ORIGINAL ARTICLE

EFFECT OF GHRELIN IN ALLEVIATING NICOTINE INDUCED OXIDATIVE STRESS IN BALB/C MICE

Hira Pervez Kiyani, Sadia Ahsin, Madiha Imran, Hira Ashraf

Department of Physiology, Foundation University Medical College, Islamabad, Pakistan

Background: Ghrelin, a hormone released from GIT mucosa is known to have antioxidant properties by increasing the levels of antioxidant enzymes and by preventing lipid peroxidation. The current study was designed to demonstrate the protective role of ghrelin against nicotine induced oxidative stress and lipid peroxidation in BALB/c mice. **Methods:** Ninety healthy, male BALB/c mice selected through non probability convenient sampling were sorted out into three groups having 30 mice each. Group I (control group) was given intraperitoneal injection of normal saline (i.p.). Group II was given nicotine at a dose of 2.5 mg/Kg body weight (i.p.), while Group III was given nicotine at a dose of 2.5 mg/Kg body weight (i.p.) along with ghrelin at a dose of 10 µg/Kg (i.p.) on alternate days for 4 weeks. On 30th day sampling was done for assessment of serum levels of oxidative stress enzymes (superoxide dismutase, glutathione reductase and catalase levels) and lipid peroxidation marker (malondialdehyde) on ELISA. Data was analysed using SPSS-24. ANOVA followed by post Hoc Tukey test were applied and $p \leq 0.05$ was considered significant. **Results:** Nicotine group showed significant decrease in serum antioxidant enzymes along with significant increase in lipid peroxidation marker. Administration of ghrelin significantly raised the antioxidant enzymes and caused decline in levels of lipid peroxidation marker. **Conclusion:** Ghrelin appears to be an antioxidant due to its ability to increase the levels of antioxidant enzymes and decrease lipid peroxidation marker in nicotine-induced oxidative stress.

Keywords: Nicotine, Ghrelin, Oxidative stress, Antioxidant enzymes, Lipid peroxidation

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INTRODUCTION

Cigarette smoking is one of the leading cause of increasing morbidity globally.[1] Nicotine is the predominant chemical amongst the constituents in cigarette smoke. Consumption forms include smoke (cigarettes, pipes, and cigars) and smokeless tobacco (chewable tobacco).[2] Nicotine is also present in insecticides, which can lead to its accidental or intentional poisoning. Nicotine damages many organs of our body like lungs, heart, liver, brain and blood vessels by increasing the production of Reactive Oxygen Species (ROS) and by causing lipid peroxidation.[3] These ROS are responsible for causing oxidative damage which includes DNA/RNA damage, oxidation of proteins and inactivation of membrane enzymes and receptors.[4–6] Smokers are at greater risk of cardiovascular diseases, chronic obstructive lung diseases, cancers, peptic ulcer, infertility and hepatotoxicity. Nicotine disrupts multiple cellular processes. Nicotine exposure produces oxidative stress in tissues by causing depletion of glutathione content and decreasing the activity of oxygen free radical scavengers, like superoxide dismutase and catalase.[7]

Limited amount of lipid peroxidation occurs naturally due to activity of reactive oxygen species (ROS) produced as a result of normal metabolic processes. High levels of ROS directly damage membrane bound lipids which results in decrease in the cell membrane fluidity and an increase in membrane permeability resulting in impairment of function of cell membrane.[8]

'Oxidative stress' results from an imbalance between the formation of ROS and the antioxidant defences. Mitochondrial enzymatic antioxidant defences like catalase (CAT), glutathione reductase (GR) and superoxide dismutase (SOD) play a significant role in abolishing the oxidative effects of ROS by scavenging them and thus preventing tissue damage.[9]

Ghrelin, a peptide hormone having 28 amino acids is released primarily by the stomach gastric cells during hunger and starvation.[10] Ghrelin is also released from other tissues like pancreas, jejunum, lungs, urogenital organs and pituitary gland.[11] Ghrelin levels peak before eating and decline after meal.[12]

Ghrelin performs multiple functions, which includes appetite stimulation, Growth Hormone secretion, increase in GI motility and secretion. Ghrelin has also shown to have anti-fibrotic, anti-inflammatory, anti-oxidant, and anti-apoptotic actions.[13,14] Ghrelin performs its antioxidant function by increasing the levels of antioxidant enzymes and by preventing lipid peroxidation.[15] Ghrelin decreases the expression of inducible nitric oxide synthase and NF-κB, both of which are responsible for production of ROS.[16]

Systemic administration of ghrelin alleviates oxidative stresses. It has been hypothesized that intraperitoneal administration of ghrelin may have protective role in alleviating oxidative stress in animals who are exposed to nicotine. The current study was

designed to determine role of ghrelin in nicotine induced oxidative stress and lipid peroxidation in BALB/c mice by estimating and comparing antioxidant enzymes (GR, CAT, SOD) and lipid peroxidation marker (MDA) levels.

MATERIAL AND METHODS

This experimental study was conducted at Foundation University School of Health Sciences, Islamabad in collaboration with National Institute of Health, Islamabad, Pakistan after approval from Ethics Review Committee of Foundation University Islamabad. The duration of study was 18 months (2019–2021).

Ninety (90) healthy male BALB/c mice of 6–10 weeks age, in the range of 25–40 g body weight were selected through non-probability convenient sampling and were divided into three groups having 30 mice each. Sample size was calculated using G power method.[17] Animals were kept at animal house of NIH a week prior to study for acclimatization to the environment (Room temperature 22±2 ºC, and 12/12 hour day/night cycle). Group I (control group; n=30) was given standardized pellet diet and 0.65% normal saline at a dose of 1 ml/Kg intraperitoneally (i.p.), Group II (Nicotine only group, n=30) was given pellet diet and i.p. nicotine injection obtained from Alfa Aesar, Johnson Matthey Company, Great Britain at a dose of 2.5 mg/Kg body weight(b.w.)/day for 4 weeks.[18,19]

Group III (Nicotine plus ghrelin group, n=30) was given pellet diet, nicotine i.p. injection at a dose of 2.5 mg/Kg b.w./day for 4 weeks along with ghrelin obtained from Abbexa Ltd Cambridge, UK at a dose of 10 µg/Kg body weight i.p. on alternate days for 4 weeks.[18–20]

On 30th day, mice were euthanized and intracardiac sampling was done for assessment of antioxidant enzymes (Superoxide dismutase, Glutathione reductase and Catalase) and lipid peroxidation marker (Malondialdehyde) in serum by ELISA.

Data was analysed on SPSS-24. Parametric test One-way Analysis of Variance (ANOVA) was applied for significant difference of means between the groups followed by post HOC Tukey test, and $p \leq 0.05$ was considered significant.

RESULTS

Group II (nicotine only group) showed evident oxidative damage with significant decrease in antioxidant enzymes levels (CAT, SOD and GR) and increase in lipid peroxidation marker (MDA) in serum ($p<0.001$) as compared to group I. (Table-1).

In Group III mice, receiving ghrelin along with nicotine, serum antioxidant enzymes and MDA levels were significantly more as compared to Group II ($p<0.001$ for each marker) (Table-1).

Statistically there was no significant difference between group I and group III in antioxidant enzymes and MDA levels ($p=0.279$ for CAT), ($p=0.517$ for SOD), ($p=0.589$ for GR) and ($p=0.978$ for MDA) (Table-1).

Table-1: Effects of nicotine, and nicotine plus ghrelin on hepatic tissue levels of glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA)

Variable	Group (Mean±SD)			ANOVA *p*-value	Group-wise comparison using post-Hoc Tukey test		
	Control (I)	Nicotine (II)	Nicotine+Ghrelin (III)		Control *vs* Nicotine (I *vs* II)	Nicotine *vs* Nicotine+Ghrelin (II *vs* III)	Control *vs* Nicotine+Ghrelin (I *vs* III)
GR pg/ml	960.40±98.82	591.80±76.89	927.17±89.76	<0.001*	<0.001*	<0.001*	0.589
SOD ng/ml	8798.58±243.75	4677.94±165.92	8712.87±198.42	<0.001*	<0.001*	<0.001*	0.517
CAT ng/ml	9.45±2.82	5.05±1.16	8.26±1.72	<0.001*	<0.001*	<0.001*	0.279
MDA ng/ml	498.15±105.63	4799.17±169.33	488.15±106.04	<0.001*	<0.001*	<0.001*	0.978

The results are Mean±SD. The group means were compared with one way ANOVA and post-hoc Tukey's HSD test. *Significant

DISCUSSION

Nicotine effects most of our body organs.[21] It has been used widely as a model agent for inducing free radical damage in animal models.[19,22] In the present study, nicotine was used to induce oxidative stress in animal model and ghrelin was evaluated for antioxidant activity against nicotine induced oxidative stress in male BALB/c mice. The results demonstrate that ghrelin significantly protected the animals from nicotine-induced oxidative stress.

In our study, manifestations of oxidative stress were observed which included significant decline in serum levels of antioxidant enzymes CAT, GR and SOD and rise in lipid peroxidation marker MDA. Nicotine administration significantly lowered the levels of antioxidant enzymes in serum as compared to the control group. Co-administration of ghrelin with nicotine significantly attenuated the decline in the antioxidant enzymes caused by nicotine. The rise in antioxidant enzymes by ghrelin proves its protective role against oxidative stress.[23]

Lipid peroxidation is one of the major manifestations of oxidative stress induced by ROS.[8] One of the markers for lipid peroxidation is MDA, which is produced by decomposition of fatty acids and is responsible for the bio molecular changes produced by lipid peroxidation. In this study, lipid peroxidation induced by nicotine administration was manifested by

increased levels of MDA in the nicotine group in serum as compared to the control group. Co-administration of ghrelin prevented the rise in MDA levels in nicotine plus ghrelin group. Several studies support the potential role of ghrelin to curtail lipid peroxidation.[24,25]

Cetin Ebru *et al* conducted a study to assess the hepatoprotective effect of ghrelin on CCl_4 induced acute liver injury in adult male Sprague-Dawley rats. In their study a single injection of CCl_4 was given to induce oxidative stress which resulted in increased MDA levels in serum and hepatic tissue. Administration of ghrelin (10 ng/Kg daily for 5 days) resulted in significant decline in MDA levels in both serum and hepatic tissue. The authors concluded that ghrelin significantly lowered MDA levels.[26] This is in line with our results of decreased MDA levels after ghrelin treatment in nicotine induced oxidative stress. It may be expected that ghrelin preserves the membrane of cells against lipid peroxidation by decreasing the concentrations of MDA due to decrease production of ROS.

Another study, conducted by Alireza Lotfi *et al*[27] to observe the effect of ghrelin on serum levels of MDA in newly hatched chicken. The authors concluded that ghrelin administration significantly lowers the levels of MDA which is in line with our study. Mahmoud Elsawy *et al*[28] studied the effect of ghrelin on antioxidant enzymes SOD and GPx in serum and hepatic tissue of a male albino diabetic rats. They concluded that in diabetes, the levels of antioxidant enzymes are reduced while treatment with ghrelin significantly raised their levels. This positive result is in line with our results of increased antioxidant enzymes levels after ghrelin treatment in nicotine induced oxidative stress. In that study only SOD and GPx were estimated however, we estimated the levels of SOD, GR, CAT and MDA levels.

Basra Deniz Obay *et al* conducted a study to observe the antioxidant effect of ghrelin on pentylenetetrazole induced oxidative stress in rat. Pentylenetetrazole decreased the levels of reduced glutathione (GSH), CAT and SOD while treatment with ghrelin prevented the fall in their levels.[29] The results of the above mentioned studies are comparable to our study in which ghrelin administration improved the levels of the antioxidant enzymes after nicotine induced oxidative stress.

To summarize, our data showed that ghrelin treatment prevents nicotine induced oxidative stress and lipid peroxidation. This deduction can be used effectively to combat oxidative stress induced by pathological conditions. Smokers are at great risk of developing oxidative stress induced injury, and they may be benefited from recombinant ghrelin supplementation to prevent the damage.

CONCLUSION

Evident effect of ghrelin in attenuating nicotine induced oxidative damage illustrates that ghrelin exhibits antioxidant properties. Co-administration of ghrelin partially restored the antioxidant enzyme levels (SOD, CAT GPx with corresponding decline in lipid peroxidation.

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Address for Correspondence:

Dr. Hira Pervez Kiyani, Demonstrator of Physiology, Foundation University Medical College, Islamabad, Pakistan.
Cell: +92-303-55530000
Email: hira.kiyani@fui.edu.pk

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HPK: Conception and design, acquisition, analysis, interpretation of data and drafting of article
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