ORIGINAL ARTICLE

ANTIOXIDANT EFFECT OF GHRELIN ON NICOTINE-INDUCED RENAL TISSUE DAMAGE IN MICE

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Background: Ghrelin is an orexiogenic peptide released from stomach. It protects multiple organs of the body by its antioxidant and anti-inflammatory properties. Ghrelin combats oxidative stress by increasing antioxidant enzymes and decreasing lipid peroxidation. This study was done to determine the nephroprotective effect of ghrelin against nicotine induced renal tissue damage in BALB/c mice by estimating tissue antioxidant enzyme levels, namely catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and lipid peroxidation marker malondialdehyde (MDA). **Methods:** This randomized control trial was conducted at Foundation University School of Health Sciences, Islamabad in collaboration with National Institute of Health, Islamabad. Total of 27 male mice from NIH were randomly allocated into 3 groups. Group I (control) received 1 ml/Kg body weight saline, Group II (nicotine only) received 2.5 mg/Kg of body weight nicotine daily and Group III (nicotine plus ghrelin) received nicotine 2.5 mg/Kg body weight each day along with ghrelin 10 µg/Kg on alternate days for 29 days via intraperitoneal administration. Renal tissue sampling was done on 30th day for estimation of tissue oxidative stress markers through ELISA. **Results:** Nicotine administration led to significantly increased levels of CAT (p<0.001), SOD (p<0.001), GR (p<0.001), and decreased MDA (p<0.001) levels in group II. Administration of ghrelin along with nicotine in Group III resulted in significant restoration (p<0.001) of antioxidant enzymes (CAT, SOD, GR) and lipid peroxidation (MDA) (p<0.001) levels. **Conclusion:** Ghrelin acted as a potent antioxidant in nicotine induced oxidative stress in renal tissue suggesting its potential nephroprotective role.

Keywords: Antioxidants, Ghrelin, Lipid peroxidation, Oxidative stress

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INTRODUCTION

Ghrelin is derived from the word 'ghre' depicting Proto-Indo-European origin meaning 'grow' based on its growth hormone releasing ability.[1] Earlier, ghrelin had only scarce known functions, including stimulation of appetite and growth hormone release. Different studies now reveal that it is a multifaceted hormone having multiple actions on different body systems.[2] It scavenges reactive oxygen by increasing the activity of various antioxidants thus helping the body to combat various diseases.[3,4] In the renal tissue ghrelin exerts its protective effect by reducing oxidative stress. Ghrelin protects kidneys from ischemic reperfusion injury by abolishing oxidative stress, decreasing inflammation and preventing apoptosis.[1,2]

Oxidative stress is altered balance between generation and degradation of reactive oxygen species in cells or decreased capacity to detoxify free radicals. Reactive Oxygen Species (ROS) are generated as a by-product of different reactions.[5] In healthy individuals production and oxidation of ROS is balanced by internal mechanism of body. In stress or diseased state, these internal mechanisms are exhausted, disrupting the natural balance leading to oxidative stress.[6,7]

Anti-oxidant are substances which ablate the damaging effects of free radicals. They are endogenous or exogenous substances. Endogenous enzymatic antioxidants include catalase, superoxide dismutase, glutathione reductase. Exogenous antioxidants include vitamin, C, E, zinc and drugs like acetylcysteine.[8] Oxidative stress has clinical implication in various disorders like cancer, diabetes, atherosclerosis, cardiovascular, hepatic and renal disorders.[6]

Nicotine, member of Solanaceae family and a natural alkaloid, has been shown to increase the production of reactive oxygen species, decrease the cell survival in various tissues including renal, ultimately causing chronic kidney disease.[9] Nicotine causes tremendous fall in antioxidant enzymes along with increased lipid peroxidation damaging renal tissue.[9–11]

Treatment with ghrelin increased CAT, SOD and GSH levels and decreased MDA levels thus nullifying oxidative damage.[12] Renal tissue damage resulting from nicotine induced oxidative stress has been well documented in literature; however, role of ghrelin in alleviating this damage has been scarcely studied.

The current study was planned to determine the antioxidant effect of ghrelin against nicotine induced oxidative stress by determination of antioxidant enzymes (CAT, SOD and GR) and lipid peroxidation (MDA) in renal tissue of Balb/c mice.

MATERIAL AND METHODS

This randomized control trial was conducted at Foundation University Medical College in collaboration with NIH Islamabad. Resource equation[13–16] (n=DF/k+1) was used to calculate sample size keeping

20% attrition whereas DF=Degree of freedom, *k*= number of groups, and *n*=number of animals per group. Total of 27 healthy male Balb/c mice were divided in three groups of 9 each and kept in separate cages in animal house. Room temperature was kept at 22 °C with 12/12 hours light/dark cycle. Intra peritoneal injections were given to all mice.[17]

Control group (I) received normal saline 1 ml/Kg body weight intraperitoneal (IP) daily.[9] Nicotine group (II) was given nicotine 2.5 mg/Kg bodyweight IP daily to cause oxidative stress in renal tissue.[9] Nicotine+ghrelin group (III) received ghrelin 10 µg/Kg (IP) every other day and nicotine 2.5 mg/Kg body weight (IP) consecutively for total duration of 29 days.[18] Dissection was done on 30th day and kidneys were removed to measure antioxidant enzymes (CAT, SOD, GR) and lipid peroxidation (MDA) in the renal tissue.

SPSS-21 was used for evaluation of mean and standard deviation of CAT, SOD, GR and MDA levels. Evaluation of significant difference among all groups was tested by ANOVA followed by Post hoc Tukey's test and $p \leq 0.05$ was considered statistically significant.

RESULTS

Comparison of control group (I) and nicotine group (II) revealed that nicotine administration resulted in significant fall in CAT, SOD, GR and rise in MDA in renal tissue ($p<0.001$) in group-II. In group-III, ghrelin administration with nicotine lead to restoration of CAT, SOD, GR and MDA levels in renal tissue ($p<0.001$) in comparison with group-II. (Table-1, 2).

Table-1: Comparison of renal tissue CAT, GR, SOD and MDA levels in study groups

Variable	Group I	Group II	Group III	*p*
Tissue CAT ng/ml	3.54	2.37	3.27	<0.001*
Tissue GR pg/ml	35.77	11.87	29.12	<0.001*
Tissue SOD ng/ml	1458.33	626.79	1350.56	<0.001*
Tissue MDA ng/ml	7.13	8.55	7.21	<0.001*

Mean was used to express the results, ANOVA was used to compare means, *Statistically significant

Table-2: Pair-wise comparison of renal tissue CAT, GR, SOD and MDA levels in study groups (*p*-values of Post Hoc Tukey's HSD test)

Variable	Group I with II	Group II with III	Group I with III
Tissue CAT (ng/ml)	<0.001*	<0.001*	0.45
Tissue GR (pg/ml)	<0.001*	<0.001*	0.07
Tissue SOD (ng/ml)	<0.001*	<0.001*	0.09
Tissue MDA (ng/ml)	<0.001*	<0.001*	0.94

*Statistically significant

DISCUSSION

Smoking affects multiple organs of the body by increasing free radical generation. Tobacco smoke leads to kidney dysfunction due to increased oxidative stress in kidneys.[19] In the current study nicotine induced oxidative stress in mice was ameliorated by ghrelin administration, depicting its antioxidant effect. We found that administration of nicotine lead to fall in antioxidant enzyme levels (CAT, GR and SOD) and increased lipid peroxidation (MDA) in nicotine group, and ghrelin administration with nicotine lead to decrease oxidative stress evident as increased levels of endogenous antioxidants (CAT, GR and SOD) and decreased lipid peroxidation marker (MDA) in ghrelin treated group. Ghrelin administration protected the renal tissue by nullifying oxidative stress.

Products of lipid peroxidation (MDA) are potent markers used for the assessment of oxidative stress and their increased levels signify oxidative damage.[20] We observed that nicotine induced oxidative damage resulted in membrane damage manifested by increased MDA levels. Administration of ghrelin along with nicotine markedly decreased lipid peroxidation as was evident by decreased MDA levels. Bademci *et al*[21] in their study done on hepatic tissue of Sprague-Dawely rats showed protective effect of ghrelin on reducing oxidative stress and lipid peroxidation. It was concluded that ghrelin administration resulted in marked reduction in MDA levels in hepatic tissue thus protecting liver from oxidative injury.[21] Akki *et al*[22] observed that administration of ghrelin resulted in marked reduction in lipid peroxidation marker (MDA) in testicular tissue. Findings of that study showed that ghrelin lowers MDA levels acting as an antioxidant.

Our study revealed that ghrelin significantly increases antioxidant enzymes and decreases lipid peroxidation. Similar fact was reported in a study on male rats with normobaric hypoxia. It was observed that ghrelin at a dose of 80 µg/Kg/day for 2 days resulted in marked improvement in anti-oxidant defense in blood and brain of rats. Treatment with ghrelin resulted in fall in MDA levels and increased total antioxidant capacity in ghrelin treated group whereas no significant difference in levels of catalase, superoxide dismutase and glutathione peroxidase was found.[23] The difference in their results from our study is probably due to fact that kidney has higher levels of antioxidant enzyme system as compared to brain tissue. Brain tissue has decreased levels of superoxide dismutase and reduced activity of catalase which resulted in insignificant change in enzyme levels in brain tissue.[23,24] In our study due to higher levels of these anti-oxidant enzymes in kidneys, the levels of CAT, SOD and GR were increased significantly in renal tissue.

Our study depicted that ghrelin protects against oxidative damage caused by nicotine by increasing antioxidant enzyme levels (CAT, SOD, GR). Similar results were obtained in a study evaluating nephropective role of ghrelin in lipopolysachride induced renal damage. That study depicted marked improvement in renal function and oxidative stress in rats receiving ghrelin+lipopolysaccharide as compared to rats receiving lipopolysachharide only. The beneficial

effect of ghrelin on renal tissue was attributable to rise in levels of CAT, SOD and gluthion peroxidase and decrease in thiobarbituric acid reactive substances.[25] The findings of that study are in concordance with our study where ghrelin administration resulted in rise in anti-oxidant enzyme levels. The difference however lies in the point that in our project MDA was used instead of thiobarbituric acid reactive substances as lipid peroxidation marker.

The fall in lipid peroxidation marker and rise in antioxidant enzymes suggests significant role of ghrelin in alleviating renal oxidative stress caused by nicotine. Our study highlights the importance of ghrelin administration to prevent nicotine induced renal damage.

CONCLUSION

Ghrelin mitigates nicotine induced oxidative stress in renal tissue by increasing antioxidant enzymes (CAT, GR, SOD) and decreasing lipid peroxidation (MDA) thus acting as a potent renal antioxidant.

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AJ: Literature search, study design and concept, data collection, data analysis, data interpretation, drafting
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NA: Drafting, proof reading
HK: Acquisition and interpretation of data

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