during a typical 45-minute episode of narghile. Several studies involving the measurement of total puff volume of a narghile smoking and showed that these smokers inhale 50 to 80 L of smoke each time they use a narghile. In contrast, cigarette smokers inhale when smoking a single cigarette about 0.5 to 0.8 L of smoke [56].

Therefore, the concurrence effects of both cigarette & narghile smoke lead to that the changing in the measured parameters of (CS&N) group is more obvious than other groups.

CONCLUSION

Based on the obtained results, one can conclude that

- Free radicals which present in tobacco smoking play a significant role in the lack of oxidative balance and this is mainly obvious in cigarette and narghile group than other studied groups .Such results indicate that the combined effect of the smoke from both cigarette and narghile together has more effect on human health than the smoke of either of narghile or cigarette alone.
- Nitric oxide seems to play antioxidants role in the studied groups.

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