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Reversal of Valproate-Induced Major Salivary Gland Changes By *Moringa Oleifera* Extract in Rats

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This study aimed to explore the potential protective impacts of *Moringa oleifera* extract on major alteration in salivary glands of rats exposed to sodium valproate (VA). Groups were defined as control, control+moringa extract, sodium valproate, and sodium valproate+moringa extract. Antioxidant and oxidant status, activities of digestive and metabolic enzymes were examined. VA treatment led to various biochemical changes in the salivary glands, including decreased levels of antioxidants like glutathione, glutathione-S-transferase, and superoxide dismutase (except for sublingual superoxide dismutase). Conversely, a decrease in alpha-amylase, alkaline and acid phosphatase, lactate dehydrogenase, protease, and maltase activities were observed. The study also demonstrated that VA induces oxidative stress, increases lipid peroxidation, sialic acid, and nitric oxide levels in the salivary glands. Total oxidant capacity was raised in all glands except in the sublingual gland. The electrophoretic patterns of proteins were similar. *Moringa oleifera* extract exhibited protective properties, reversing these VA-induced biochemical changes due to its antioxidant and therapeutic attributes. This research suggests that moringa extract might serve as an alternative treatment approach for individuals using VA and experiencing salivary gland issues, although further research is necessary to confirm these findings in human subjects.

Introduction

The main task of the salivary glands is to produce saliva, which contains enzymes that aid digestion and protect against microbes, and to moisten the mouth. Drugs for the treatment of various diseases can cause adverse effects such as salivary gland dysfunction, salivary gland hypofunction, sialorrhea (excessive salivation), and dry mouth.[1]

Valproic acid/sodium valproate (VA), or 2-propyl pentanoic acid, is a branched short-chain fatty acid derived from valeric acid. Besides being used in the treatment of epilepsy, VA is also an effective drug for use in psychiatric disorders such as anxiety, bipolar disorder, and migraine.[2] VA is generally well tolerated and has proven to be relatively safe, but it has also been found to have many side effects.[3] Clinical side effects of VA are indigestion, weight gain, dysphoria, fatigue, dizziness, drowsiness, hair loss, headache, nausea, sedation, and tremor. In addition, it has been suggested that VA causes sialadenosis and dry mouth,[4] and that VA treatment inhibits the growth of salivary gland tumors.[5] There is evidence demonstrating that VA can modulate oxidative stress in a tissue-specific way.[6] It has not yet been fully established whether VA can alter oxidative state in major salivary glands.

Moringa oleifera (*M. oleifera*) belongs to the Moringaceae family, every part of the moringa plant, including seeds, leaves, and fruit, is valuable for the development of drugs and food products. *M. oleifera* leaves contain minerals such as calcium, potassium, zinc, magnesium, iron, and copper. It is also rich in beta-carotene, vitamin C, vitamin E, protein, polyphenols, and a known good source of natural antioxidants. Extracts obtained from *M. oleifera* exhibit many nutraceutical and pharmacological properties such as anti-inflammatory, antioxidant, anticancer, hepatoprotective, neuroprotective, blood sugar, and lipid-lowering effects. Their ability to perform these beneficial functions is strongly associated with flavonoids or bioactive isothiocyanates.[7]

Although it is widely used in food and drugs, there are few studies on clinical trials demonstrating the therapeutic effect of *M. oleifera* leaf and other parts of the plant in humans.[8]

Literature relating to the effect on *M. oleifera* on salivary glands of VA individuals is scarce. Moreover, evidence demonstrating that VA can induce oxidative stress in a tissue-specific way is present. Therefore this study investigated the effects of *M. oleifera* extract on the salivary glands of VA treated Sprague Dawley rats, especially the changes in biochemical compositions.

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Results and Discussion

This study focused on investigating the effects of VA and *M. oleifera* extract on salivary glands, highlighting a potential research gap in this area. VA is widely used as an antiepileptic and mood-stabilizing drug and also inhibits histone deacetylase activity. Although it has been reported to prevent the growth of salivary gland tumors,[5] VA has some clinical side effects including sialadenosis and dry mouth.[4,9] It has been observed to cause oxidative stress in tissues by oxidant/antioxidant imbalance, trigger inflammatory reactions, and autoimmune reactions.[2,10] Therefore, conducting research on compounds that can counteract the adverse effects of VA has become important. The various pharmacological effects of *M. oleifera* leaves have been demonstrated to come from its abundant active components like flavonoids and polyphenols.[7]

The dose of *M. oleifera* extract (0.3 g/kg/day) and the treatment period (2 weeks) used in the present study have been previously shown to be safe and effective.[11,12] The use of a 70% ethanolic extract is supported by previous research, indicating its strong antioxidant activity.[13–15]

The results regarding the antioxidant parameters of salivary glands are presented in Table 1. The groups included in the study are as follows: control (C), control + moringa extract (CM), sodium valproate (VA), and sodium valproate + moringa extract (VM). Glutathione (GSH), glutathione-S-transferase (GST) and boron levels in the salivary glands decreased significantly in the VA group compared to the control groups (C and CM) and increased significantly in the VM group compared to the VA group. When the salivary glands' catalase (CAT) activities were compared, no significant difference was found between the groups ($p > 0.05$). Superoxide dismutase (SOD) activity in the parotid and submandibular salivary glands decreased significantly in the VA group compared to the control groups (C and CM) and increased significantly in the VM group compared to the VA group. However, there was no significant difference in SOD activity between the groups in the sublingual salivary gland. Total antioxidant capacity (TAC) values in the parotid and sublingual salivary glands decreased significantly in the VA group compared to the control groups (C and CM), and increased significantly in the VM group compared to the VA group. However, no significant difference was found in the TAC value between the groups in the submandibular salivary gland ($p > 0.05$).

GSH is an antioxidant that can prevent oxidative stress by capturing free radicals and ROS species.[16] It also prevents the destruction of important biomolecules such as lipids by stopping the formation of lipid peroxide.[17] In this study, it was found that the amount of GSH decreased in the VA group compared to the C group. The decrease in GSH in the salivary glands is an indicator of the damage caused by VA. Increased intracellular ROS and reactive VA metabolites may consume

Table 1. Comparison of GSH, GST, CAT, SOD, TAC, and boron values in salivary glands between groups.

	Parotid Salivary Gland					
Group	GSH (mg/g T)	GST (U/g T)	CAT (kU/g T)	SOD (kU/g T)	TAC (μmol/g T)	Boron (ppm)
C	0.569 ± 0.004	1.978 ± 0.075	1.631 ± 0.070	0.114 ± 0.005	2.926 ± 0.150	4.223 ± 0.113
CM	0.569 ± 0.009	2.179 ± 0.110	1.448 ± 0.148	0.106 ± 0.006	3.243 ± 0.117	4.267 ± 0.106
VA	$0.524 \pm 0.006^{c,g}$	$1.355 \pm 0.047^{a,f}$	1.408 ± 0.148	$0.078 \pm 0.008^{b,e}$	$1.903 \pm 0.147^{d,h}$	$3.215 \pm 0.096^{b,f}$
VM	0.558 ± 0.006^{m}	2.049 ± 0.259^{k}	1.496 ± 0.154	0.105 ± 0.009^{k}	$2.506 \pm 0.092^{f,k}$	4.813 ± 0.348^{r}
P_{ANOVA}	< 0.0001	< 0.01	> 0.5	< 0.01	< 0.0001	< 0.0001
	Submandibular Salivary Gland					
Group	GSH (mg/g T)	GST (U/g T)	CAT (kU/g T)	SOD (kU/g T)	TAC (μmol/g T)	Boron (ppm)
C	0.311 ± 0.015	2.924 ± 0.105	14.890 ± 0.180	0.109 ± 0.005	4.583 ± 0.097	3.183 ± 0.112
CM	0.315 ± 0.012	2.975 ± 0.182	14.240 ± 0.440	0.116 ± 0.005	4.650 ± 0.075	3.713 ± 0.258
VA	$0.263 \pm 0.005^{a,f}$	$1.979 \pm 0.249^{b,f}$	14.140 ± 0.357	$0.091 \pm 0.003^{a,f}$	4.584 ± 0.105	$2.109 \pm 0.132^{b,h}$
VM	0.305 ± 0.005^{k}	2.743 ± 0.127^{k}	14.220 ± 0.437	0.114 ± 0.004^{m}	4.865 ± 0.051	3.685 ± 0.192^{r}
P_{ANOVA}	< 0.01	< 0.01	> 0.5	< 0.01	> 0.05	< 0.0001
	Sublingual Salivary Gland					
Group	GSH (mg/g T)	GST (U/g T)	CAT (kU/g T)	SOD (kU/g T)	TAC (μmol/g T)	Boron (ppm)
C	1.044 ± 0.038	4.313 ± 0.382	7.566 ± 0.527	0.071 ± 0.004	2.253 ± 0.143	3.250 ± 0.089
CM	1.040 ± 0.042	4.716 ± 0.239	8.421 ± 0.476	0.079 ± 0.003	2.423 ± 0.176	3.426 ± 0.134
VA	$0.873 \pm 0.031^{a,e}$	$3.021 \pm 0.219^{a,g}$	7.174 ± 0.330	0.074 ± 0.003	$1.569 \pm 0.187^{a,f}$	$2.780 \pm 0.127^{a,f}$
VM	1.059 ± 0.050^{k}	4.355 ± 0.211^{m}	7.455 ± 0.519	0.080 ± 0.002	2.288 ± 0.174^{k}	3.532 ± 0.103^{n}
P_{ANOVA}	< 0.01	< 0.001	> 0.05	> 0.05	< 0.01	< 0.001

Values were given as mean ± standard error. T: Tissue, GSH: Glutathione, GST: Glutathione-S-transferase, CAT: Catalase, SOD: Superoxide dismutase, TAC; Total antioxidant capacity, C: Control group, CM: Moringa extract given control group, VA: Sodium valproate group, VM: Sodium valproate given moringa extract group, $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, $^{d}p < 0.0001$ compared to C, $^{e}p < 0.05$, $^{f}p < 0.01$, $^{g}p < 0.001$, $^{h}p < 0.0001$ compared to CM, $^{k}p < 0.05$, $^{m}p < 0.01$, $^{n}p < 0.001$, $^{r}p < 0.0001$ compared to VA.

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GSH levels, leading to lysosomal damage and oxidative stress.[18] Also, there was a significant increase in salivary gland GSH levels in the VM group compared to the VA group. Abdel-Daim et al., showed that moringa extract administration inhibited $CoCl_2$-induced nephrotoxicity and increased GSH levels in renal tissue.[19]

GST, an enzyme catalyzing the conjugation of electrophiles harmful to cells with GSH, plays a crucial role in protecting against carcinogens, drug toxicities, and various cellular oxidative damage.[20] In the present study, GST activities in salivary glands were significantly reduced due to VA-induced toxicity. Consistent with these findings, it has been shown that VA toxicity causes a decrease in GST activities in tissues such as small intestine,[21] pancreas,[22] and lens.[23] Due to the antioxidant properties of moringa extract, GST activities in the VM group were significantly increased in salivary glands compared to the VA group.

SOD and CAT are antioxidant enzymes that prevent free radical formation and are also known as primary defense antioxidant enzymes. While SODs convert superoxide radicals to less harmful hydrogen peroxide, CAT renders hydrogen peroxide harmless by converting it to oxygen and water.[24] In this study, VA-induced oxidative stress significantly decreased SOD activity in the parotid and submandibular salivary glands, except for sublingual salivary glands. The decrease in CAT activity was not significant. In previous studies, the SOD and CAT activities of various rat tissues such as lung,[25] brain,[26,27] lens,[23] liver[28] and pancreatic tissues[22] have been reported to be significantly lower in VA-treated groups compared to control groups. In parallel with the present findings, VA-induced liver toxicity caused decreased SOD activity in the VA group, no significant change was detected in CAT activity.[29] On the other hand, SOD activities increased significantly in the VM group compared with the control group of the present study. There was no difference in CAT activity in terms of salivary glands. The increased SOD in conjunction with the administration of moringa extract may have contributed to effective defense against oxidative stress. In studies investigating the impact of *M. oleifera* on the heart tissue under diabetic conditions[30] and on the cerebral cortex in an experimental Alzheimer's rat model,[31] the administration of moringa extract led to increase SOD and CAT activities. In the study of Ertik et al., while *M. oleifera* leaf extract increased SOD activity in muscle tissue, it decreased CAT activity.[32] The variations in the results of the studies may have arisen from tissue diversity or differences in the dose, method of administration, and frequency of dosing of moringa.

There is no previous study in the literature examining salivary gland boron levels. The study by Coban et al., revealed a decrease in boron levels in rat plasma, kidney, brain, and liver tissues, which was associated with oxidative stress.[33] Various studies have shown that boron plays a role in energy and lipid metabolism.[34] It has been suggested that boron and boron-containing molecules can be used as natural anticancer agents or potential drugs in the daily diet.[35] In experimental studies, boron has been shown to reduce oxidative stress and damage in cells.[36–38] In the present study, the salivary glands' boron concentration decreased significantly in the VA group compared to the C group. It was observed that the amount of boron increased significantly with the administration of moringa extract. The boron level in the moringa extract used was approximately 9 ppm, while its level in the feed given to the rats was approximately 9 ppm. The boron level in the drinking water of rats in this study could not be determined since it was below the detection limit of the carmine method. However, in a previous study, the amount of boron in the drinking water of the Marmara region was 0.08 ppm.[39] All groups in the present study were provided with the same food and drinking water source. This suggests that the boron intake through food and water would be similar across all groups. Therefore, the increase in boron level may be due to the boron content of the *M. oleifera* plant.

The findings relating to oxidant parameters of the salivary glands are given in Table 2. Lipid peroxidation (LPO), sialic acid (SA), and nitric oxide (NO) values in salivary glands increased significantly in the VA group compared to the control groups (C and CM), and significantly decreased in the VM group compared to the VA group. When the tissue factor (TF) activities in the salivary glands were compared, no significant difference was found between the groups ($p > 0.05$). Total oxidant capacity (TOC) values in the parotid and submandibular salivary glands increased significantly in the VA group compared to the control groups (C and CM) and decreased significantly in the VM group compared to the VA group. However, there was no significant difference in TOC value between the groups in the sublingual salivary gland ($p > 0.05$).

LPO occurs when ROS targets polyunsaturated fatty acids. Malondialdehyde (MDA) is an important marker for the effect of oxidative stress on LPO.[40] The main indicator of VA-induced toxicity is a decrease in GSH and an inverse increase in LPO.[18,41] There is no study in the literature examining the effect of VA on LPO in salivary glands, but in experimental studies conducted in other tissues, an increase in MDA has been reported after VA administration. In the study of Heidari et al., it was found that GSH reserves and tissue antioxidant capacity decreased, while LPO increased in kidney tissues of rats given VA.[42] The findings of this study support the previous findings. LPO increased significantly in salivary glands in the VA group compared to group C, and GSH decreased. Additionally, with the administration of moringa extract, GSH levels increased in the VM group compared to the VA group, while LPO levels decreased significantly. Hence, moringa may have counteracted and decreased VA-induced defects through its antioxidant properties.

SA is a potent antioxidant that combats oxidative damage caused by H_2O_2. It is susceptible to attack by superoxide and related ROS. Higher levels of SA in tissues are often associated with increased ROS levels.[43,44] Previous research has shown that valproate administration increases SA levels in the brain tissue of rats.[27] In this study, the administration of moringa extract significantly reduced the SA levels that had been elevated by VA. This reduction is likely due to the antioxidant properties of the compounds in moringa, which helped scavenge ROS species and mitigate the increase in SA levels induced by VA.

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Table 2. Comparison of LPO, SA, NO, TF, and TOC values in salivary glands between groups.

Group	LPO (μmol MDA/g T)	SA (mg/g T)	NO (μmol/g T)	TF (sec)	TOC (μmol/g T)
Parotid Salivary Gland					
C	0.373 ± 0.020	1.779 ± 0.164	0.174 ± 0.013	51.67 ± 1.970	0.800 ± 0.013
CM	0.286 ± 0.022	1.718 ± 0.097	0.169 ± 0.010	53.00 ± 2.228	0.669 ± 0.028^{a}
VA	0.539 ± 0.028^{c,g}	2.301 ± 0.127^{a,f}	0.218 ± 0.011^{a,e}	53.13 ± 2.748	0.951 ± 0.057^{a,h}
VM	0.438 ± 0.021^{g,k}	1.661 ± 0.052^{m}	0.178 ± 0.004^{k}	51.75 ± 1.319	0.634 ± 0.020^{b,r}
P_{ANOVA}	< 0.0001	< 0.01	< 0.01	> 0.05	< 0.0001
Submandibular Salivary Gland					
Group	LPO (μmol MDA/g T)	SA (mg/g T)	NO (μmol/g T)	TF (sec)	TOC (μmol/g T)
C	0.103 ± 0.003	1.814 ± 0.129	0.142 ± 0.015	27.50 ± 1.722	0.284 ± 0.009
CM	0.104 ± 0.003	1.575 ± 0.088	0.124 ± 0.004	27.38 ± 1.742	0.261 ± 0.014
VA	0.120 ± 0.006^{a,e}	2.298 ± 0.097^{a,g}	0.181 ± 0.004^{a,g}	26.50 ± 0.906	0.326 ± 0.011^{a,g}
VM	0.104 ± 0.006^{k}	1.483 ± 0.113^{r}	0.147 ± 0.005^{k}	27.00 ± 0.866	0.258 ± 0.005^{n}
P_{ANOVA}	< 0.01	< 0.0001	< 0.001	> 0.05	< 0.001
Sublingual Salivary Gland					
Group	LPO (μmol MDA/g T)	SA (mg/g T)	NO (μmol/g T)	TF (sec)	TOC (μmol/g T)
C	0.153 ± 0.006	5.935 ± 0.172	0.156 ± 0.005	47.00 ± 1.803	0.405 ± 0.004
CM	0.134 ± 0.018	5.743 ± 0.230	0.134 ± 0.006^{a}	47.13 ± 1.552	0.405 ± 0.003
VA	0.240 ± 0.016^{c,h}	7.413 ± 0.236^{c,h}	0.179 ± 0.003^{a,h}	48.63 ± 1.426	0.414 ± 0.004
VM	0.183 ± 0.015^{k}	5.546 ± 0.230^{r}	0.120 ± 0.006^{c,r}	45.38 ± 0.653	0.404 ± 0.003
P_{ANOVA}	< 0.0001	< 0.0001	< 0.0001	> 0.05	> 0.05

Values were given as mean ± standard error. T: Tissue, LPO: Lipid peroxidation, MDA: Malondialdehyde, SA: Sialic acid, NO: Nitric oxide, TF: Tissue factor, TOC: Total oxidant capacity, C: Control group, CM: Moringa extract given control group, VA: Sodium valproate group, VM: Sodium valproate given moringa extract group, $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ compared to C, $^{e}p < 0.05$, $^{f}p < 0.01$, $^{g}p < 0.001$, $^{h}p < 0.0001$ compared to CM, $^{k}p < 0.05$, $^{m}p < 0.01$, $^{n}p < 0.001$, $^{r}p < 0.0001$ compared to VA.

NO has both prooxidant and antioxidant effects in lipid peroxidation. Besides inhibiting lipid oxidation, NO can directly neutralize ROS.[45] The administration of VA caused an increase in NO values in the salivary glands of the VA group compared to the C group. This may be as a result of inflammation and inflammatory responses resulting from the accumulation of ROS and toxic VA metabolites by overexpression of nitric oxide synthase or stimulation of the NO-citrulline cycle.[46] In the VM group, NO values in the salivary glands decreased, an indication of the effective role of *M. oleifera* extract in reducing VA toxicity. This outcome is consistent with previous studies reporting that antioxidant agents such as alpha-lipoic acid[47] and edaravone[48] could reduce the effect of VA. In addition, *M.oleifera* extract decreased NO levels and other oxidants in muscle,[32] liver[11,49] and kidney tissues.[50] The present outcomes are also consistent with these findings.

TF is the main initiator of the coagulation system. An adequate hemostatic clot is essential for the normal wound healing process.[51] In the present research, no difference was found in terms of TF activity in salivary glands.

While TOC provides information about the level of oxidants and ROS in a biological sample, TAC is used to measure the total antioxidant status.[52] The increase in TOC values and the decrease in TAC values indicate oxidative stress or increased susceptibility to oxidative damage.[53] TOC values increased significantly while TAC values decreased in the salivary glands of group VA of this study. This phenomenon has also been reported in various tissue types exposed to VA in other studies.[42,47,54] This situation may have been caused by the decrease in GSH levels, GST and SOD activities, as well as increase in oxidative stress due to VA. Administration of moringa extract improved TAC and TOC values in the VM group due to its antioxidant activity. In the study of Imran et al., it was observed that *M. oleifera* extract showed similar trend in nerve tissue as observed in the present study.[55]

The activities of digestive (α-amylase, protease, and maltase) and metabolic enzymes (alkaline phosphatase and acid phosphatase (ALP, ACP), and lactate dehydrogenase (LDH)) in the salivary glands of all groups are shown in Table 3. Protease activity was not detected in the sublingual salivary gland. Administration of VA to the rats significantly changed the activities of the salivary glands' digestive and metabolic enzymes. All enzyme activities increased in the VA group compared to the C group. The administration of moringa extract to the VM group significantly reduced these increases in all salivary glands when compared to the VA group. Moringa extract administration to the CM group caused a significant increase in sublingual α-amylase while sublingual ALP and maltase activity decreased significantly compared to the C group.

Alpha-amylase and maltase are important enzymes for carbohydrate metabolism. Many researchers indicate the ele-

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Table 3. Comparison of α-amylase, protease, maltase, ALP, ACP and LDH activities of groups in salivary glands.

	Parotid Salivary Gland					
Group	α-Amylase (U/g T)	Protease (U/g T)	Maltase (mU/g T)	ALP (mU/g T)	ACP (mU/g T)	LDH (mU/g T)
C	300.30 ± 0.92	70.44 ± 1.95	487.00 ± 22.17	59.02 ± 3.18	1132.00 ± 37.96	47.62 ± 1.55
CM	299.80 ± 0.26	61.60 ± 1.40	728.00 ± 27.31^{d}	85.56 ± 2.13^{a}	1418.00 ± 83.99^{a}	47.62 ± 0.01
VA	306.60 ± 0.15^{d,h}	106.20 ± 2.58^{d,h}	762.20 ± 25.57^{d}	278.50 ± 10.14^{d}	2002.00 ± 65.04^{d,h}	107.80 ± 3.99^{d,h}
VM	298.40 ± 0.59^{r}	78.42 ± 4.89^{e,r}	620.80 ± 14.76^{a,e,m}	55.70 ± 3.56^{f,r}	1532.00 ± 52.01^{c,r}	47.62 ± 2.07^{r}
P_{ANOVA}	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	Submandibular Salivary Gland					
Group	α-Amylase (U/g T)	Protease (U/g T)	Maltase (mU/g T)	ALP (mU/g T)	ACP (mU/g T)	LDH (mU/g T)
C	141.60 ± 1.15	46.83 ± 2.67	59.08 ± 6.54	166.50 ± 7.13	950.40 ± 160.00	23.81 ± 1.16
CM	151.40 ± 0.33^{d}	36.75 ± 4.22	97.89 ± 17.33	105.60 ± 12.44	1383.00 ± 406.70	23.81 ± 0.87
VA	151.30 ± 0.17^{d}	92.72 ± 3.87^{d,h}	204.00 ± 11.64^{d,h}	331.30 ± 41.66^{b,g}	2953.00 ± 227.50^{d,f}	47.62 ± 0.94^{d,h}
VM	146.80 ± 2.07^{d,g,m}	35.78 ± 1.29^{r}	117.70 ± 6.87^{a,r}	136.30 ± 10.41^{m}	1249.00 ± 119.20^{n}	29.76 ± 3.20^{r}
P_{ANOVA}	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	Sublingual Salivary Gland					
Group	α-Amylase (U/g T)	Protease (U/g T)	Maltase (mU/g T)	ALP (mU/g T)	ACP (mU/g T)	LDH (mU/g T)
C	300.90 ± 0.73	N/A	232.80 ± 7.36	43.40 ± 0.93	1567.00 ± 77.83	47.62 ± 1.55
CM	303.30 ± 0.29^{a}	N/A	60.85 ± 5.92^{d}	60.76 ± 2.29^{d}	897.10 ± 46.73^{c}	47.63 ± 1.71
VA	304.10 ± 0.73^{b}	N/A	418.00 ± 11.83^{d,h}	60.76 ± 0.25^{d}	2358.00 ± 96.52^{d,h}	71.43 ± 3.50^{d,h}
VM	297.90 ± 0.13^{b,h,r}	N/A	206.30 ± 1.86^{h,r}	52.46 ± 0.54^{c,f,n}	1659.00 ± 53.43^{h,r}	45.62 ± 0.73^{r}
P_{ANOVA}	< 0.0001	N/A	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Values were given as mean ± standard error. ALP:Alkaline phosphatase, ACP: Acid phosphatase, LDH: Lactate dehyrogenase, C: Control group, CM: Moringa extract given control group, VA: Sodium valproate group, VM: Sodium valproate given moringa extract group, N/A: Not applicable. $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, $^{d}p < 0.0001$ compared to C, $^{e}p < 0.05$, $^{f}p < 0.01$, $^{g}p < 0.001$, $^{h}p < 0.0001$ compared to CM, $^{k}p < 0.05$, $^{m}p < 0.01$, $^{n}p < 0.001$, $^{r}p < 0.0001$ compared to VA.

vated activities of α-amylase in different complications.[56] In addition, α-amylase stores and activities are strongly affected by the sympathetic/parasympathetic activity which is closely related to the salivary area. It was observed that sympathetic nerve excitation was related to the loss of acinar granules which contain α-amylase.[57] Findings indicates that a relationship exist between sympathetic/parasympathetic activity and VA administration[9] Once α-amylase hydrolyzes α-1,4 linkages of starch, glucose, and maltose are formed. Thus, elevated maltase activity might have been induced by the increased levels of maltose as a result of increased α-amylase action. Elevated salivary amylase activity and/or hyperamylasemia are associated with tumor, pneumonia, and use of various drugs.[58] In line with these, elevated α-amylase and maltase activities in the VA treated group was observed in the present study. Acidosis (due to ketoacidosis) also plays a role on salivary amylase elevation. This condition is also vital for LDH activity. VA is a strong and negative effector on mitochondrial respiration rate, it alters beta-oxidation of biomolecules and promotes lactic acidosis.[59,60] Furthermore, increased salivary LDH levels in diabetic patients, which is linked to distorted energy metabolism has been reported.[61] In this study, increased LDH activities was observed in all the three glands after VA administration. Moringa decreased α-amylase, maltase, and LDH in all the salivary glands. The α-amylase inhibitory effect of moringa can be attributed to its rich flavonoid, phenolic, and sulfur contents. Formulations containing these phytochemicals are shown to be inhibitors of α-amylase.[62,63] Magaji et al., proved that the methyl alcohol extracts (MetOH) of moringa leaves, roots, and seeds have excellent inhibition effect on α-amylase.[64] In the present study, the ethanolic extract of moringa administered as a protective agent decreased α-amylase activity in the VA group, thereby supporting previous reports. Ertik et al., showed that moringa extract was a potent inhibitor of LDH both *in vitro* and *in vivo* in VA-induced muscle injury.[32] In another report by Magaji et al., it was shown that moringa had a powerful antioxidant capacity by scavenging nitrite and N,N-dimethyl-p-phenylenediamine ($DMPD^{+}$) radicals, and also showed ferric reducing power.[65] Thus, it can be suggested that the inhibition of α-amylase, maltase, and LDH in all salivary glands of the VM group may be related to the antioxidant activity of moringa.

ACP and ALP are enzymes that hydrolyze organophosphates at different pH levels. Their altered activities are associated with many toxicities. Some reports associates their sensitivity in media exposed to the toxic chemical compounds.[66,67] In addition, phosphatases are markers of programmed cell death in salivary systems of various types of insects.[68,69] In the present study, the ACP and ALP activities of the VA group were increased in all parts of the salivary glands as compared to the control group. Furthermore, elevated protease activities in

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parotid and submandibular glands of VA given group was detected when compared to the control group. As regards the importance of protein homeostasis protection particularly in mitochondria,[70] it can be assumed that VA may have increased protease activity by damaging mitochondria processes via increasing oxidative stress. Moringa administration decreased ACP and ALP activities in all salivary glands and protease activities in parotid and submandibular glands. This protective effect may be associated with the antioxidant effect of moringa.

The protein bands obtained by SDS-PAGE were in the same position for all samples, the molecular weight ranges from 29–140 kDa in all investigated salivary glands (Figure 1). There were decreases and increases in some protein bands. No difference was found between the groups in terms of protein bands in the parotid salivary glands. The densities of the proteins with 56, 35, and 33 kDa molecular weights in the parotid salivary gland were much more than those of submandibular and sublingual salivary glands. In the submandibular salivary gland, the density of the protein bands with higher molecular weight (80, 98,140 kDa) seemed to be increased in the VA group compared to the C, CM, and VM groups. On the other hand, a decrease was observed in the density of 74, 56, 51, and 36 kDa protein bands in the VA group compared to the C, CM, and VM groups. The density of the 56 and 51 kDa protein bands in the submandibular and sublingual salivary glands was noticeably less than those in the parotid salivary glands.

The densities of the protein bands in the sublingual salivary gland were lower than in both parotid and submandibular salivary glands. In the moringa extract given groups (CM and VM), the intensities of 134, 51, 45, 34, and 28 kDa protein bands were decreased as compared those of the C and VA groups. Proteins whose densities changed should be investigated in detail.

Conclusions

VA causes oxidative damage to major salivary glands that synthesize and secrete saliva. It interferes with oral homeostasis by altering the quality and quantity of saliva. The fact that *M. oleifera* extract was found to be effective in the regression of VA-induced changes and it may be an alternative for the development of different treatment approaches for people using VA and having salivary gland problem.

The limitation of our study is the lack of salivary parameters that could support the findings of oxidative damage caused by VA and the ameliorating effects of *M. oleifera* on oxidative damage. Another is the absence of histological findings.

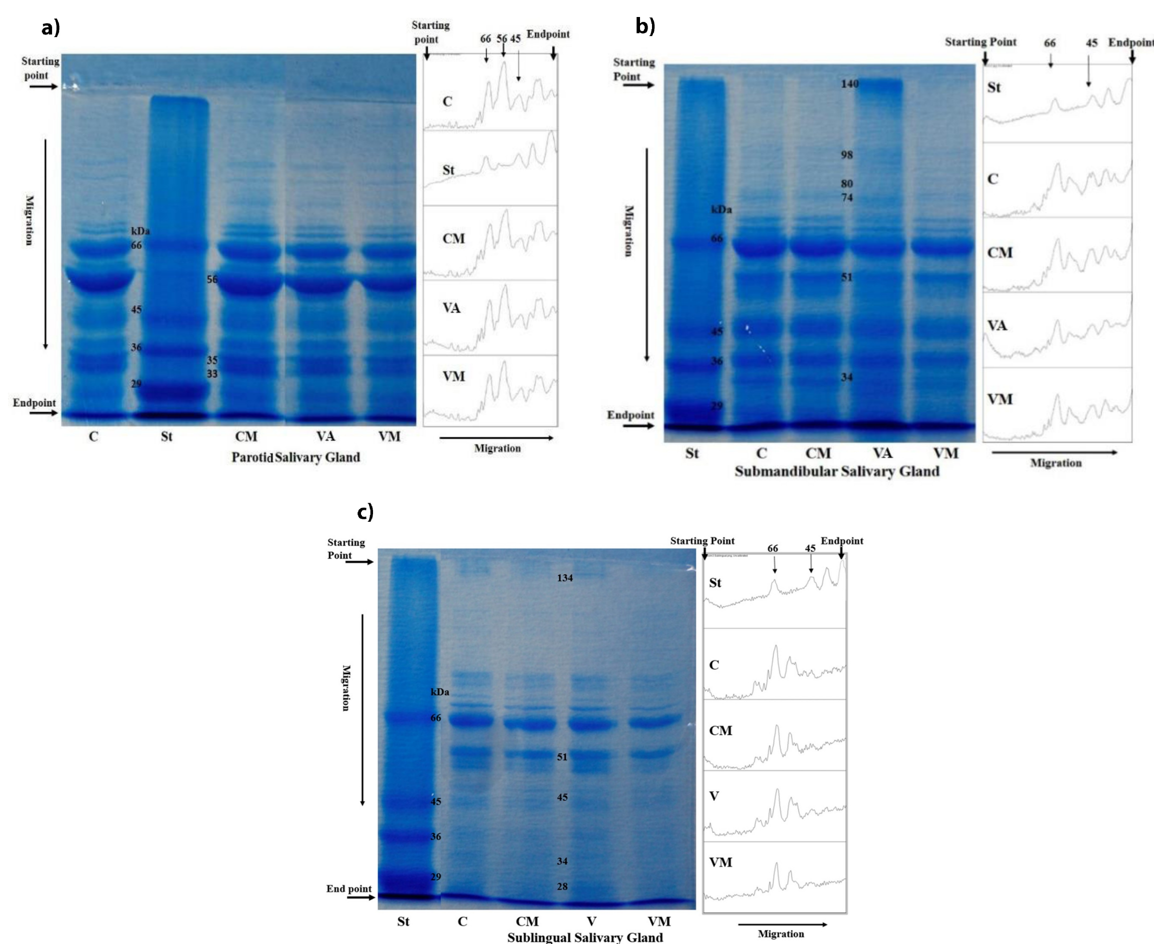

Figure 1. SDS-PAGE patterns of salivary gland proteins. a) Parotid salivary gland b) Submandibular salivary gland c) Sublingual salivary gland

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Experimental Section

Chemicals

The chemicals used in this study were of analytical grade and were obtained from Merck (Darmstadt, Germany), Sigma-Aldrich (St. Louis, MO, USA) and Fluka (Buchs, Switzerland) companies. The sodium valproate was purchased from Merck (Darmstadt, Germany).

Plant Materials

Fresh *M. oleifera* leaves were collected from farms in Sokoto town of Sokoto State, Nigeria in 2019. At the Botany Unit of Biological Sciences Department, Usmanu Danfodiyo University Sokoto, the plant was identified and authenticated by a taxonomist; Umar Abdullahi (PhD), followed by deposition of voucher specimens in the university herbarium (Herbarium number: UDUS/VS/2011/31).

Preparation of Moringa Leaf Extract

M. oleifera leaves were dried in the shade before being pulverized. The resulting powdery plant leaves were packed in paper envelopes and stored in polyethylene bags to prevent contamination and moisture absorption. To prepare 70% ethanol extract, 100 g of plant leaves were put into the Soxhlet device. 150 mL of 70% ethanol was added and refluxed until at least 20 siphons were formed. The solvent was removed in an evaporator. The resulting residue was weighed, transferred to Eppendorf tubes, and stored at −20 °C until use.

Animals

Three-months-old Sprague Dawley female rats weighing 250–300 g were used in this study which was approved by Marmara University Experimental Animals Ethics Committee (Decision No: 60.2021mar). The experimental animals were housed in the animal room which has optimum temperature (20 °C ±2), humidity, and light/dark (12 h light/12 h dark) conditions. All rats were orally fed pellet-type rat food and fresh tap water.

Groups

The rats were divided into four groups (8 animals per group); control (C, 0.9% NaCl given orally for 15 days), control + moringa extract (CM, a single dose of *M. oleifera* 70% ethanolic leaf extract (0.3 g/kg/day) given for 15 days orally), sodium valproate (VA, a single dose of sodium valproate (0.5 g/kg/day) given orally for 15 days) and valproic acid + moringa extract (VM, sodium valproate + *M. oleifera* 70% ethanolic leaf extract given at the same dose and for the same duration orally). On the 16th day, fasted rats were sacrificed under anesthesia. Parotid, submandibular, and sublingual salivary glands were taken and homogenized. The homogenates were stored in a deep freezer (at −80 °C) until the experimental day.

Biochemical Parameters

The homogenates of salivary glands were centrifuged and the supernatants were used for the analysis of all biochemical parameters except TF. The homogenate was used directly for TF analysis.

For salivary glands' GSH determination, the absorbance of the colored product of the reaction of Ellman's reagent 5–5'dithiobis (—2-nitrobenzoic acid) (DTNB) and sulfhydryl groups was spectrophotometrically recorded at 412 nm and calculated using the extinction coefficient ($1.36 \times 10^4\ M^{-1} cm^{-1}$).[71]

The levels of SA were evaluated using the method of Warren.[72] For the assay, the salivary glands samples were first incubated at 80 °C for 1 hour with 0.1 N H_2SO_4 and the hydrolysate was used for analysis. The absorbances obtained from the samples were determined at 549 nm.

The absorbance of the pink color produced at the end of the reaction between the LPO product MDA and thiobarbituric acid (TBA) was evaluated spectrophotometrically to determine LPO.[73]

To measure NO levels, nitrate was converted to nitrite with vanadium (III) chloride. The complex diazonium compound was produced based on the reaction of nitrite sulfanilamide with N- (1-Naphthyl) ethylenediamine dihydrochloride in an acidic medium. The colored complex formed was measured spectrophotometrically at 540 nm.[74]

GST activity was measured by the spectrophotometric determination of the absorbance at 340 nm belonging to the product formed by conjugation of GSH with 1-chloro-2,4-dinitro-benzene (CDNB).[75]

CAT activity was determined based on the H_2O formation reaction from H_2O_2. It was noted by the decrease in the absorbance obtained at 240 nm.[76]

SOD activity was determined by riboflavin-sensitized photooxidation of ortho-dianisidine.[77] Ortho-dianisidine oxidation, by the reaction with riboflavin, is induced by SOD. The increase in absorbance depends on the SOD concentration. Finally, the colored product's absorbance was determined spectrophotometrically at 460 nm.

TAC is used to provide insights into the development and treatment of oxidative-stress-related disorders. Antioxidants in the sample reduce dark blue-green colored ABTS radical to colorless reduced ABTS form. The change in absorbance at 660 nm is related to the total antioxidant level of the sample. The assay is calibrated with a stable antioxidant standard solution which is traditionally named Trolox Equivalent is a vitamin E analog.[78]

TOC provides information about the level of oxidants and ROS in a biological sample. Total oxidants present in the sample oxidize the ferrous ion-chelator complex to ferric ion. The oxidation reaction is prolonged by enhancer molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with chromogen in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Equiv./L).[79]

TF activity of the salivary glands was evaluated according to the method of Ingram and Hills.[80] In Quick's one-stage method, the TF activity of homogenate was measured using pooled plasma obtained from healthy subjects. Since the clotting time is inversely proportional to the activity of TF, the lengthening of the clotting time shows decreased TF activity.

Boron measurements were performed according to the carminic acid method of Hatcher and Wilcox[81] with a few modifications.[82] First, samples were burned in a high-temperature furnace. The boron determination was carried out with a spectrophotometric method based on the measurement of the absorbance at 585 nm of the colored complex formed as a result of the reaction of boron

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and carminic acid in the presence of sulfuric acid. Boric acid was used as the boron standard.

The alpha amylase activity was determined according to Miller.[83] In this method, the reducing sugars that were formed by the hydrolysis of starch were determined with dinitrosalicylic acid.

The protease activity was assayed by the casein digestion method of Singh et. al.[84] The absorbance of tyrosine residues which were formed by the degradation of casein (1%) by the presence of protease was determined at 280 nm.

Maltase activity was determined according to the methodology by Dahlqvist.[85] The method is based on the measurement of glucose released from the maltose by the enzymes. Absorbance was determined at 420 nm.

ALP and ACP activities were determined by the method of Walter and Schutt.[86] ALP and ACP are the enzymes that catalyze the hydrolysis of 4-nitrophenyl phosphate to 4-nitrophenol due to the pH of the reaction medium. Under optimum conditions for measurements of the ALP and ACP activities, the absorbance was monitored at 405 nm.

LDH activity was assayed by the method of Bais and Philcox.[87] This method is based on the ability of LDH to convert pyruvate to lactate in the presence of coenzyme-reduced nicotinamide adenine dinucleotide (NADH). The absorbance of the samples was measured spectrophotometrically at 340 nm.

The total protein (TP) levels in the samples were determined using the method of Lowry et al.,[88] and bovine serum albumin was used as the protein standard. Copper ions were applied to the rats salivary gland samples in an alkaline environment. They were then reduced with a phosphomolybdic–phosphotungstic acid reagent (Folin reagent). At 500 nm, the intensity of the blue color was evaluated spectrophotometrically. The intensity of the blue color formed is proportional to the protein concentration.

Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was also carried out according to the Laemmli method.[89] The salivary gland homogenates mixed in SDS gel loading buffer (Tris–HCl, SDS, mercaptoethanol, bromophenol blue, glycerol). Then, the samples were heated for five min in a boiling water bath, and equivalent protein concentrations (20 μg total protein) per lane of the gel loaded on 7.5% SDS polyacrylamide gels (with 4.5% separating gel). Protein size in kilo Daltons (kDa) was determined by comparing the relative mobility of each band with the banding pattern of a colored protein standard of known molecular size. The mixture of bovine albumin (66 kDa), egg albumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (rabbit muscle, 36 kDa), and bovine carbonic anhydrase (29 kDa) as protein molecular weight markers were used as standard protein mixture. Electrophoresis was carried out at 30 mA/gel for one hour, at 20 °C using Bio-Rad Mini-PROTEAN Tetra Vertical Electrophoresis Cell. For quantitative measurements, high-resolution test photographs were taken using a Canon EOS 700D camera with an 18-55 mm lens for evaluation of protein bands after electrophoresis and the images were exported as jpeg files. Densitometric plots of protein bands were drawn using Image J software.[90]

Statistical Analyses

Biochemical results were statistically evaluated via GraphPad Prism 9.0. The values were expressed as means ± standard error. Since there is a normal distribution the results were evaluated using an unpaired t-test and analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. The value of $p<0.05$ was considered statistically significant.

Author Contributions

Conceptualization: A.Y., R.Y.; Methodology: E.C., B.A.T, S.O., I.B.T., U.F.M.; Validation: A.Y., R.Y., O.S., B.A.T., E.C., S.O., I.B.T., U.F.M.; Formal analysis: A.Y., R.Y., O.S., B.A.T., S.O.; Investigation: E.C., U.F.M., I.B.T., B.A.T., S.O.; Resources: A.Y., R.Y., O.S.; Data Curation: A.Y., R.Y., O.S., B.A.T., E.C., S.O., I.B.T., U.F.M.; Writing – original draft preparation A.Y., I.B.T.; Writing – review and editing: A.Y., R.Y., O.S., B.A.T., E.C., S.O., I.B.T., U.F.M.; Visualization: E.C., B.A.T., S.O.; Supervision: A.Y., R.Y., O.S.; Project administration: A.Y., E.C.; Funding acquisition: A.Y.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

All relevant data are included in the manuscript further questions can be directed to the corresponding author.

Keywords: antioxidant-oxidant parameters · *Moringa oleifera* · salivary gland · SDS-PAGE · sodium valproate

[1] L.B. Ungureanu, I. Grădinaru, C.M. Ghiciuc, C. Amălinei, G.L. Geleţu, C.G. Petrovici, R.Ş. Stănescu, *Medicina* **2023**, *59*, 1692.
[2] Y. Ghodke-Puranik, C. F. Thorn, J. K. Lamba, J. S. Leeder, W. Song, A. K. Birnbaum, R. B. Almand, T. E. Kleind, *Genomics* **2013**, *23*, 236–241.
[3] M. Kostrouchova, Z. Kostrouch, M. Kostrouchova, *Folia Biol.* **2007**, *53*, 37–49.
[4] A. Wolff, R.K. Joshi, J. Ekström, D. Aframian, A. M. L. Pedersen, G. Proctor, N. Narayana, A. Villa, Y. W. Sia, A. Aliko, R. McGowan, A. Ross Kerr, S. B. Jensen, A. Vissink, C. Dawes, *Drugs R&D* **2017**, *17*, 1–28.
[5] H. Nagai, M. Fujioka-Kobayashi, G. Ohe, K. Hara, N. Takamaru, D. Uchida, T. Tamatani, K. Fujisawa, Y. Miyamoto, *Oncol. Rep.* **2014**, *31*, 1453–1458.
[6] R. N. de Freitas, L. G. L. da Silva, G. A. Fiais, D. S. de Biagi Ferreira, A. S. C. Veras, G. R. Teixeira, S. H. P. Oliveir, R. C. M. Dornelles, A. C. de Melo Stevanato Nakamune, W. D. Fakhouri, A. H. Chaves-Neto, *Arch. Oral Biol.* **2023**, *155*, 105805.
[7] X. Su, G. Lu, L. Ye, R. Shi, M. Zhu, X. Yu, Z. Li, X. Jia, L. Feng, *RSC Adv.* **2023**, *13*, 24353–24384.
[8] A.K. Dhakad, M. Ikram, S. Sharma, S. Khan, V.V. Pandey, A. Singh, *Phytother. Res.* **2019**, *33*, 870–903.
[9] P. Mauz, K. Mörike, E. Kaiserling, S. Brosch, *Acta Oto-Laryngol.* **2005**, *125*, 386–391.
[10] M. O. Iqbal, M. Manzoor, A. Mumtaz, R. Riaz, S. Arshad, I. A. Khan, U. Javaid, Z. Manzoor, S. H. Munawar, S. Andleeb, M. M. Ahmed, A. Aslam, *Biomed. Pharmacother.* **2022**, *150*, 112953.

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[11] A.E. El-Hadary, M.F. Ramadan, *J. Food Biochem.* **2019**, *43*, e12704.
[12] F. T. Mthiyane, P. V. Dludla, K. Ziqubu, S. X. Mthembu, N. Muvhulawa, N. Hlengwa, B. B. Nkambule, S. E. Mazibuko-Mbeje, *Front. Pharmacol.* **2022**, *13*, 940572.
[13] P. Nobossé, E.N. Fombang, C.M. Mbofung, *Food Sci. Nutr.* **2018**, *6*, 2188–2198.
[14] B. Vongsak, P. Sithisarn, S. Mangmool, S. Thongpraditchote, Y. Wongkrajang, W. Gritsanapan, *Ind. Crops Prod.* **2013**, *44*, 566–571.
[15] Y. Wang, Y. Gao, H. Ding, S. Liu, X. Han, J. Gui, D. Liu, *Food Chem.* **2017**, *218*, 152–158.
[16] B. Niu, K. Liao, Y. Zhou, T. Wen, G. Quan, X. Pan, C. Wua, *Biomaterials* **2021**, *277*, 121110.
[17] A. Pompella, A. Visvikis, A, Paolicchi, V. De Tata, A.F. Casini, *Biochem. Pharmacol.* **2003**, *66*, 1499–1503.
[18] E. Devaraj, M. Uthirappan, *Environ. Toxicol. Pharmacol.* **2022**, *95*, 103967.
[19] M. M. Abdel-Daim, S. R. Khalil, A. Awad, E. H. Abu Zeid, R. A. El-Aziz, H. A. El-Serehy, *Nutrients* **2020**, *12*, 1031.
[20] Y. Kim, S. J. Cha, H.-J. Choi, K. Kim, *Oxid. Met.* **2017**, *2017*, 5049532.
[21] S. Oktay, B. Alev, S. Tunali, E. Emekli-Alturfan, T. Tunali-Akbay, L. Koc-Ozturk, R. Yanardag, A. Yarat, *Hum. Exp. Toxicol.* **2015**, *34*, 654–661.
[22] S. Oktay, B. Alev-Tüzüner, S. Tunalı, E. Ak, E. Emekli-Alturfan, T. Tunalı-Akbay, L. Koç-Öztürk, Ş. Çetinel, R. Yanardağ, A. Yarat, *Marmara Pharm. J.* **2017**, *21*, 570–577.
[23] S. Tunali, S. Kahraman, R. Yanardag, *Hum. Exp. Toxicol.* **2015**, *34*, 904–910.
[24] O. Ighodaro, O. Akinloye, *Alexandria Med. J.* **2018**, *54*, 287–293.
[25] F. Oztay, S. Tunali, O. Kayalar, R. Yanardag, *J. Biochem. Mol. Toxicol.* **2020**, *34*, e22602.
[26] S. Chaudhary, S. Parvez, *Neurosci.* **2012**, *225*, 258–268.
[27] I. B. Turkyilmaz, N. Altas, I. Arisan, R. Yanardag, *J. Biochem. Mol. Toxicol.* **2021**, *35*, e22855.
[28] M.A. Ibrahim, R.I. Abdel-Karim, H.G. Tamam, A.A. Mohamed, F.A. Wani, *Mansura J. Forensic. Med. Clin. Toxicol.* **2017**, *25*, 33–49.
[29] O. F. Koroglu, M. Gunata, N. Vardi, A. Yildiz, B. Ates, C. Colak, L. H. Tanriverdi, H. Parlakpinar, *Tissue Cell* **2021**, *72*, 101526.
[30] B. Aju, R. Rajalakshmi, S. Mini, *Heliyon* **2019**, *5*, e02935.
[31] R. Ganguly, R. Hazra, K. Ray, D. Guha, *Ann. Neurosci.* **2005**, *12*, 33–36.
[32] O. Ertik, U. F. Magaji, O. Sacan, R. Yanardag, *Drug Chem. Toxicol.* **2023**, *46*, 1212–1222.
[33] F. K. Coban, S. Ince, I. Kucukkurt, H. H. Demirel, O. Hazman, *Drug Chem. Toxicol.* **2015**, *38*, 391–399.
[34] A. Doğan, S. Demirci, H. Abdik, O. F. Bayrak, S. Güllüoğlu, E. C. Tüysüz, O. Gusev, A. A. Rizvanov, E. Nikerel, F. Şahin, *Metabolism* **2017**, *69*, 130–142.
[35] I. Uluisik, H. C. Karakaya, A. Koc, *J. Trace Elem. Med. Biol.* **2018**, *45*, 156–162.
[36] S. Meacham, S. Karakas, A. Wallace, F. Altun, *Open Miner. Process J.* **2010**, *3*, 36–53.
[37] F.H. Nielsen, C.D. Hunt, L.M. Mullen, J.R. Hunt, *FASEB J.* **1987**, *1*, 394–397.
[38] K. Warrington, *Ann. Bot.* **1923**, *37*, 629–672.
[39] R. Kuru, S. Yilmaz, O. Sacan, R. Yanardag, A. Yarat, F. Sahin, *Toxicol. Environ. Chem.* **2020**, *102*, 240–249.
[40] C. Mas-Bargues, C. Escrivá, M. Dromant, C. Borrás, J. Viña, *Arch. Biochem.* **2021**, *709*, 108941.
[41] M. Raza, A. Al-Bekairi, A. Ageel, S. Qureshi, *Pharmacol. Res.* **1997**, *35*, 153–157.
[42] R. Heidari, F. Jafari, F. Khodaei, B. Shirazi Yeganeh, H. Niknahad, *Nephrol. (Carlton)* **2018**, *23*, 351–361.
[43] H. Eguchi, Y. Ikeda, T. Ookawara, S. Koyota, N. Fujiwara, K. Honke, P. G. Wang, N. Taniguchi, K. Suzuki, *Glycobiology* **2005**, *15*, 1094–1101.
[44] J. Yadav, A. K. Verma, R. K. Garg, K. Ahmad, A. A. Mahdi, S. Srivastava, *Exp. Gerontol.* **2020**, *141*, 111092.
[45] S.M. Andrabi, N.S. Sharma, A. Karan, S.S. Shahriar, B. Cordon, B. Ma, J. Xie, *Adv. Sci.* **2023**, *10*, 2303259.
[46] D. Hayashi, T. Okubo, T. Suzuki, Y. Miyazaki, K. Tanaka, M. Usami, T. Takizawa, *Nitric Oxide* **2021**, *106*, 35–44.
[47] I. B. Turkyilmaz, B. Bilgin Sokmen, R. Yanardag, *J. Biochem. Mol. Toxicol.* **2020**, *34*, e22580.
[48] C. Celik, B. Bayrak, N. Hacihasanoglu Cakmak, R. Yanardag, *J. Pharm. Res.* **2022**, *26*, 52–62.
[49] A. A. Mousa, H. A. I. El-Gansh, M. A. A. Eldaim, M. A. E. Mohamed, A. H. Morsi, H. S. El Sabagh, *Environ. Sci. Pollut.* **2019**, *26*, 32488–32504.
[50] A. S. Akinrinde, O. Oduwole, F. J. Akinrinmade, F. B. Bolaji-Alabi, *Afr. Health Sci.* **2020**, *20*, 1382–1396.
[51] M. Hoffman, *Semin. Thromb. Hemostasis* **2018**, *44*, 142–150.
[52] X.-F. Du, L.-L. Zhang, D.-Z. Zhang, L. Yang, Y.-Y. Fan, S.-P. Dong, *Onco Targets Ther.* **2018**, *11*, 6767–6775.
[53] T. A. Imro'ati, T. Sugihartono, B. Widodo, E. P. Nefertiti, I. Rovian, I. G. N. Wibawa, *Open Access Maced. J. Med. Sci.* **2021**, *9*, 584–589.
[54] R. Omidipour, L. Zarei, M. B. Boroujeni, A. Rajabzadeh, *BioMed Res. Int.* **2021**, *2021*, 8839898.
[55] M. Imran, G. Hussain, A. Hameed, I. Iftikhar, M. Ibrahim, R. Asghar, I. Nisar, T. Farooq, T. Khalid, K. Rehman, M. A. Assiri, *Metabolites* **2022**, *12*, 1242.
[56] C. Peyrot des Gachons, P.A. Breslin, *Curr. Diabetes Rev.* **2016**, *16*, 102.
[57] C. Delporte, A. Bryla, J. Perret, *Int. J. Mol. Sci.* **2016**, *17*, 166.
[58] O. Akinfemiwa, M. Zubai, T. Muniraj, 'Amylase' **2022** https://www.ncbi.nlm.nih.gov/books/NBK557738/ (accessed 10 November 2023).
[59] C. W. Kim, H. J. Lee, D. Ahn, R. E. Go, K. C. Choi, *Toxicol. Rev.* **2022**, *38*, 511–522.
[60] T. Komulainen, T. Lodge, R. Hinttala, M. Bolszak, M. Pietilä, P. Koivunen, J. Hakkola, J. Poulton, K. J. Morten, J. Uusimaa, *Toxicology* **2015**, *331*, 47–56.
[61] B. Malicka, K. Skoskiewicz-Malinowska, U. Kaczmarek, *Medicine (Baltimore)* **2016**, *95*,e5211.
[62] M.U. Ahmed, A. Ibrahim, N.J. Dahiru, H.S. Mohammed, *Clin. Med. Insights Endocrinol. Diabetes* **2020**, *13*, 1179551420963106.
[63] G. Hasbal Çelikok, T. Yılmaz Özden, Ö. Saçan, A. Can, R. Yanardağ, *Istanbul J. Pharm.* **2022**, *52*, 73–79.
[64] U.F. Magaji, O. Sacan, R. Yanardag, *S. Afr. J. Bot.* **2020**, *128*, 225–230.
[65] U.F. Magaji, O. Sacan, R. Yanardag, *Rom. Biotechnol. Lett.* **2022**, *27*, 3208–3214.
[66] A. P. Basseto, F. T. A. Neves, S. F. Toledo, T. L. Silva, M. A. R. Buzalaf, F. A. C. Faria, J. M. Granjeiro, *Rev. FOB.* **2001**, *9*, 49–54.
[67] K. J. Dal Prá, A. de Fatima Moraes da Silva, A. H. Chaves-Neto, A. M. P. Soubhia, *Clin. Oral Invest.* **2020**, *24*, 4283–4290.
[68] H. E. Jones, I. D. Bowen, *Cell Biol. Int.* **1993**, *17*, 305–315.
[69] E. C. Silva-Zacarin, G. A. Tomaino, M. R. Brocheto-Braga, S. R. Taboga, R. L. De Moraes, *J. Biosci.* **2007**, *32*, 309–328.
[70] K. Szczepanowska, A. Trifunovic, *FEBS J.* **2022**, *289*, 7128–7146.
[71] E. Beutler, in ' *Red cell metabolism: A Manual of Biochemical Methods*', Ed. E. Beutler, Grune and Stratton Inc, New York, **1975**, pp. 112–114.
[72] L. Warren, *J. Biol. Chem.* **1959**, *234*, 1971–1975.
[73] A. Ledwozyw, J. Michalak, A. Stepien, A. Kadziolka, *Clin. Chim. Acta* **1986**, *155*, 275–284.
[74] K.M. Miranda, M.G. Espey, D.A. Wink, *Nitric Oxide* **2001**, *5*, 62–71.
[75] W. H. Habig, W. B. Jakoby, *Methods Enzymol.* **1981**, *77*, 398–405.
[76] H. Aebi, *Methods Enzymol.* **1974**, *105*, 121–126.
[77] A. A. Mylroie, H. Collins, C. Umbles, J. Kyle, *Toxicol. Appl. Pharmacol.* **1986**, *82*, 512–520.
[78] O. Erel, *Clin. Biochem.* **2004**, *37*, 277–285.
[79] O. Erel, *Clin. Biochem.* **2005**, *38*, 1103–1111.
[80] G. I. C. Ingram, M. Hills, *Thromb. Haemost.* **1976**, *36*, 237–238.
[81] J. T. Hatcher, L. V. Wilcox, *Anal. Chem.* **1950**, *22*, 567–569.
[82] R. Kuru, S. Yilmaz, G. Balan, B. Alev Tuzuner, P. N. Tasli, S. Akyuz, F. Y. Ozturk, Y. Altuntas, A. Yarat, F. Sahin, *J. Trace Elem. Med. Biol.* **2019**, *54*, 191–198.
[83] G. L. Miller, *Anal. Chem.* **1959**, *31*, 426–428.
[84] S.K. Singh, S.K. Singh, V.R. Tripath, S.K. Garg, *Afr. J. Biotechnol.* **2011**, *10*, 12257–12264.
[85] A. Dahlqvist, *Anal. Biochem.* **1968**, *22*, 99–107.
[86] K. Walter, C. Schütt, in ' *Methods of Enzymatic Analysis*' Ed. H.U. Bergmeyer, Academic Press, Inc. New York and London, **1974**. pp. 856–860.
[87] R. Bais, M. Philcox, *Eur. J. Clin. Chem. Clin. Biochem.* **1994**, *32*, 639–655.
[88] O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **1951**, *193*, 265–275.
[89] U. K. Laemmli, *Nature* **1970**, *227*, 680–685.
[90] C. Schneider, W. Rasband, K. Eliceiri, *Nat. Methods.* **2012**, *9*, 671–675.

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