DOI: https://doi.org/10.53350/pjmhs2023171171

ORIGINAL ARTICLE

Ghrelin: A Potent Nephro-protective Agent against Nicotine Induced Kidney Damage in Mice

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ABSTRACT

Background: Kidneys are the major organs responsible for elimination of toxic metabolites from the body. Apart from this kidneys play a vital role in maintaining body fluid and electrolyte balance, blood pressure regulation, red blood cells production and vitamin D activation. **Aim**: To evaluate role of ghrelin in ameliorating nicotine induced kidney damage by estimating renal function test and serum antioxidant enzymes in BALB/c mice.
Methodology: It was a Randomized Control Trial. Healthy male BALB/c mice (n=27) were taken from NIH Islamabad and sorted into 3 groups (nine each). Group I was labeled as control group and given intra-peritoneal normal saline for 29 days. Group II was labeled as nicotine group and received intra-peritoneal nicotine for 29 days. Group III was labeled as ghrelin + nicotine and received intra-peritoneal ghrelin on alternate days and nicotine daily for 29 days. On 30th day intra-cardiac sampling was done for estimation of renal function test and serum antioxidant enzyme levels. Data was evaluated by using SPSS version 23. One way ANOVA and post hoc tukey's test were applied. **Results:** In group II, nicotine administration led to decrease level of antioxidants enzymes and significant deterioration of renal function test in comparison with group I. In group III, administration of ghrelin along with nicotine prevented the fall in antioxidant enzymes and restored renal function test back to normal.
Practical Implication: Ghrelin a newly discovered peptide has shown to have anti-oxidant properties and thus has potential to reduce the onset and progression of different diseases. It can directly remove reactive oxygen species or can increase the levels of anti-oxidant enzymes thus nullifying oxidative damage.
Conclusion: It was concluded that ghrelin by virtue of its anti-oxidant properties protects against nicotine induced kidney damage as reflected through restoration of serum antioxidant enzymes and renal function test.
Keywords: Ghrelin, Oxidative Stress, Nicotine, Anti-oxidant Enzymes.

INTRODUCTION

Kidneys are the major organs responsible for elimination of toxic metabolites from the body. Apart from this kidneys play a vital role in maintaining body fluid and electrolyte balance, blood pressure regulation, red blood cells production and vitamin D activation. Partial cessation of kidney function results in deranged urea and creatinine levels depending on extent of viable renal tissue. After total loss of renal function in many species, death is reported within a week[1,2].

According to World Health Organization renal disease has been categorized as most neglected chronic disease[3]. It imparts major load on the economy of both developed and under developed countries[3]. Renal disease can result due to multiple risk factors including diabetes, hypertension, obesity, high cholesterol and smoking. Also, prolong and inappropriate use of certain medications like aspirin, ibuprofen, some herbal supplements and iodine contrast have a detrimental effect on renal tissue.[4] Deleterious effects of smoking on renal function occur not only in renal patients but also in healthy adults. In cigarette, more than 4000 components are present and among them highly toxic nitrogen containing alkaloid is nicotine which has been shown to cause kidney dysfunction[5,6].

Nicotine, a member of solanaceace family is the most commonly consumed psychoactive substance worldwide[7-9]. Main products containing nicotine are cigarettes, pipes, cigar, snuff tobacco, chewable tobacco and nicotine replacement therapies available in transdermal patches, nasal sprays, gums and tablets. In kidneys, nicotine by acting on cell surface nicotinic acetylcholine receptors stimulates NADPH oxidase, leading to increased level of reactive oxygen species (ROS) causing renal damage.[9,10] Nicotine administration also leads to decrease in levels of endogenous enzymatic antioxidants like SOD, CAT, GSH and increased lipid peroxidation markers (MDA). Decrease in antioxidant enzymes accompanied with increase in ROS levels causes' glomerular and tubular damage leading to inflammation and fibrosis of kidneys[8-12].

Ghrelin is a orixiogenic peptide released mainly from gastric mucosa. Majority of the ghrelin in circulation is thought to

Received on 17-08-2022
Accepted on 26-12-2022

secreted from stomach although small amounts are also secreted from other tissues like pituitary, lungs and breast.[13,14] Ghrelin has strong anti-oxidant effect in various organs of the body thus protecting them from oxidative damage. It scavenges reactive oxygen as well as reactive nitrogen species by increased levels of anti-oxidant enzymes and directly removing free radicals thus preventing multiple diseases[15,16]. Free radicals are the reactive compounds produced naturally in the human body. They have multiple deleterious effects on lipids, proteins and DNA. To combat these harmful effects body has a complex protective antioxidant system[17]. Ghrelin, a potent anti-oxidant, protects various organs of the body from oxidative damage by increasing the levels of anti-oxidant enzymes (catalase, superoxide dismutase and glutathione reductase)[15]. Oxidative stress induced by nicotine has been reversed by various substances having antioxidant properties like vit c, reverstal and *Menthe Spicata*[18-20]. Ghrelin a newly discovered peptide has shown to have anti-oxidant properties and thus has potential to reduce the onset and progression of different diseases. It can directly remove reactive oxygen species or can increase the levels of anti-oxidant enzymes thus nullifying oxidative damage. Due to the lack of local data regarding the role of ghrelin as anti-oxidant in minimizing nicotine induced renal pathology, we planned current study.

The objective of the study was to evaluate role of ghrelin in ameliorating nicotine induced kidney damage by estimating renal function test and serum antioxidant enzymes in BALB/c mice.

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.

Total of 27 BALB/c mice which were healthy and free of disease were included in the study. Mice that develop disease during study were excluded. They were acclimatized 1 week prior

to start of study. Mice were kept at animal house of NIH and given free access to water. They were fed with the diet ad libitum. Room temperature was maintained at 22±2 ºC. Mice were acclimatized 1 week prior to start of study and divided into 3 groups, each having 9 mice. Group 1 (Control group) received daily intraperitoneal normal saline at dose of 1ml/kg for 29 days[8]. Group 2 (nicotine only group) received daily 2.5mg/kg intraperitoneal nicotine for 29 days[8]. Group 3 (nicotine + ghrelin group received daily 2.5mg/kg intraperitoneal nicotine for 29 days and 10 µg/kg intraperitoneal ghrelin on alternate days for 29 days[21].

On 30th day, mice were anesthetized and intra cardiac sampling was done. This sample was transferred into 3.8% citrated vacutainers for plasma separation by centrifugation Samples were centrifuged at 4000 rpm for duration of 15 minutes. Serum was used for analysis of renal function test and antioxidant enzyme analysis. Renal function tests (urea and creatinine) were done on separated serum through auto analyzer (Selectra E Fully Automatic Chemistry Analyzer). To measure level of antioxidant enzymes in serum mouse GR, SOD and CAT kits (Glory science Co.) were used. Color changes were measured spectrophotometrically and absorbance was noted. Standard curves were used for analysis of antioxidant enzyme levels in the sample.

Statistical analysis: Data will be entered and analyzed in SPSS version 23.0. For continuous variables mean and standard deviation was calculated. For evaluation of significant difference among control and experimental groups one way analysis of variance (ANOVA) was applied on the data followed by post-hoc tukey's test. Difference in groups was considered significant with *p* value ≤0.05.

RESULTS

One way ANOVA revealed significant difference in serum urea and creatinine levels ($p<0.001$) between group means. Comparison of group II with group I by post hoc tukey's test depicted that nicotine administration resulted in increase in levels of serum urea and creatinine ($p<0.001$) in group II. Co administration of ghrelin and nicotine in group III lowered serum urea and creatinine levels ($p<0.001$) in comparison with group II. In group I and group III serum urea levels ($p=0.189$) and creatinine ($p=0.97$) were not different statistically. Summary of results obtained after nicotine and ghrelin administration on serum urea and creatinine levels was shown in table-1.

Comparison of group I with group II revealed statistically significant decrease in antioxidant enzymes and increase in lipid peroxidation in serum ($p<0.001$) of group II. In group III and group II comparison, administration of ghrelin and nicotine significantly restored levels of antioxidant enzymes and lipid peroxidation in serum ($p<0.001$) of group III as shown in table-2.

Table-1: Effect of nicotine and nicotine+ ghrelin administration on serum urea and creatinine levels

Variable	Groups			P value	P value		
	Group I	Group II	Group III		Control vs Nicotine	Nicotine vs Nicotine+ Ghrelin	Control vs Nicotine+ Ghrelin
Urea(mg/dl)	18.56±1.23	32.89±3.66	20.89±2.76	<0.001*	<0.001*	<0.001*	0.189
Creatinine(mg/dl)	0.40±0.09	0.83±0.12	0.41±0.11	<0.001*	<0.001*	<0.001*	0.975

*Statistically Significant.

Table-2: Effect of nicotine and nicotine+ ghrelin on serum levels of CAT, GR, SOD and MDA

Variable	Groups			*p*-value (ANOVA)	*p*-value (pairwaise Comparison)		
	Group I	Group II	Group III		Group I and II	Group II and III	Group I and III
CAT(ng/ml)	8.24±0.82	4.00±0.8	7.3±0.96	<0.001*	<0.001*	<0.001*	0.086
GR(ng/ml)	1965.27±118.18	1620.56±101.55	1912.07±188.07	<0.001*	<0.001*	<0.001*	0.578
SOD(ng/ml)	13001.93±128.48	8876.56±97.78	13072±82.77	<0.001*	<0.001*	<0.001*	0.294
MDA(ng/ml)	383.11±38.75	5959.11±140.06	466.56±74.46	<0.001*	<0.001*	<0.001*	0.167

*Statistically Significant

DISCUSSION

Ghrelin a newly discovered peptide has shown to have anti-oxidant properties and thus has potential to reduce the onset and progression of different diseases. It can directly remove reactive oxygen species or can increase the levels of anti-oxidant enzymes thus nullifying oxidative damage.[15] Current study showed that ghrelin improved renal function and protected kidneys of BALB/c from oxidative damage caused by nicotine due to its anti-oxidant properties by increasing expression of anti-oxidant enzymes.

In 2017, a study was done on human lens epithelial cells to explore the antioxidant properties of ghrelin. Oxidative injury caused by H_2O_2 resulted in marked decrease in levels of enzymatic anti-oxidants SOD and CAT and ghrelin administration restored anti-oxidant enzymes level back to normal[15]. Results of this study are in line with our study except for the difference in the reagent. They used H_2O_2 for oxidative damage and current study used nicotine.

In a rat seizure model, pentylenetetrazole induced oxidative stress resulted in decreased levels of anti-oxidant enzymes. Ghrelin administration alleviated the oxidative injury by increasing the levels of SOD, CAT and GSH. This study concluded that treatment with ghrelin increased the levels of antioxidant enzymes[22]. Results of this study are in line with our results that ghrelin alleviates oxidative stress by increasing levels of anti-oxidant enzymes.

In the present study nicotine administration resulted in increased levels of serum urea and creatinine, where co-administration of ghrelin reverted the serum urea and creatinine back to normal by restoring renal function and thus attenuating nicotine induced renal damage.

In mice with ischemic acute renal failure, treatment with ghrelin at a dose of 100ug/kg resulted in marked improvement in renal function as evident by lowered levels of serum urea and renal injury score[23]. These findings are in accordance with our results depicting renoprotective role of ghrelin by partially restoring renal function back towards normal.

In 2014, a study conducted on rat model of acute renal injury due to sepsis showed protective effect of ghrelin on kidneys. Cecal ligation and puncture (CLP) method was used to induce renal damage resulting in deranged renal function test (urea and creatinine). Administration of ghrelin markedly improved renal function[24]. The findings of this study support our results that ghrelin restored renal damage markers back to normal as comparison of group I with group III did not show any significant difference.

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

CONCLUSIONS

It was concluded that nephro-protective effect of ghrelin against nicotine induced kidney damage was due to its potent anti-oxidant properties. Administration of nicotine resulted in deranged renal function tests and decreased levels of serum antioxidant enzymes. Ghrelin co-administration improved renal function tests and restored antioxidant enzymes thus mitigating nicotine induced kidney damage.

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

Conflict of interest: None
Funding: None

REFERENCES

1. Finco DR. Chapter 17 - Kidney Function. In: Kaneko JJ, Harvey JW, Bruss ML, editors. Clinical Biochemistry of Domestic Animals (Fifth Edition). San Diego: Academic Press; 1997. p. 441-84.
2. Fraser SD, Blakeman T. Chronic kidney disease: identification and management in primary care. Pragmat Obs Res. 2016;7:21-32.
3. Luyckx VA, Tonelli M, Stanifer JW. The global burden of kidney disease and the sustainable development goals. Bull World Health Organ. 2018;96(6):414-22D.
4. Razmaria AA. JAMA PATIENT PAGE. Chronic Kidney Disease. JAMA. 2016;315(20):2248.
5. Salahshoor M, Mohamadian S, Kakabaraei S, Roshankhah S, Jalili C. Curcumin improves liver damage in male mice exposed to nicotine. J Tradit Complement Med. 2016;6(2):176-83.
6. Arany I, Grifoni S, Clark JS, Csongradi E, Maric C, Juncos LA. Chronic nicotine exposure exacerbates acute renal ischemic injury. Am J Physiol Renal Physiol. 2011;301(1):F125-33.
7. Siqueira LM, Committee On Substance USE, Prevention. Nicotine and Tobacco as Substances of Abuse in Children and Adolescents. Pediatrics. 2017;139(1).
8. Salahshoor MR, Roshankhah S, Motavalian V, Jalili C. Effect of Harmine on Nicotine-Induced Kidney Dysfunction in Male Mice. Int J Prev Med. 2019;10:97.
9. Zheng CM, Lee YH, Chiu IJ, Chiu YJ, Sung LC, Hsu YH, et al. Nicotine Causes Nephrotoxicity through the Induction of NLRP6 Inflammasome and Alpha7 Nicotinic Acetylcholine Receptor. Toxics. 2020;8(4).
10. Jain G, Jaimes EA. Nicotine signaling and progression of chronic kidney disease in smokers. Biochem Pharmacol. 2013;86(8):1215-23.
11. Sengupta B, Sahihi M, Dehkhodaei M, Kelly D, Arany I. Differential roles of 3-Hydroxyflavone and 7-Hydroxyflavone against nicotine-induced oxidative stress in rat renal proximal tubule cells. PLoS One. 2017;12(6):e0179777.
12. Zahran WE, Emam MA. Renoprotective effect of Spirulina platensis extract against nicotine-induced oxidative stress-mediated inflammation in rats. Phytomedicine. 2018;49:106-10.
13. Spiridon IA, Ciobanu DGA, Giusca SE, Caruntu ID. Ghrelin and its role in gastrointestinal tract tumors (Review). Mol Med Rep. 2021;24(3).
14. Akalu Y, Molla MD, Dessie G, Ayelign B. Physiological Effect of Ghrelin on Body Systems. Int J Endocrinol. 2020;2020:1385138.
15. Bai J, Yang F, Dong L, Zheng Y. Ghrelin Protects Human Lens Epithelial Cells against Oxidative Stress-Induced Damage. Oxid Med Cell Longev. 2017;2017:1910450.
16. Akki R, Raghay K, Errami M. Potentiality of ghrelin as antioxidant and protective agent. Redox Rep. 2021;26(1):71-9.
17. Finaud J, Lac G, Filaire E. Oxidative stress. Sports Med. 2006;36(4):327-58.
18. Hassan KA, Ahmed MA, Hassanein KM, Waly H. Ameliorating effect of vitamin C and selenium against nicotine induced oxidative stress and changes of p53 expression in pregnant albino rats. Journal of Advanced Veterinary and Animal Research. 2016;3(4):321-31.
19. Saad AB, Rjeibi I, Brahmi N, Elaloui E, Zouari N. Nicotine-induced oxidative stress, testis injury, AChE inhibition and brain damage alleviated by Mentha spicata. Inflammopharmacology. 2020;28(4):939-48.
20. Hall S, Dixit M, Arany I. Resveratrol Attenuates Nicotine-mediated Oxidative Injury by Inducing Manganese Superoxide Dismutase in Renal Proximal Tubule Cells. In Vivo. 2017;31(4):551-5.
21. Mao Y, Zhang S, Yu F, Li H, Guo C, Fan X. Ghrelin Attenuates Liver Fibrosis through Regulation of TGF-beta1 Expression and Autophagy. Int J Mol Sci. 2015;16(9):21911-30.
22. Obay BD, Tasdemir E, Tumer C, Bilgin H, Atmaca M. Dose dependent effects of ghrelin on pentylenetetrazole-induced oxidative stress in a rat seizure model. Peptides. 2008;29(3):448-55.
23. Takeda R, Nishimatsu H, Suzuki E, Satonaka H, Nagata D, Oba S, et al. Ghrelin improves renal function in mice with ischemic acute renal failure. J Am Soc Nephrol. 2006;17(1):113-21.
24. Khowailed A, Younan SM, Ashour H, Kamel AE, Sharawy N. Effects of ghrelin on sepsis-induced acute kidney injury: one step forward. Clin Exp Nephrol. 2015;19(3):419-26.