DOI: https://doi.org/10.53350/pjmhs2216283

ORIGINAL ARTICLE

Ameliorating effect of Ghrelin on nicotine induced oxidative stress in liver tissue morphology in BALB/c mice

HIRA PERVEZ KIYANI, SADIA AHSIN, MADIHA IMRAN, HIRA AYAZ
Department of Physiology, Foundation University, Islamabad, Pakistan
Correspondence to Dr. Hira Pervez Kiyani, Email: hirakiyani1@gmail.com Tel:+92-303-5553000

ABSTRACT

Background: Nicotine, a naturally occurring alkaloid is the predominant chemical among the constituents in cigarette smoke. Consumption forms include smoke (cigarettes, pipes and cigars) and smokeless tobacco (chewable tobacco).
Aim: To determine the effect of ghrelin in nicotine induced liver toxicity in BALB/c mice.
Study Design: Randomized Control Trial.
Methodology: Present study enrolled 90 male BALB/c mice Group I (control group) was given standardized laboratory diet and intraperitoneal injection (i.p) of normal saline. Group II was given standardized laboratory diet and nicotine at a dose of 2.5mg/ kg body weight (i.p), while Group III was given standardized laboratory diet plus nicotine a dose of 2.5mg/ kg body weight (i.p) along with ghrelin at a dose of 10µg/kg on alternate days for 4 weeks. On 30th day sampling was done for hepatic tissue oxidative stress enzymes (superoxide dismutase, glutathione reductase and catalase levels), and histology of liver tissue for assessment of hepatic tissue damage and recovery.
Results: Nicotine group showed evident hepatic damage with significant increase in liver oxidative stress markers. On histological examination, liver showed mild to moderate grade necro-inflammation. Administration of ghrelin partially restored the oxidative stress markers and inflammatory histological changes due to nicotine induced toxicity.
Conclusion: We concluded that Ghrelin appears to be hepatoprotective due to its antioxidant and anti-inflammatory properties.
Keywords: Nicotine, Ghrelin, Oxidative Stress, Antioxidant Enzymes and Necro-Inflammation.

INTRODUCTION

Cigarette smoking is the leading cause of increasing morbidity worldwide[1]. Nicotine, a naturally occurring alkaloid is the predominant chemical among the constituents in cigarette smoke. Consumption forms include smoke (cigarettes, pipes and cigars) and smokeless tobacco (chewable tobacco)[2]. It is also an essential part of insecticides, which can lead to accidental or deliberate poisoning. Nicotine affects many organs of our body like lungs, liver, heart, blood vessels, brain and urogenital organs by increasing the synthesis of Reactive Oxygen Species (ROS) and lipid peroxidation[3]. These ROS in turn initiate and promote oxidative damage. Smokers are at greater risk for cardiovascular diseases, respiratory disorders, cancer (lung, pancreas, liver, breast, bladder, oral, larynx, esophagus, stomach and kidney), peptic ulcers and gastroesophageal reflux disease (GERD), male impotence, infertility and hepatotoxicity[4]. In liver, nicotine causes necrosis and damages the cell membrane of hepatocytes leading to release of hepatic cytosomal enzyme into the serum[5].

Excessive production of ROS can pose a threat to cells by causing oxidative stress and lipid peroxidation which can lead to DNA/RNA damage, oxidation and inactivation of proteins (both structural and enzymatic)[6,7]. Liver is one of the major organ attacked by ROS[8]. In liver, the formation of metabolic products of nicotine by 5-hydroxylation further contributes to the generation of ROS.[9] Liver parenchymal cells (hepatocytes), *Kupffer* cells (macrophages), hepatic stellate cells and endothelial cells are all subjected to oxidative stress induced hepatocellular injury.

Ghrelin is a 28 amino acid peptide hormone Ghrelin is released primarily by the gastric "enteroendocrine cells" and "epsilon cells" of pancreas during hunger and starvation. Ghrelin levels rise to maximum before eating and decline after food intake[10]. It performs a wide array of functions, which includes its orexigenic effect, Growth Hormone-releasing effect, effect on energy metabolism and GI motility. In addition to these, ghrelin has shown to have anti- fibrotic, anti-inflammatory, anti-oxidant, and anti-apoptotic actions. Ghrelin also increases muscle mass and stimulates bone formation[11,12]. It acts as an antioxidant in liver by increasing the levels of antioxidant enzymes and preventing lipid peroxidation[13]. It also decreases the production of ROS in liver by decreasing the expression of inducible nitric oxide synthase and NF-κB[14,15].

Received on 23-08-2021
Accepted on 13-01-2022

The objective of the study was to determine the effect of ghrelin in nicotine induced liver toxicity in BALB/c mice.

METHODOLOGY

Present study was conducted at Foundation University Islamabad in collaboration with National Institute of Health. Ninety healthy male BALB/c mice of age 6-10 weeks weighing 25-40g were selected through non probability convenient sampling and sorted out into three groups of 30 mice each. Female, diabetic or mice weighing more than 40g were excluded from the study. Animals were kept at animal house a week prior to study for acclimatization to the environment and were given an environment of 22±2ºC room temperature and 12hr light/dark cycle. Group I (control group; n=30) received standard pellet diet and 0.65% normal saline 1ml/kg intraperitoneally (i.p), Group II (Nicotine only group; n=30) received pellet diet plus nicotine injection i.p at a dose of 2.5mg/kg b.w/day for 4 weeks. Group III (Nicotine plus ghrelin group; n=30) was given pellet diet, nicotine i.p injection at a dose of 2.5mg/kg b.w/day for 4 weeks and ghrelin at a dose of 10µg/kg b.w i.p on alternate days for 4 weeks. On 30th day, mice were sacrificed and liver tissue sampling was done for oxidative stress enzyme (Superoxide dismutase, Glutathione reductase and Catalase) in hepatic tissue. A sample of liver tissue was sent for histopathological analysis of hepatic tissue damage and recovery.
Statistical Analysis: Data was collected and entered by using SPSS 24. One way ANOVA applied for significant difference of means between the groups followed by post HOC Tukey test. P-value of ≤0.05 was taken as significant.

RESULTS

Group II showed evident hepatic damage with significant decrease in antioxidant enzymes levels (CAT, SOD and GR) in liver tissue ($p<0.001$ for CAT), ($p<0.001$ for SOD) and ($p<0.001$ for GR) as compared to group I as shown in table-1. Histological analysis of liver tissue showed mild to moderate grade of necrosis and inflammation in group II. The total score of the HAI index of each mice of nicotine group was between 4 and 11. Group III mice, receiving ghrelin along with nicotine, liver tissue antioxidant enzymes levels were significantly more as compared to group II (($p<0.001$ for each marker) as shown in table 1.

Histological analysis showed partial reversal of necro-inflammation (minimal to mild grade) HAI score between 0-4 (Table 2)

Table 1: Effects of nicotine and nicotine plus ghrelin on hepatic tissue levels

Variable	Group means ± SD			ANOVA P-value	Group-wise comparison using post-Hoc Tukey test		
	Control	Nicotine	Nicotine plus ghrelin		Control vs nicotine (I vs II)	Nicotine vs nicotine plus ghrelin (II vs III)	Control vs nicotine plus ghrelin (I vs III)
GR pg/ml	337.79±52.23	182.34±76.89	328.26±73.77	<0.001*	<0.001*	<0.001*	0.889
SOD ng/ml	1648.67±172.20	920.91±103.97	1526.52±180.88	<0.001*	<0.001*	<0.001*	0.110
CAT ng/ml	6.30±1.0	4.46±0.61	5.95±1.08	<0.001*	<0.001*	<0.001*	0.578

*Statistically significant

Table-2: Grading of necro-inflammation in liver by modified Hai index

Groups / Parameters	Control group	Nicotine group	Nicotine plus ghrelin group
1.Periportal or Periseptal Interface Hepatitis	Absent **(Score 0)-** 100%	Absent **(Score 0)-**42. % **Score 1**-21.4% **Score 2**-14.2% **Score 3**-21.4%	Absent **(Score 0)-**85.7% **Score 1**-14.2%
2.Confluent Necrosis	Absent (100%)- **Score 0**	Absent **(Score 0)-**35.7% **Score 2**-42.8% **Score 3**-14.2% **Score 4**-7.14%	Absent (Score **0**)-92.8% **Score 3**-7.2%
3.Focal Lytic Necrosis, Apoptosis and Focal Inflammation	Absent (**Score 0)-**78.5% **Score 1**-21.5%	Absent **(Score 0)-**7.14% **Score 2**-92.8%	Absent **(Score 0)-**35.7% **Score 1**-50% **Score 2**-14.2%
4.Portal Inflammation	Absent- **(Score 0)-**78% Mild **(Score 1)**-22%	Mild **(Score 1**)-21.4% Moderate (**Score 3**)-42.8% Marked **(Score 4)-**35.7%	Absent **(Score 0)-**35.7% Mild (**Score 1**)-35.7% Moderate **(Score 2)-**28.5%
Total score (range)	0-3	4-11	0-4
Grade	Minimal	Mild-moderate	Minimal-mild

Figure-1: a&b) Normal liver histology with hepatic lobules

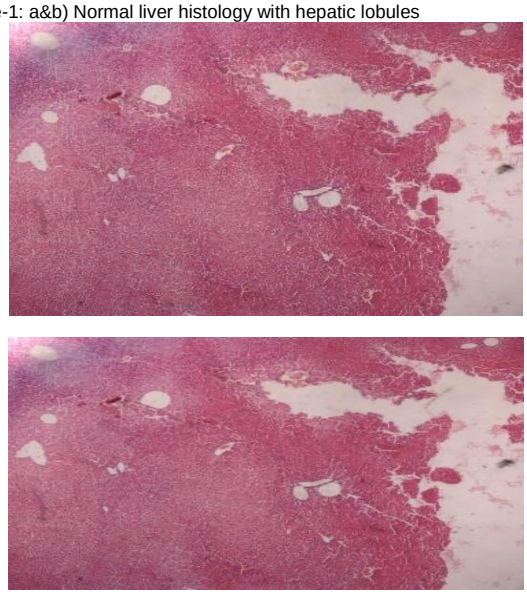
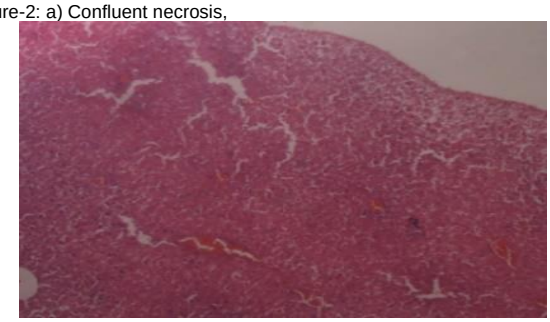
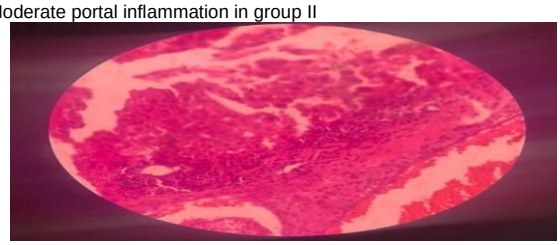


Figure-2: a) Confluent necrosis,



b) Moderate portal inflammation in group II



DISCUSSION

In our study, manifestations of oxidative stress were observed which included significant decline in the level of hepatic tissue antioxidant enzymes CAT, GR and SOD. Nicotine administration significantly lowered the levels of antioxidant enzymes in hepatic tissue as compared to the control group. Ghrelin co-administration with nicotine significantly ameliorated the decline in the antioxidant enzymes caused by nicotine. The rise in antioxidant enzymes by ghrelin reflects it protective role against oxidative stress as these enzymes catalyze the detoxification of ROS.[15]

In another study, the effect of ghrelin on antioxidant enzymes SOD and GPx in hepatic tissue of a male albino diabetic rat. He concluded that in diabetes, the levels of antioxidant enzymes are reduced while treatment with ghrelin significantly raised their levels[16].

Increase in the levels of antioxidant enzymes after ghrelin administration was also observed in a study carried out in 2013 in Egypt by Shereen M. Samir *et al*. The author studied the role of ghrelin in exhaustive exercise- induced oxidative stress in Sprague- Dawley rat brain and liver. It was concluded that pretreatment with ghrelin augments the antioxidant defense against exhaustive exercise-induced oxidative stress injury in rat's brain and liver by increasing the levels of reduced GSH and antioxidant enzymes CAT and SOD[17]. These results are in line with our study where ghrelin treatment seemed to have played role in maintenance of balance in ROS and antioxidant enzymes.

In our study, control group had normal morphology with no necrosis and inflammation. The nicotine group exhibited mild to moderate piecemeal necrosis as well as confluent necrosis of zone 3 in most areas along with occasional portal-central (P-C) bridging. There were 1-4 foci of focal lytic necrosis and focal inflammation per 10x and portal inflammation was moderate to marked in the nicotine only group. There was no periportal or periseptal interface hepatitis (piecemeal necrosis), no confluent necrosis, one or less focus of focal/ lytic necrosis and absent-mild portal inflammation in the nicotine plus ghrelin treated group. Our study proves that ghrelin does have a role in protecting liver from oxidative stress injury induced by nicotine.

In 2010, Masoumeh Golstan Jahromi *et al* studied the effect of ghrelin on acetaminophen induced liver injury. The authors observed the effect of ghrelin on only one parameter i.e confluent necrosis, which was induced by injecting acetaminophen. Confluent necrosis was not seen in the ghrelin treated group.[18] This result is however in congruence with our study where ghrelin prevented the development of confluent necrosis.

CONCLUSION

We concluded that Ghrelin appears to be hepatoprotective due to its antioxidant and anti-inflammatory properties. As co-administration of ghrelin partially restored the antioxidant enzyme levels with corresponding improvement in liver tissue histology.

Authors' Contribution: HPK&SA: Conceptualized the study, analyzed the data, and formulated the initial draft., **MI&HA:** Contributed to the histomorphological evaluation.

Acknowledgement: All authors are thankful to Allah Subhanao Taála, Mr Hussain Ali, animal house incharge at NIH National Institute of Health) Islamabad and Dr Faiza Kazi, Head of pathology department Foundation University Islamabad for their help and guidance during this study.

Limitations: Our study had limitations like financial constraints, lack of resources and lack of genetic workup.

Conflict of Interest: None to declare

Financial Disclosure: None

REFERENCES

1. Samet JM. Tobacco smoking: the leading cause of preventable disease worldwide. Thorac Surg Clin. 2013;23(2):103-12.
2. Mundel T. Nicotine: Sporting Friend or Foe? A Review of Athlete Use, Performance Consequences and Other Considerations. Sports Med. 2017;47(12):2497-506.
3. Khademi F, Totonchi H, Mohammadi N, Zare R, Zal F. Nicotine-Induced Oxidative Stress in Human Primary Endometrial Cells. Int J Toxicol. 2019;38(3):202-8.
4. El-Zayadi AR. Heavy smoking and liver. World J Gastroenterol. 2006;12(38):6098-101.
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. Nita M, Grzybowski A. The Role of the Reactive Oxygen Species and Oxidative Stress in the Pathomechanism of the Age-Related Ocular Diseases and Other Pathologies of the Anterior and Posterior Eye Segments in Adults. Oxid Med Cell Longev. 2016;2016:3164734.
7. He L, He T, Farrar S, Ji L, Liu T, Ma X. Antioxidants Maintain Cellular Redox Homeostasis by Elimination of Reactive Oxygen Species. Cell Physiol Biochem. 2017;44(2):532-53.
8. Sanchez-Valle V, Chavez-Tapia NC, Uribe M, Mendez-Sanchez N. Role of oxidative stress and molecular changes in liver fibrosis: a review. Curr Med Chem. 2012;19(28):4850-60.
9. Ateyya H, Nader MA, Attia GM, El-Sherbeeny NA. Influence of alpha-lipoic acid on nicotine-induced lung and liver damage in experimental rats. Can J Physiol Pharmacol. 2017;95(5):492-500.
10. Feng Y, Wang N, Ye X, Li H, Feng Y, Cheung F, et al. Hepatoprotective effect and its possible mechanism of Coptidis rhizoma aqueous extract on carbon tetrachloride-induced chronic liver hepatotoxicity in rats. J Ethnopharmacol. 2011;138(3):683-90.
11. Li S, Tan HY, Wang N, Zhang ZJ, Lao L, Wong CW, et al. The Role of Oxidative Stress and Antioxidants in Liver Diseases. Int J Mol Sci. 2015;16(11):26087-124.
12. Khatib N, Gaidhane S, Gaidhane AM, Khatib M, Simkhada P, Gode D, et al. Ghrelin: ghrelin as a regulatory Peptide in growth hormone secretion. J Clin Diagn Res. 2014;8(8):MC13-7.
13. Makris MC, Alexandrou A, Papatsoutsos EG, Malietzis G, Tsilimigras DI, Guerron AD, et al. Ghrelin and Obesity: Identifying Gaps and Dispelling Myths. A Reappraisal. In Vivo. 2017;31(6):1047-50.
14. Nagaya N, Uematsu M, Kojima M, Date Y, Nakazato M, Okumura H, et al. Elevated circulating level of ghrelin in cachexia associated with chronic heart failure: relationships between ghrelin and anabolic/catabolic factors. Circulation. 2001;104(17):2034-8.
15. Goodman ZD. Grading and staging systems for inflammation and fibrosis in chronic liver diseases. J Hepatol. 2007;47(4):598-607.
16. Pessione F, Ramond MJ, Njapoum C, Duchatelle V, Degott C, Erlinger S, et al. Cigarette smoking and hepatic lesions in patients with chronic hepatitis C. Hepatology. 2001;34(1):121-5.
17. Cetin E, Kanbur M, Cetin N, Eraslan G, Atasever A. Hepatoprotective effect of ghrelin on carbon tetrachloride-induced acute liver injury in rats. Regul Pept. 2011;171(1-3):1-5.
18. Golestan Jahromi M, Nabavizadeh F, Vahedian J, Nahrevanian H, Dehpour AR, Zare-Mehrjardi A. Protective effect of ghrelin on acetaminophen-induced liver injury in rat. Peptides. 2010;31(11):2114-7.