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ORIGINAL ARTICLE

Ghrelin Ameliorates Nicotine induced Renal Damage in Balb/c mice: RCT

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ABSTRACT

Background: Kidneys are the major organs responsible for elimination of toxic metabolites from the body.
Aim: To determine role of ghrelin in mitigating nicotine induced renal damage in Balb/c mice by assessing serum creatinine levels, renal tissue MDA levels and renal tubular histological changes.
Methodology: It was a Randomized Control Trial. Healthy male BALB/c mice (n=27) through non probability convenience sampling were taken from NIH Islamabad and sorted into 3 groups (nine each). Group I was labeled as control group and given intraperitoneal normal saline for 29 days. Group II was labeled as nicotine group and received intraperitoneal nicotine for 29 days. Group III was labeled as ghrelin + nicotine and received intraperitoneal ghrelin on alternate days and nicotine daily for 29 days. On 30th day intracardiac sampling was done for estimation of serum creatinine. After intra cardiac sampling dissection was done to obtain renal tissue sample for lipid peroxidation and histological analysis. Data was evaluated by using SPSS version 23. One way ANOVA and post hoc tukey's test were applied.
Results: In group 2, nicotine administration markedly elevated serum creatinine and tissue MDA levels whereas ghrelin administration along with nicotine in group 3 restored serum creatinine and tissue MDA levels back to normal. Renal tubular changes caused by nicotine in group-2 are also mitigated by ghrelin and nicotine administration in group-3.
Practical Implication: Ghrelin a newly discovered peptide has shown to have anti-oxidant properties and nephro-protective effect thus has potential to reduce renal damage. It can restored creatinine levels thus minimizes renal damage.
Conclusion: It was concluded that ghrelin protects kidneys against nicotine induced renal damage by restoring serum creatinine, lipid peroxidation marker and histological changes.
Keywords: Ghrelin, Lipid Peroxidation, Nicotine, Nephro-Protection, Anti-Oxidant and MDA.

INTRODUCTION

Primary marker for kidney damage in humans and rodents is creatinine. Creatinine, a chief indicator of renal function is produced in the body from the breakdown of creatine and phosphocreatine. In the liver transamination of various amino acids like arginine, glycine and methionine yields creatinine. Creatinine circulates in the body and is converted into phosphocreatine in muscle. It is endogenously produced and freely filtered by glomeruli.[1] Two physiological processes that contribute to creatinine handling are glomerular filtration and secretion by proximal tubule[2].

Nicotine induced oxidative stress leads to increased free radical production thus damaging cellular membranes resulting in glomerular necrosis and tubular damage impairing filtration and excretion and thus increasing serum creatinine levels.[3,4] Nicotine is an alkaloid which has shown to cause renal injury by increasing renal oxidative stress resulting in damage to renal proximal tubule cells[5]**.** Kidney damage induced by nicotine is manifested by increased levels of creatinine accompanied by increased lipid peroxidation products melandialdehyde (MDA)[6,7].

Ghrelin a newly discovered peptide released mainly (60-70%) from gastric mucosa, small amounts are also released from hypothalamus, pituitary, lung and adrenal cortex.[8] Ghrelin has many physiological actions including secretion of growth hormone, promotion of the appetite signal, and stimulation of gastrointestinal activity. In addition, ghrelin exerts a powerful protective effect against damage of the heart, gastrointestinal tract, liver, nervous system, and kidney[9]. Ghrelin exerts its potential protective effects through inhibition of oxidative stress, modulation of inflammation and apoptosis.[10] Studies reveal its role as an anti-inflammatory, anti-oxidant, anti-apoptotic and anti-fibrotic in different tissues[11-15].

Ghrelin protects the cells against oxidative stress by decreasing reactive oxygen species. It combats oxidative injury by increasing expression of antioxidant enzymes and directly scavenging free radicals.[13] Oxidative stress particularly effect mitochondria because of absence of histone protein in its DNA thus it is readily broken down by reactive oxygen species. Renal proximal tubular cells contain large number of mitochondria and rely on energy produced by mitochondria. Oxidative stress induced mitochondrial injury lead to proximal tubular cell insult and dysfunction. Treatment with ghrelin has shown significant decrease in ROS production from mitochondria and increase in their number thus mitigating renal damage.[16] Oxidative stress induced renal injury caused by angiotensin has been shown to be reversed in rats, treated with ghrelin by increasing activity of antioxidant enzymes[16].

Another study showed protective effect of ghrelin in cisplantin induced renal damage in mice[17]. Due to lack of local data regarding this effect of ghrelin as well as literature search did not reveal role of ghrelin in nicotine induced renal damage for which current study was planned. Results of this study added local literature with nephro-protective effect of ghrelin but still more studies are recommended.

The objective of the study was to determine role of ghrelin in mitigating nicotine induced renal damage in Balb/c mice by assessing serum creatinine levels, renal tissue MDA levels and renal tubular histological changes.

METHODOLOGY

This study was a RCT conducted at the department of physiology foundation university school of health sciences, Islamabad and National Institute of Health Islamabad. Ethical review certificate was obtained prior to start of study from foundation university Islamabad. Study population was mice (n=27) through non probability convenience sampling. Mice were acclimatized for 1 week before the start of study and then divided into 3 groups. Group 1 was control group and received intraperitoneal normal saline daily at a dose of 1ml/kg for 4 weeks. Group 2 was nicotine group and received intraperitoneal nicotine daily at a dose of 2.5mg/kg for 4 weeks. Group 3 was ghrelin and nicotine group and received intraperitoneal nicotine daily at a dose of 2.5mg/kg and ghrelin on alternate day at a dose of 10µg/kg for 4 weeks.

After 4 weeks mice were anesthetized and intra-cardiac sampling was done. Sample was transferred to labeled bottles and then centrifuged for serum separation. In serum creatinine levels

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were checked by E selectra analyzer. After intra-cardiac sampling, dissection was carried out for removal of both kidneys. One kidney was put in tissue sample bottle containing 10% formaldehyde for histological examination. Renal tissue sample was preserved in 10% formaldehyde for 24 hours before slide preparation. The tissue slide sections were cut keeping 4-6µm thickness, followed by paraffin embedding and staining with hematoxylin and eosin dye. They were observed under light microscope for histological analysis and comparison among the 3 groups. Qualified histopathologist assessed the renal damage. Renal damage was assessed by examining 40 cortical fields (x100 magnification) in renal tubules. Tubular injury was defined as tubular dilatation, tubular atrophy and loss/sloughing of basement membrane.

Second kidney was placed in labeled sample bottles containing 25ml of PBS for lipid peroxidation analysis. Second kidney was grinded manually with mortar and pestle. After grinding, sample was homogenized with an electric whisk and then transferred to pre-labeled bottles for MDA analysis by ELISA.

Statistical analysis: Data will be entered and analyzed in SPSS version 23.0. Quantitative variables like serum creatinine and MDA levels were presented as mean and standard deviation. Qualitative variables were presented as frequency and percentage. ANOVA followed by post hoc tukey test which provided information about statistically significant difference between the groups with *p* value ≤0.05.

RESULTS

In renal tissue, levels of serum creatinine ($p<0.001$) between group means showed siginificant difference by One way ANOVA. Result of post hoc tukey test illustrated that nicotine administration led to increase in serum creatinine ($p<0.001$) in group-2 as compared to group-1. In group-3 with co-administration of ghrelin the levels of creatinine were lower in serum as compared to group-2. In group-1 and group-3 levels of serum creatinine ($p=0.97$) were not statistically significant as shown in figure-1.

Figure-1: Graphical representation of mean values for serum creatinine levels in group 1, 2 and 3.

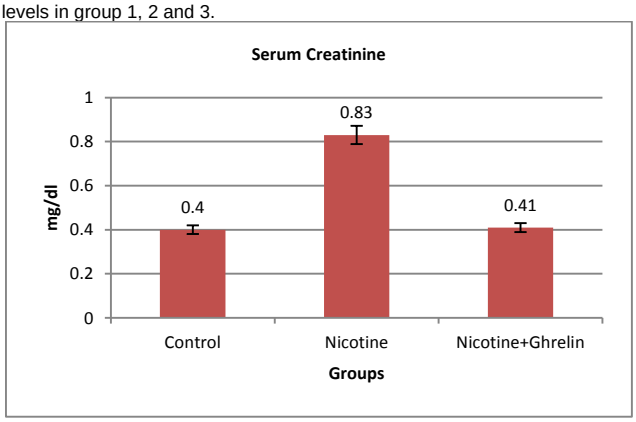


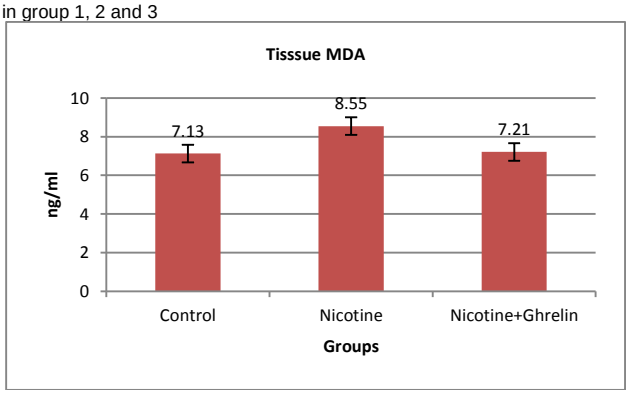
Table-1: Grading of renal tubular injury in group 1, 2 and 3

Grading of Tubular damage	Groups		
	Control group	Nicotine group	Nicotine plus ghrelin group
Grade 0	100%	-	33%
Grade 1	-	-	44%
Grade 2	-	-	22%
Grade 3	-	22%	-
Grade 4	-	44%	-
Grade 5	-	33%	-

MDA levels were increased significantly by nicotine administration and co-administration of ghrelin with nicotine restored levels of MDA in group-3. No significant difference in MDA levels between group-1 and group-3 as shown in figure-2.

Renal tubular injury was assessed by a grading system ranging from 0 to 5. Grade 0 was considered normal with no tubular injury and grade 5 showed severe renal tubular injury.

Figure-1: Graphical representation of mean values for TISSUE MDA levels in group 1, 2 and 3

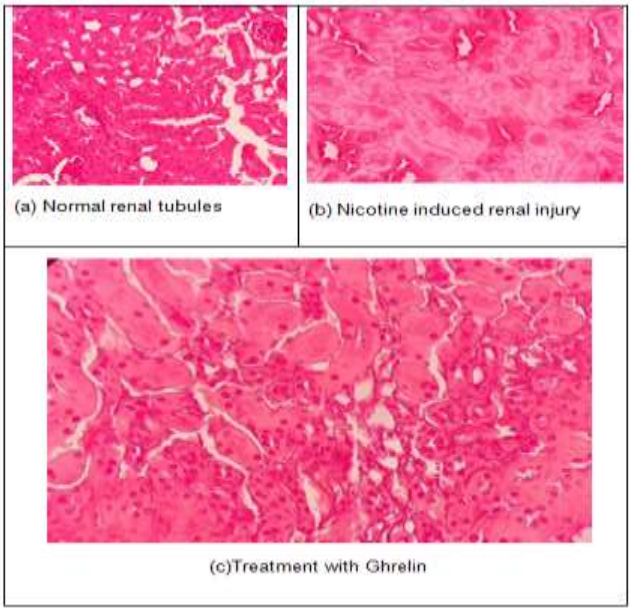


Control group (1): The control group showed no evidence of renal tubular injury. 9(100% mice (n=9) in control group exhibited grade 0 renal injury which is no tubular injury.

Nicotine group (2): Nicotine administration damaged the renal tubules as shown in table-1. Grade 5 injury was seen in 3(33%) of mice 4(44%) mice showed grade 4 renal damage and 2(22%) demonstrated grade 3 renal damage.

Nicotine plus ghrelin group (3): Ghrelin administration with nicotine prevented severe renal tubular injury. In group III, 3(33%) mice showed grade 0 tubular injury, 4(44%) had grade 1 tubular injury and 2(22%) exhibited grade 2 tubular injury. Hostological changes were shown in figure-3.

Figure 3: Histology of renal tubules (a) Normal renal tubules (b) Nicotine induced tubular damage (c) Treatment with ghrelin



DISCUSSION

Ghrelin is a small peptide having potent nephroprotective properties. It protects the kidneys by restoring renal function test, lipid peroxidation and renal tubular damage. Results of present study depicted that ghrelin administration in mice restored serum creatinine levels back to normal, ameliorated rise in MDA levels and mitigated tubular damage caused by nicotine.

Nicotine seemed to have exerted its effects on renal tissue by increasing the production of ROS, increasing lipid peroxidation and renal tubular damage causing deranged renal function test. Our study results depicted that nicotine administration resulted in increased lipid peroxidation (MDA) and serum creatinine levels in nicotine treated group and co-administration of ghrelin lowered lipid peroxidation marker (MDA) and restored creatinine back towards normal in ghrelin treated group. It not only restored the renal damage biochemical markers but also prevented histological damage as shown by reduction in tubular damage in group III.

MDA is final product of lipid peroxidation and its increased level denotes oxidative damage. Results of current study depicted that nicotine administration increases lipid peroxidation as evident by increased MDA levels in nicotine group. Administration of ghrelin restores MDA levels back to normal. In 2021, a study was done on liver of rats to explore role of ghrelin in cisplatin induced liver toxicity, Comparison between ghrelin and ghrelin + cisplatin group revealed that ghrelin admistration lowers MDA level and protects liver tissue from oxidative injury. Findings of this study are in line with our study that ghrelin treatment lowers MDA level[18].

Serhan *et al* in 2019 conducted a study on protective effect of ghrelin in rats with partial ureteral obstruction. Findings of this study showed ghrelin through its anti-inflammatory and anti-oxidant properties protect renal tissue. Ghrelin treatment resulted in lowering of lipid peroxidation as reflected in decrease in level of MDA marked and improvement in serum creatinine levels[19]. The results of this study are comparable to our study in which ghrelin administration decreased lipid peroxidation marker after nicotine induced oxidative stress in renal tissue.

A study was conducted on heaptic tissue of mice by Khordad Elnaz *et al.* in 2021 to evaluate hepatoprotective effect of ghrelin against cyclophosphamide induced hepatic damage. This study depicted marked improvement in MDA level and oxidative stress in mice receiving ghrelin+ cyclophosphamide as compared to rats receiving cyclophosphamide only. The beneficial effects of ghrelin on hepatic tissue are attributable to fall in MDA levels. [20] The results of this study are in concordance with our study where ghrelin administration resulted in fall in lipid peroxidation..

In the present study nicotine administration resulted in increased levels creatinine and renal tubular damage where co-administration of ghrelin reverted the creatinine and tubular changes back to normal thus attenuating nicotine induced renal damage.

In 2021, a study was done on to explore effect of ghrelin in doxorubicin induced nephropathy in rats. Treatment with ghrelin resulted in marked decline in serum creatinine levels. These findings are in accordance with our results depicting nephroprotective role of ghrelin by restoring renal function back towards normal.

In a study conducted by Ryo Takeda in 2006, ischemic/reperfusion induced renal failure caused damage to renal tubules and glomeruli, thus increasing renal injury score. Administration of ghrelin reverted the histologic changes in renal tubules back to normal by decreasing renal injury score.[21] These findings are similar to our study results.

In the nut shell, ghrelin mitigated changes in renal tissue caused by nicotine. Preparations of recombinant ghrelin may provide potential benefits to patients with chronic kidney disease. Smoking induced renal damage can be ameliorated by ghrelin administration.

Limitations of study: Single centre study with financial constrains, small sample size and limited resources.

CONCLUSIONS

It was concluded that ghrelin protects kidneys against nicotine induced renal damage by restoring serum creatinine, lipid peroxidation marker and histological changes.

Author's contribution: AJ&AJ: Overall supervision, write up and literature review, **MN&SR:** Statistics application, analysis literature review, help in write up, **ZS:** Literature review help in write-up.
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