

## POTENTIAL EFFECT OF METFORMIN ON SALIVARY GLANDS OF RATS EXPOSED TO CHRONIC UNPREDICTABLE MILD STRESS

Laila E Amin\*  and Mahmoud El Sherbeny\*\* 

### ABSTRACT

**Background:** Exposure to mild, uncontrollable, and persistent stress strongly correlates with several mental and physical problems. This study aimed to assess the ameliorative effect of metformin (MET) on the parotid salivary gland of rats exposed to chronic unpredictable mild stress (CUMS).

**Material and methods:** A total of 40 male albino rats were used in the study and divided into four groups (n = 10 each): control group, CUMS group: exposed to CUMS for four weeks, fluoxetine (FLX) group: exposed to CUMS for four weeks and treated orally with 10 mg/kg/day FLX for two weeks, and MET group: exposed to CUMS for four weeks and treated orally with 50 mg/kg MET daily for 2 weeks. The specimens from parotid salivary glands were obtained and processed for histological and ultrastructural examinations, and expressions of proliferating cell nuclear antigen (PCNA) and neurotrophin-3 (NT-3) gene using real-time polymerase chain reaction.

**Results:** Exposure to CUMS significantly reduced body weight. Histologically, CUMS-induced hydropic degeneration in acinar cells, and the ultrastructural findings showed the acinar cells with many intracellular vacuoles, irregular, shrunken nuclei with condensed chromatin, deteriorated mitochondria, and expanded rough endoplasmic reticulum. Besides reducing immunoexpression of PCNA with the highest level of NT-3 gene expression, treatment with MET improves body weight, restores the histological architecture of salivary glands, and protects against neurodegenerative disorders as it reduces the NT-3 expression.

**Conclusion:** Treatment with MET, as an antidepressant drug, is a more effective approach than treatment with FLX in the short term.

**KEYWORDS:** Depression; Metformin; Fluoxetine; Parotid Gland; Proliferation.

\* Associate professor of Oral Biology, Faculty of Dentistry, Mansoura University; Horus University, Egypt.

\*\* Associate professor of Oral Pathology, Faculty of Dentistry, Mansoura University; Horus University, Egypt.

## INTRODUCTION

Depression is the most prevalent debilitating mental disorder, with an increased occurrence and death rate. The accumulative outcome of stress can cause allostatic overload, according to sustained persistent or recurrent stimulation of effectors. The allostatic load has been linked to several diseases, including circulatory disorders, diabetic nephropathy, chronic renal disease, obesity, and depression <sup>(1)</sup>.

Chronic unpredictable mild stress (CUMS) is a well-known technique for simulating clinical depression. The CUMS model is a commonly used animal model of depression to study the processes that underpin depression. The chronic mountain sickness rat model is a widely used animal model of depression because it simulates depression in the face of social environmental stress and may more closely resemble the development of depression in humans than other models <sup>(2)</sup>.

Stress has also been shown to affect oral health. Using unhealthy coping strategies for stress (such as smoking, consuming alcohol, and comfort eating) increases the risk of periodontal disease, dental caries, and oral cancer. Biological changes brought about by stress are often represented as risk factors for oral diseases. Furthermore, stress has been reported to cause salivary changes, which may increase the risk of dental caries <sup>(3)</sup>.

The primary purpose of salivary glands is to produce saliva, which aids in food digestion and swallowing, as well as chewing and antimicrobial activity. Salivary glands play an important role as they release several growth factors. Additionally, saliva contains several components extracted from the blood. The quantity and consistency of salivary products are related to the protection of oral health. Therefore, the presence of salivary products may indicate the state of overall health or disease. The autonomic nervous system controls salivary gland innervation and secretion, influencing salivary protein concentration and flow rate. The salivary gland function may be altered by persistent stress, which can increase the risk of dental caries <sup>(4)</sup>.

Selective serotonin reuptake inhibitors (SSRIs) are the commonly used drugs for managing major depressive cases. However, the effectiveness of SSRIs varies, with 60%–70% of patients experiencing no remission and 30%–40% showing no meaningful response. Fluoxetine (FLX) is one of the most widely used SSRIs and changes the molecular and behavioral depression-like phenotype along with the superiority of the existing life. FLX therapy contributed to a deterioration of depression-like endpoints, such as an elevation in the hedonistic activity and a decrease in neurogenesis <sup>(5)</sup>.

The effect of SSRIs on brain plasticity and sensitivity to the surroundings provides novel possibilities for restoring antidepressant effectiveness by enhancing the quality of the patients' living environment. Furthermore, metformin (MET) is a commonly prescribed medicine for type 2 diabetes and other metabolic syndromes <sup>(6)</sup>. It passes through the blood-brain barrier. Therefore, it controls the neuronal outlines at the peripheral and central levels together. Although the underlying molecular mechanisms of MET action are still unclear, preclinical studies showed that it improves brain flexibility by growing long-standing potentiation in the hippocampal region and modifying neurotrophic levels, such as the brain-derived neurotrophic factor (BDNF) <sup>(7)</sup>.

In ribonucleic acid (RNA) sequencing, persistent stress makes major histopathologic changes. Downregulated genes from chronically stressed classes were considerably enriched for many biological processes in cell proliferation-related keywords, according to a Gene Ontology review. PCNA (proliferating cell nuclear antigen) was used as a cell proliferation indicator. Immunohistochemistry studies showed that long-term stress resulted in a substantial decrease in PCNA mRNA and protein expression. PCNA, a supplementary protein of DNA polymerase  $\delta$ , rises through the late part of the G1 phase of the mitotic cell cycle, increases in the S phase, and declines through the G2 and M phases. These variations depend on the level of DNA synthesis, DNA repair,

sister chromatid exchange, and cohesion. They are involved in cell cycle regulation and play a key role in meiosis <sup>(8)</sup>.

Neurotrophic factors, such as BDNF, nerve growth factor, neurotrophin-3 (NT-3), and NT-4, are proteins that play a significant role in developing the growth of neurons and are critical for preserving and controlling the integrity and function of neurons all over the life <sup>(9)</sup>.

This study aimed to investigate the role of FLX and MET in the histologic architecture and morphological changes of the parotid salivary glands in CUMS-induced rats. The PCNA detection immunohistochemistry ratio is a valuable indicator for evaluating the proliferating properties of cells in tissue specimens. Additionally, NT-3 gene expression via real-time polymerase chain reaction (RT-PCR) was assessed to investigate the neuronal survival rate.

## MATERIAL AND METHODS

### Animal design

This study used a total of 40 adult male albino rats weighing 150–200 gm. was used in this study. The rats were housed in separate cages in a light-controlled room with a 12:12 h light-dark cycle and fixed temperature ( $\pm 22^{\circ}\text{C}$ ) and qualified moisture (65%–70%). Ordinary rodent food and tap water were provided. The experimental processes were approved by the Animal Care and Use Committee, Mansoura University, Egypt, code number MU-ACUC (DENT.R.22.11.4).

The rats were weighed before the beginning and end of the experimentation. They were divided into four groups ( $n = 10$  each): group I (negative control group), group II (CUMS group): CUMS-induced rats were kept without any treatment, group III (FLX group): CUMS-induced rats were treated orally with 10 mg/kg/day FLX for two weeks, and group IV (MET group): CUMS-induced rats were treated orally with 50 mg/kg MET daily for 2 weeks. For CUMS induction, groups II, III, and IV

were exposed to CUMS for 4 weeks, as designated by Doron et al. <sup>(10)</sup>.

### Drugs

FLX was purchased from Eli Lilly and Co. It was available in the form of capsules, each containing 20 mg of FLX hydrochloride. The powder content of each capsule was added aseptically to drinking water to prepare a 240 mg/L FLX solution so that the rats received 10 mg/kg/day orally using an oral gavage syringe. The dose is equal to the dosage used for humans <sup>(11)</sup>.

Metformin treatment: Metformin was gained MET was obtained from Sigma-Aldrich (St. Louis, MO, USA) and liquefied in saline. Rats were orally administered 50 mg/kg MET daily for 2 weeks <sup>(12)</sup>.

### CUMS procedure

The CUMS action was approved for 4 weeks consistent with the following circumstances: food deprivation (24 h), water deprivation (24 h), 45° cage tilting (24 h), crowded housing (24 h), restriction in an empty water bottle (4 h), noise (20 min), tail clamping (1 min), forced swimming (10 min), and day-night reversal (12 h/12 h). The protocols were haphazardly arranged to allow the rats to obtain one of them daily to confirm that the process was changeable <sup>(10)</sup>.

### RT-PCR

RT-PCR was performed at Mansoura Experimental Research center, Mansoura. The parotid glands were preserved in liquid nitrogen to be used for extraction and isolation of RNA to quantify NT-3 via RT-PCR.

### Histological processing

Hematoxylin and eosin (H&E). The parotid glands were removed. The right side was fixed immediately in 10% neutral buffered formalin. Then, the samples were rinsed with running water, dried in ascending concentrations of alcohol, and transferred to xylene to clear the samples from the

alcohol. Previously, the samples were embedded in paraffin wax and mounted in the center of the paraffin wax blocks. Paraffin-embedded tissue blocks were cut into 5-micron thick sections and placed on glass slides for histological investigation by H&E stain.

Ultrathin sections of salivary glands. Small sections of an average size of 1 mm<sup>3</sup> from the parotid glands were made using a very sharp blade and then rapidly fixed in a 3% phosphate-buffered glutaraldehyde solution for 1–2 hours (primary fixation). Sections were cleaned and fixed in 1% buffered osmium tetroxide at 4°C for 1–2 hours. Sections were dehydrated in ascending concentrations of ethanol (50%, 70%, and 90%) and then embedded in oven-dried gelatin capsules using fresh epoxy resin. Semithin sections (1–2 microns) were prepared and stained with toluidine blue to be examined under a light microscope. Ultrathin sections (0.06 microns) were cut using ultramicrotome and glass knives and then mounted on copper grids. The sections were finally examined using the transmission electron microscope (JEOL 1000) at different magnifications and photographed using a charge-coupled device electron microscope unit at Electron Microscope Unite, Faculty of Science, Mansoura University, Egypt.

Immunohistochemical stains. The sections were stained using the streptavidin-biotin-peroxidase immunohistochemical technique, an indicator of cell proliferation PCNA polyclonal antibodies (Thermo Fisher Scientific, Product no. PA5-27214, 1:100 dilution) used in this study.

Images were taken using a digital camera attached to an Olympus BX-51 microscope (Tokyo, Japan). The immunoexpression of the examined antibodies (expressed as percentage area) was evaluated in 30 fields at a magnification of 200× using Pro Plus image analysis software version 6.0. The histopathological evaluation was performed by a histopathologist who was blind to the experimental groups. The group assignment at each stage of the

experiment was known to the primary investigator. The collected data were tallied, coded, and examined using a social science statistical analysis tool.

## RESULTS

### Body weight

The control group (negative control) had the highest body weight (246.33 ± 10.29), while the CUMS group had the lowest body weight (135 ± 8.41). The body weight of the FLX and MET groups (136.45 ± 0.91 and 154.66 ± 0.46, respectively) was higher than that of the CUMS group but lower than that of the control group. Statistically, the analysis of variance test showed a general significant difference in body weights among all study groups.

Moreover, Tukey's post hoc test for multiple comparisons showed a significant difference between the control group and the CUMS, FLX, and MET groups. Also, a significant difference was observed between the CUMS group and the MET group. However, no significant difference was observed between the CUMS group and the FLX group (Table 1).

TABLE (1) Showed means ± STD of the body weights of rats of the studied groups at the end of experiments.

Body weight	Mean ±STD				P
	Control	CUMS	FLX	MET	
	246.20 ±0.54	134.98 ±0.98	136.45 ±0.91	154.66 ±0.46	
P1		<0.0001	<0.0001	<0.0001	<0.0001
P2			0.034	<0.0001	
P3				<0.0001	

*STD: standard deviation P: Probability*

*Test used: ANOVA followed by posthoc tukey for multiple comparisons*

*P1: Significance relative to Group I*

*P2: Significance relative to Group II*

*P3: Significance relative to Group III*

**H&E Histological results**

In the control group, the parotid salivary glands consisted of densely packed serous acini, which appeared rounded and small in size with narrow lumens and a well-formed duct system in between. In the CUMS group, the sections showed hydropic degeneration with ballooning-shaped acini and loss of cell outlines with numerous vacuoles. In the FLX group, the parotid glands showed multiple minute intracellular vacuoles with pyknotic nuclei and increasing spaces between acini with many dilated blood vessels. In the MET group, the parotid glands maintained their normal histological architecture, consisting of densely packed serous acini with basally located nuclei and some minute intracellular vacuoles (Figure 1).

**Immunohistochemical results**

Statistically, the ANOVA test showed a general significant difference in PCNA expression among all study groups. Also, Tukey’s post hoc test for multiple comparisons showed a significant difference between the control group and the CUMS and FLX groups. Also, a significant difference was

observed between the CUMS group and the MET group. However, no significant difference was observed between the control group and the MET group, between the CUMS group and the FLX group, and between the FLX group and the MET group, respectively (Figure 1, Table 2).

TABLE (2): Showed means± STD of the PCNA expression in the parotid gland of the studied groups.

	Group 1	Group 2	Group 3	Group 4	p
Mean	0.14	0.06	0.08	0.11	
± STD	±.012	±0.010	±0.011	±.011	
P1	.000	.000	.000	.005	<0.0001
P2		.000	.032	.000	
P3				.006	

*STD: standard deviation P: Probability*  
*Test used: ANOVA followed by posthoc tukey for multiple comparisons*  
*P1: Significance relative to Group I*  
*P2: Significance relative to Group II*  
*P3: Significance relative to Group III*

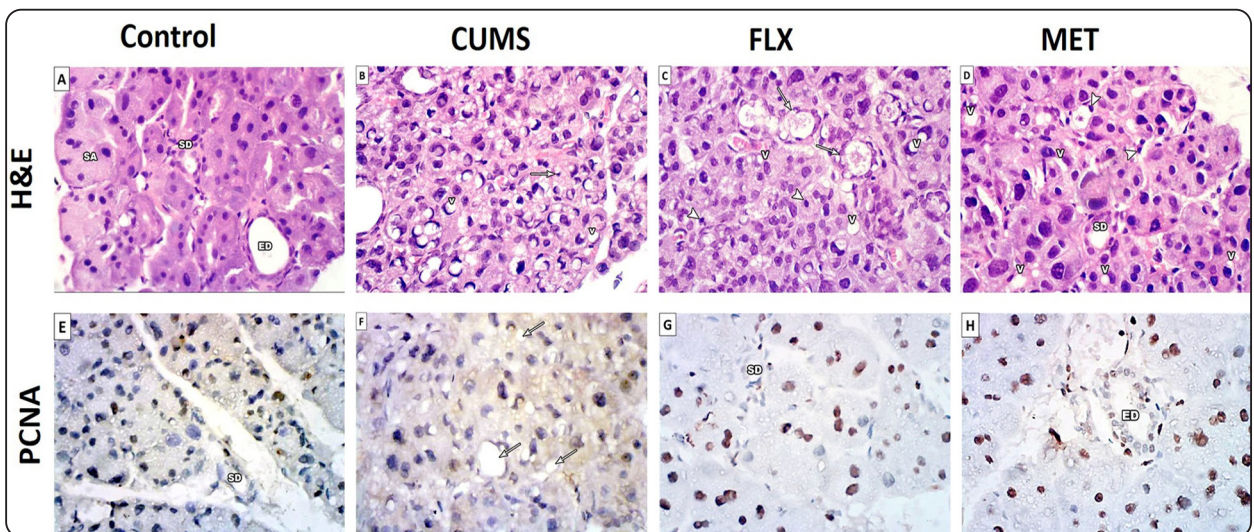


Fig. (1) Photomicrograph (A) Parotid glands formed of densely packed serous acini (SA), intralobular striated duct (SD) and excretory ducts (ED) between the gland’s lobules. (B) Serous acini appeared with pyknotic nuclei (arrow) and many intracellular vacuoles (V). (C) The parotid gland showed loss of acinar outlines (arrow) and multiple minute intracellular vacuoles (V). (D) The gland consisted of densely packed serous acini with basally located nuclei (arrow) and some intracellular vacuoles (V) (H&E, X400). (E-H) positive immunopexpression of PCNA in the nucleus of serous acini and some of striated duct outline (SD) (Immunoperoxidase staining with anti- PCNA antibody, DAB chromagen X400).

### Ultrastructural examination

In the control group, serous acinar cells appeared with euchromatic nuclei, densely packed electron-dense homogeneous secretory granules, parallel cisternae of rough endoplasmic, various morphology mitochondria, and proper desmosomal attachments between cells. In the CUMS group, the serous acini appeared with an irregular nuclear outline and condensed peripheral heterochromatin, and numerous dilated cisternae of rough endoplasmic reticulum were prominent with swollen and degenerated forms of mitochondria. Some vacuoles were apparent in the cytoplasm. In the FLX group, the serous acini appeared with an irregular nuclear outline, condensed heterochromatin, degenerated areas of lateral cytoplasm, secretory materials of highly varying electron density, and a large number of vacuoles. In the MET group, the nucleus of serous cells appeared with peripheral condensed heterochromatin. The secretory granules showed homogeneous electron-dense secretions. The rough endoplasmic reticulum appeared vesiculated with

dilated cisternae, and some cytoplasmic vacuoles can be seen (Figure 2).

### NT-3 gene expression

NT-3 gene was expressed in all study groups. The CUMS group had the highest level of NT-3 gene expression ( $46.14 \pm 08.72$ ). The control, FLX, and MET groups nearly had the same level of expression ( $26.14 \pm 0.72$ ,  $26.29 \pm 0.69$ , and  $26.53 \pm 0.53$ , respectively).

Statistically, the ANOVA test showed a significant difference in NT-3 gene expression among all study groups. Moreover, Tukey's post hoc test for multiple comparisons showed a significant difference between the control group and the CUMS group. Also, a significant difference was observed between the CUMS group and the FLX and MET groups. However, no significant difference was observed between the FLX group and the MET group and between the control group and the FLX and MET groups (Table 3).

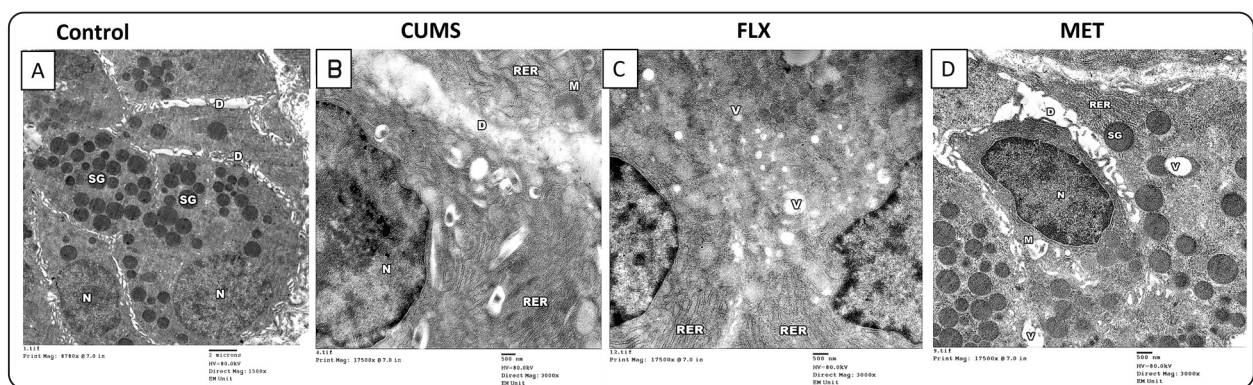


Fig. (2) Representative high-resolution electron microscopic images for (A) control group revealed the serous cells formed of round basally located euchromatic nuclei (N) with abundant homogenous secretory granules (SG), parallel arrays of rough endoplasmic reticulum (RER), oval-shaped mitochondria (M) with intact desmosomal attachments between cells (D). (B) CUMS Group showed heterochromatic nuclei (N) with irregular nuclear outlines and multiple number of heterogenous densities of secretory granules, dilated cisternae of rough endoplasmic reticulum (RER) with enlarged swollen mitochondria (M) and widen destructed desmosomal attachments between cells (D). (C) FLX Group showed irregular nucleus (N) with chromatin aggregated into dense mass, dilated rough endoplasmic reticulum (RER), cytoplasmic vacuoles (V). (D) MET Group showed elongated euchromatic nuclei (N), homogenous dense secretory granules (SG), some vacuoles (V) intact desmosomal attachments between cells (D) (Scale bar: 500 nm).

TABLE (3): Showed means± STD of the NT-3 expression in the parotid gland of the studied groups.

	Means± STD of the NT-3 expression				P
	Control	CUMS	FLX	MET	
	26.14 ±0.72	46.14 ±0.72	26.29 ±0.69	26.53 ±0.53	
<b>P1</b>		<0.0001	0.98	0.7	<0.0001
<b>P2</b>			<0.0001	<0.0001	
<b>P3</b>				0.9	

*STD: standard deviation P: Probability*  
*Test used: ANOVA followed by posthoc tukey for multiple comparisons*  
**P1: Significance relative to control group**  
**P2: Significance relative to CUMS group**  
**P3: Significance relative to FLX group**

**DISCUSSION**

Chronic mild stress is most expected to cause metabolic, hormonal, and immune system changes. Chronic stress causes cognitive dysfunction, behavioral changes, oxidative stress, and immune system suppression. Several methods can be used to cause depression in laboratory animals. In the laboratory, stressors such as forced swimming, tail shock, immobilization, cold, and ether stress are used (13). In this study, the rats were kept under stressful conditions for four weeks, which is thought to be long enough to cause depression in the salivary glands. FLX is a commonly selective serotonin reuptake blocker antidepressant that can improve the behavioral and biochemical changes induced by CUMS (5).

The results showed that CUMS-induced rats showed a substantial reduction in body weight. These results are consistent with those reported by Bhatnagar et al. (2014), who found that weight loss could be due to a decrease in daily food consumption rather than changes in food intake-related genes. Furthermore, stress-induced weight loss can be due to food consumption reduction first and then later continued by growth in energy level

and body temperature through CUMS. The lower body weight of stressed animals reflected earlier studies and may have resulted from a grouping of influences containing an increased caloric requisite to keep body temperature after sleep deficiency and wet bedding (14).

In this study, FLX-treated rats showed a significant reduction in body weight. Other researchers, such as Hajizadeh et al. (2016), found that FLX has inhibitory effects on the gastrointestinal tract and lowers the height of the intestinal villus, resulting in reduced intestinal absorption and depletion of essential nutrients. FLX also has an effect on the hypothalamic appetite centers, which lowers appetite and reduces calorie intake (15).

The MET group lost significantly more weight than the control group. MET regulates glucose levels by lowering hepatic glucose consumption, improving peripheral insulin sensitivity, and preventing glucose absorption in the GI tract. MET aids in weight loss by reducing calorie intake while having little effect on total daily energy expenditure. Surprisingly, meal size decreases within the first few weeks of MET therapy, but the number of meals decreases over time. MET raised leptin levels when prompting AMP-activated protein kinase (AMPK) action in the skeletal muscle and liver, according to Barnea et al. (2012). These results were related to the stimulation of hepatic casein kinase 1-a and skeletal muscle casein kinase 1-e, implying that numerous tissues' circadian clock pathways are impacted by MET, which also helps regulate energy metabolism (16).

The current histological findings indicated morphological changes in the parotid gland after exposure to CUMS, such as the loss of acinar outlines and excessive hydropic degeneration with intracellular vacuoles. According to Ayuob et al. (2019), CUMS exposure results in the formation of vacuoles in serous acini and a reduction in the size of some SMG and parotid gland acini (17). These results are consistent with those who demonstrated that

repetitive stress, even of brief length, can affect the glandular tissues such as the adrenal cortex and testes directly or indirectly. Stress induces vacuolization, disorganization of the secretory granules, indented nuclei, and areas missing cell boundaries, according to the researchers. Immobilization stress, lipid peroxidation, and protein oxidation have also been linked in other studies. The enhanced excretion of corticosterone, serotonin, and catecholamine, which play a role in lipid peroxidation, clarified the degenerative changes caused by immobilization stresses <sup>(18)</sup>.

The histological results of the FLX-treated group showed degenerative changes in the serous acini and duct system. Chronic use of psychotropic drugs has been linked to hypertrophy in serous acini, which is characterized by expanded cellular outlines and accumulation of secretory granules. Since FLX is a selective serotonin reuptake inhibitor, it can increase serotonin availability in the synaptic gap, altering acetylcholine essential to the salivary glands' muscarinic receptors (M3). As a result, FLX can decrease salivary secretion. Antidepressants appear to have little effect on saliva development. However, they interfere with the binding of certain proteins <sup>(19)</sup>.

In this study, the MET-treated group revealed decreased atrophy of most serous acini with variable degrees of cytoplasmic vacuolation. This is in agreement with Kim et al. (2019), who reported that MET decreases inflammation in salivary glands and maintains saliva flow. Additionally, MET lowers the levels of the mRNA and proteins for interleukin-6 (IL-6), tumor necrosis factor- $\alpha$ , and IL-17 in the salivary glands. In an animal model of Sjögren's syndrome, MET also lowers effector T cells while increasing regulatory T cells and adjusting B-cell differentiation <sup>(20)</sup>.

According to several studies, the primary effect of MET is to inhibit mitochondrial complex I. Mitochondrial complex I plays a significant role in cellular reactive oxygen species (ROS) development.

A blockage of this complex has been reported to cause a decrease in reactive species production due to reduced electron transport from NADH plus H<sup>+</sup>. Therefore, MET lowers endogenous ROS levels in mitochondria <sup>(21)</sup>.

After exposure to CUMS, statistical analysis of the proliferating index PCNA showed that cellular proliferation was significantly reduced. The results indicated that chronic mild stress disturbs the proliferative activity by causing changes in the neuroendocrine system, as evidenced by a previous study that found that PCNA-positive cells were far less in hamsters subjected to four consecutive days of immobilization stress in every area than in the control group <sup>(22)</sup>.

The main action of the body to stress is the stimulation of the hypothalamo-hypophyseal-adrenocortical system (HHAS) through the progress of an extensive spectrum of physiological effects, comprising an increase in corticosteroid secretion and reduction of immune functions. The principal action in the working of the HHAS stimulated by stress is performed by adrenocorticotrophic hormones. The stress caused alterations in the neuroendocrinological system with physiological variations. For example, the release of glucocorticoid, catecholamine, testosterone, or a mixture of these hormones prevents the proliferation of the epidermis *in vivo*. However, the hormone system accountable for the decrease in proliferative activity is still unknown <sup>(23)</sup>.

The proliferation index was found to be non-significantly higher in the FLX-treated community. SSRIs had no outcome on saliva flow, most likely owing to their deficiency of anticholinergic activity. The serotonin receptor action present in the peripheral microcirculation may cause a flow reduction. This finding is consistent with that of Mattioli et al. (2011), who found that using FLX for 30 days resulted in a reduction in the activated flow of saliva, elevation of cell volume, and proliferation in the number of MECs in rats <sup>(19)</sup>.



The addition of MET scavenged ROS induced by increased blood glucose level. Oxidative stress reduced the adenosine triphosphate synthesis and mitochondrial transcriptional activity caused by elevated glucose levels and affected physiological processes such as proliferation. In other studies, AMP-activated protein kinase (AMPK) has been implicated in the control of fasting, inflammation, stress, and other central nervous system responses. In mice subjected to persistent stress, phosphorylated AMPK (pAMPK) reduction is linked to depression-like behaviors. Furthermore, several studies showed that MET increases pAMPK levels, which activates AMPK for its effects on type 2 diabetes, cancer, and other diseases. However, whether MET alleviates depressive symptoms through an AMPK-dependent pathway is unclear <sup>(24)</sup>.

In this study, the CUMS group had high levels of NT-3 mRNA gene expression within the parotid glands. The altered expression of BDNF in the brains of stressed animals verified that BDNF plays a crucial part in the stress response and that neuropathic and inflammatory pain processes are linked to BDNF. The biological effects of BDNF are mediated by two transmembrane receptors, p75NTR (pan-selective p75 neurotrophin receptor) and tropomyosin receptor kinase B (TrkB or tyrosine receptor kinase B), and potential stressors that can dramatically increase stress include cold, loud, restraint, and forced swimming. Earlier studies showed that a few of these stresses can also affect an experimental animal's ability to transmit pain <sup>(25)</sup>.

According to preclinical models, MET modifies the release of neurotrophic factors like BDNF by activating AMPK and cAMP-response element binding protein (CREB) <sup>(26)</sup>. Additionally, MET increases CREB phosphorylation and supports histone acetylation to boost BDNF production while increasing the plasticity of neural architecture. MET has also been shown to lessen DNA damage, endogenous ROS, and IGF-1 levels <sup>(27)</sup>.

## CONCLUSION

Based on the outcomes of the obtained data, the present study exhibited that MET ameliorates depressive-like status caused from exposure to chronic stress by employing a hopeful antioxidant, anti-inflammatory and antidepressant-like effects. MET subsequently improves the structural changes made by stress in the salivary glands that might be due to up-regulation of salivary NT-3 expression. We also recommend future studies to test the efficacy of MET in improving stress-related salivary alterations in humans.

## Author contribution

Laila E. Amin: conceptualization, methodology, writing—original draft, writing—review and editing, investigation, formal analysis, and visualization. Mahmoud El Sherbeny: conceptualization, methodology, writing—original draft, writing—review and editing, investigation, formal analysis, and visualization.

## Declaration of competing interest

The authors report no competing interest.

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