Hepatoprotective Potentials of Ghrelin and/or N-Acetyl Cysteine in Thioacetamide Induced Chronic Liver Injury in Rats

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Abstract

Background: Ghrelin is a gut hormone that has protective effects on many tissues' injury while its role in chronic liver injury (CLI) remains unclear as well as N-acetyl cysteine (NAC) has powerful health benefits derive from its ability to restore intracellular levels of glutathione (GSH), which is the body’s most powerful antioxidant.

Objective: This study was designed to detect the hepatoprotective potentials of ghrelin or NAC and their combination in thioacetamide (TAA) induced CLI in rats and the possible mechanisms involved.

Material and Methods: Forty male rats were divided into five groups: Group I: Control; Group II: Chronic liver injury (CLI); Group III (CLI+Ghrelin); Group IV: (CLI+NAC); Group V: (CLI+Ghrelin+NAC). Liver enzymes, albumin, bilirubin and tumor necrosis factor alpha (TNF-α) were measured in serum. Nitric oxide (NO), Malondialdehyde (MDA), glutathione peroxidase enzyme (GSH-Px) and hydroxyproline were assessed in the liver tissue. Histopathological examinations were also performed for detection of inflammation and fibrosis.

Results: Results showed that Ghrelin and NAC significantly decreased liver enzymes, MDA, NO metabolites, bilirubin, hydroxyproline and TNF-α levels with increased GSH-Px and albumin levels. Combination of Ghrelin and NAC leads to more improvement in all parameters reaching the control level. Furthermore histopathological examination showed that Ghrelin has more protective effect on CLI than NAC, while their combination showed the most protective effect.

Conclusion: These findings suggested that Ghrelin and NAC have promising hepatoprotective potential against TTA induced chronic liver injury by their anti-oxidant, anti-inflammatory and anti-fibrotic effects. As well as Ghrelin has more protective effect than NAC in histopathological examination.

Key Words: Chronic liver injury – Ghrelin – NAC – Oxidative stress – TNF-α – Fibrosis.

Introduction

THIOACETAMIDE (TAA) is a thiono-sulphur-containing compound, which has been frequently used in industry in the past. Administration of one dose of TAA leads to acute hepatic toxicity, while chronic exposure causes hepatic cirrhosis and possible development of liver tumors. After metabolic activation of TAA by cytochrome P450, TAA-intermediates and reactive oxygen species (ROS) can covalently bind to biologically important molecules and increase cellular oxidative stress, lipid peroxidation, and deplete glutathione [1]. Both necrosis and apoptosis appear in the process of cell death after TAA application [2].

Hepatic fibrosis is the result of the wound-healing response of the liver to repeated injury. After an acute liver injury (e.g., viral hepatitis), parenchymal cells regenerate and replace the necrotic or apoptotic cells. This process is associated with an inflammatory response and a limited deposition of extracellular matrix (ECM) proteins. If the hepatic injury persists, then eventually the liver regeneration fails, and hepatocytes are substituted with abundant ECM, including fibrillar collagen. The distribution of this fibrous material depends on the origin of the liver injury. As fibrotic liver diseases advance, disease progression from collagen bands to bridging fibrosis to frank cirrhosis occurs [3].

The influences of nitric oxide (NO) on hepatic injury are controversial, mainly derived from its diverse functions. NO may be protective or toxic at various concentrations. NO is generated from L-arginine by a reaction which is catalyzed by three different nitric oxide synthases (NOSs). The constitutive NO synthases are the endothelial NO synthase (eNOS) and the neuronal NO synthase. Both synthesize low amounts of NO and regulate
physiological NO homeostasis and cellular signaling. In contrast, the inducible form of NO synthase (iNOS) produces high amounts of NO induced by cytokines, such as interferon-gamma (INF-γ), tumor necrosis factor-alpha (TNF-α) or interleukin 1-alpha (IL-1α) [4].

So the mechanisms of CLI induced by TAA are oxidative stress, increase in cytokines as TNF-α that leads to increase in iNOS all these leads to increase in hydroxyproline as an index of fibrosis and disturbance of liver function in the form of increase of hepatic enzymes as aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) with increase in bilirubin level as a disturbance of excretory function of the liver and decrease in albumin level as a disturbance of secretory function of the liver.

Ghrelin is a gut hormone (28-amino-acids) firstly discovered as a potent growth hormone secretagogue. Moreover, plays a major role in the regulation of food intake. Recently, peripheral effects such as cytoprotection, vasodilatation and regulation of energy balance have been also attributed to ghrelin. The primary site of ghrelin synthesis is the stomach but ghrelin transcripts have been detected in many other organs including the liver, bowel, pancreas, kidneys, lungs, etc. [5].

Many studies have revealed that ghrelin may be an antioxidant and anti-inflammatory agent in many organs such as the rat ovary [6], erythrocytes, and brain of rats [7]. However, its role as a possible hepatoprotectant still remains unclear. Ghrelin has been reported to have several effects through nitric oxide (NO) modulation in other organs, such as ghrelin induced feeding by increasing nitric oxide synthase (eNOS) in the hypothalamus [8], and regulation of gastric acid secretion [9].

The use of antioxidants can minimize the oxidative stress and contribute to the therapeutic in cirrhotic livers. Among the various antioxidants N-acetylcysteine (NAC) is a glutathione precursor that increases glutathione levels in hepatocytes [10]. Increased glutathione levels, in turn, limit the production of reactive oxygen species (ROS) which cause hepatocellular injury [11].

NAC is a small molecule which is freely filterable and has prompt access to the intracellular compartments. This drug has a diversity of applications, largely because of the chemical properties of the thiol moiety present in its structure. The ability of the reduced thiol moiety to sweep reactive oxygen species is well-established with NAC [12]. In addition to this marked antioxidant capacity, it acts as an antidote for intoxication by acetaminophen, as well as in adult respiratory distress syndrome, cystic fibrosis and chronic bronchitis as a mucolytic agent [13].

Material and Methods

Animals:

This study was conducted on 40 adult male albino rats, weighing between 180 and 200g. Animals were fed a standard diet and housed in the animal laboratory at the medical research center at Benha Faculty of Medicine. All animals were acclimatized for 2 weeks prior to experimentation.

The animal experiments described below comply with the ethical principles and guidelines for the care and use of laboratory animals adopted by the Research Ethics Committee of the Faculty of Medicine, Benha University, Benha, Egypt.

Drugs and chemicals:

1- Thioacetamide was purchased from (Epico Co., Cairo, Egypt) in the form of light yellow powder supplied in a dark brown bottle. The calculated TAA dose was dissolved in hot saline and an injected volume of 1ml per rat was given i.p. Control rats were injected the same amount of saline only.

2- Ghrelin was purchased from (Sigma-Aldrich, St Louis, MO, USA) in the form of a white powder. The calculated dose was dissolved in distilled water; given subcutaneously (S.C.) with an injected volume of 1ml.

3- NAC: Was purchased from (Sigma Aldrich) in the form of White to light yellow cast powder. It was freshly prepared by dissolving in distilled water before use by heating. Was given i.p. in a volume of 1ml.

Groups of the experiment:

The animals were randomly divided into 5 groups each consisted of 8 rats as follow: Group (I): Control group rats were injected with saline (1ml i.p.) twice a week for 6 weeks. Group (II): CLI group rats received TAA (200mg/kg/i.p.) twice a week for 6 weeks [14]. Group (III): CLI+Ghrelin Group; CLI was induced by the same dose of TAA as Group II, plus rats received treatment of S.C. Ghrelin (10ng/kg/day) for 6weeks [15]. Group (IV): CLI+NAC. CLI was induced by the same dose of TAA as Group II, plus rats received NAC treatment of i.p. at a dose of 10mg/kg/day for 6 weeks [13]. Group (V): CLI+Ghrelin+NAC. CLI rats received combined treatment with Ghrelin and NAC.
Experimental procedure:
After the last treatment, rats were fasted for 12h and subjected to sodium thiopental anesthesia (40mg/kg, i.p.). The body weights of the animals in each group were determined. And the abdomen was opened medially. Then blood samples harvested from the heart. Blood samples were centrifuged (4000g, 15min) and Serum samples were used for the measurement of aspartate transferase (AST), alanine transferase (ALT), alkaline phosphatase (ALP), albumin and bilirubin as markers of hepatic injury; as well as the pro-inflammatory marker tumor necrosis factor-alpha (TNF-α) was measured. The liver from each animal was weighed for determination of the liver weight/body weight ratio. Also, liver middle lob tissue was fixed in a buffered formaldehyde solution (10%) and stained with Hematoxylin & Eosin (H & E) and Masson trichrome for histological analysis. Weighed samples of liver tissue (0.5g) were placed in 1.5ml microfuge tubes and homogenized using an electrical homogenizer [16]. The obtained rat liver homogenate was immediately frozen at −20 °C for biochemical measurement of reduced glutathione (GSH) content, malondialdehyde (MDA) concentration, hydroxyproline level and for determination of NO metabolites.

Liver index calculation:
Liver weight index was calculated according to Sahar El-Swefy, et al. [17] as follow: (Liver weight / Body weight x 100).

Assessment of liver function:
Serum AST, ALT ALP levels were measured clorimetrically according to Young [18], Murray [19] and Belfield and Goldberg [20] respectively. Determination of total bilirubin and albumin using commercially available colorimetric diagnostic kits according to manufacturers’ instructions [21].

Determination of tumor necrosis factor α by solid phase ELISA:
TNF-α was determined by the Quantikine Rat/Mouse Immunoassay ELISA commercial kit according to the manufacturer's instructions (Ray Biotech Inc., USA) Values were expressed as pg/ml [22].

Determination of NO metabolites:
Since NO is a very liable molecule its measurement in the biological sample is very difficult. In an aqueous solution, NO reacts with molecular oxygen and accumulates in the plasma as nitrite and nitrate ions. Therefore, the stable oxidation end products of NO, nitrite and nitrate, can be readily measured in biological fluids and have been used in vivo and in vitro as indicators of nitric oxide production. So homogenates tested for NO metabolites (nitrite and nitrate) with the Griess Micro Assay method [23].

Assessment of oxidative stress:
GSH-Px was measured according to [24] and the results were expressed as units/mg liver tissue. Lipid peroxidation contents (LPO) in the form of MDA level were measured by a modified method of [25] and the results were expressed as nmoles TBARS/mg liver tissue.

Hydroxyproline content assay for detection of fibrosis:
Hydroxyproline in tissue hydrolysates is a direct measure of the amount of collagen or gelatin present. A colorimetric assay was performed according to [26]. The Hydroxyproline content was expressed as micrograms per gram of wet liver.

Histopathological evaluation for detection of fibrosis:
The liver was dissected and fixed in 10% formalin solution at room temperature. All fields in each section were examined for congested central vein, vacuolated hepatocyte using H & E and fibrosis using Masson trichrome stains.

Statistical analysis:
Data are expressed as means±SE. Significance of results was set at \( p < 0.05 \). Comparison between different groups was carried out using one-way ANOVA followed by a Tukey-Kramer multiple comparisons test [27]. Statistical tests were carried out using GraphPad Instat software, version 3.10 (GraphPad Software Inc., San Diego, Calif., USA).
(p<0.001) in hydroxyproline in comparison to CLI that treated with NAC alone in group (IV). Combination of Ghrelin and NAC in group (V) caused significant decrease (p<0.05) in liver weight, liver weight index and hydroxyproline in comparison to group III and group IV.

**Table (1): Comparison of the effect of Ghrelin or NAC and their combination on body weight, liver weight, liver weight index and Hydroxyproline content in liver tissue among the experimental groups (mean ±SD).**

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (gm)</th>
<th>Liver weight (gm)</th>
<th>Liver weight index %</th>
<th>Hydroxyproline content in liver tissue (µg/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: Control</td>
<td>186.75±11.68</td>
<td>5±0.35</td>
<td>2.69±0.05</td>
<td>70.8±3</td>
</tr>
<tr>
<td>Group II: CLI</td>
<td>191±9.77</td>
<td>7.7±0.33 *</td>
<td>4±0.14 *</td>
<td>302.3±7.1 *</td>
</tr>
<tr>
<td>Group III: CLI+Ghrelin</td>
<td>196±4.69</td>
<td>5.8±0.27 #</td>
<td>2.98±0.14 #</td>
<td>105±5.3 #</td>
</tr>
<tr>
<td>Group IV: CLI+NAC</td>
<td>195±7.29</td>
<td>5.5±0.3 #</td>
<td>2.9±0.2 #</td>
<td>129±2 #</td>
</tr>
<tr>
<td>Group V: CLI+Ghrelin+NAC</td>
<td>191.9±7.7</td>
<td>4.9±0.3 # @&amp;</td>
<td>2.6±0.18 # @&amp;</td>
<td>76±3.3 # @&amp;</td>
</tr>
</tbody>
</table>

* : Significant difference (p<0.001) compared with normal control.
#: Significant difference (p<0.001) compared with CLI group.
@: Significant difference (p<0.001) compared with CLI+Ghrelin group.
&: Significant difference (p<0.05) compared with CLI+NAC.

**Effect of ghrelin or NAC or their combination on liver enzymes, bilirubin and albumin:**

Table (2) showed that administration of TAA in group (II) caused hepatocellular injury as evidenced by a significant increase (p<0.001) in ALT, AST, ALP and bilirubin levels and significant decrease (p<0.001) in albumin level compared to the Control Group (I). Treatment of CLI with Ghrelin or NAC or their combination as in groups (III), (IV) and (V) respectively caused significant decrease (p<0.001) in liver enzymes and bilirubin and significant increase in the serum albumin level (p<0.001) compared to the CLI untreated Group (II). Combination of Ghrelin and NAC in group (V) caused significant decrease (p<0.05) in all parameters in comparison to group III and group IV.

**Table (2): Comparison of the effect of Ghrelin on serum markers of liver tissue injury and secretory and synthetic functions of the liver among the experimental groups (mean ±SD).**

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>Total serum bilirubin (Mg/dl)</th>
<th>Albumin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: Control</td>
<td>77.5±2.95</td>
<td>99.6±3.3</td>
<td>132.3±2.93</td>
<td>0.85±0.03</td>
<td>3.89±0.34</td>
</tr>
<tr>
<td>Group II: CLI</td>
<td>143.36±3.09*</td>
<td>122.45±2.5*</td>
<td>225.2±10.5*</td>
<td>1.32±0.06*</td>
<td>2.79±0.26*</td>
</tr>
<tr>
<td>Group III: CLI+Ghrelin</td>
<td>90.32±3.77#</td>
<td>102.58±3.09#</td>
<td>175.7±3.88#</td>
<td>0.99±0.07#</td>
<td>4.03±0.13#</td>
</tr>
<tr>
<td>Group IV: CLI+NAC</td>
<td>88.3±3.4#</td>
<td>99.6±3.4#</td>
<td>175.3±4.5#</td>
<td>1.06±0.09#</td>
<td>4±0.18#</td>
</tr>
<tr>
<td>Group V: CLI+Ghrelin+NAC</td>
<td>79.5±2.9@&amp;</td>
<td>95.8±2.9@&amp;</td>
<td>140.3±5.5@&amp;</td>
<td>0.84±0.03@&amp;</td>
<td>4.02±0.05@</td>
</tr>
</tbody>
</table>

* : Significant difference (p<0.001) compared with normal control.
#: Significant difference (p<0.001) compared with CLI injury group.
@: Significant difference (p<0.001) compared with CLI+Ghrelin group.
&: Significant difference (p<0.001) compared with CLI+NAC.

**Effect of ghrelin or NAC or their combination on serum TNF-α and hepatic MDA, GSH-Px and NO metabolites:**

As shown in Table (3), administration of TAA in group (II) caused significant increase (p<0.001) in serum TNF-α, hepatic MDA and NO metabolites with significant decrease (p<0.001) in hepatic GSH-Px compared to the control group (I). Treatment of CLI with Ghrelin or NAC or their combination as in groups (III), (IV) and (V) respectively caused significant decrease (p<0.001) in serum TNF-α, hepatic MDA and NO metabolites with significant increase (p<0.001) in GSH-Px compared with CLI group (II). There were significant decrease in TNF-α and NO metabolites in CLI treated with Ghrelin (group III) by comparing it with CLI treated with NAC (group IV). Treatment of CLI with NAC (group IV) caused significant increase in GSH-Px when compared with CLI treated with Ghrelin (group III).
Table (3): Comparison of the effect of Ghrelin or NAC or their combination on serum TNF-α and hepatic MDA, GSH-Px and NO metabolites among the experimental groups. (mean±SD).

<table>
<thead>
<tr>
<th>Group</th>
<th>TNF-α (pg/dl)</th>
<th>MDA (nmol/g protein)</th>
<th>GSH-Px (U/mg protein)</th>
<th>NO Metabolites (µM/gr Wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: Control</td>
<td>44.34±3.38</td>
<td>3.36±0.09</td>
<td>22.62±0.37</td>
<td>142.59±3.77</td>
</tr>
<tr>
<td>Group II: CLI group</td>
<td>137.3±3.98*</td>
<td>5.41±0.12*</td>
<td>17.34±0.55*</td>
<td>207.8±8.76*</td>
</tr>
<tr>
<td>Group III: CLI+Ghrelin</td>
<td>77.51±3.05#</td>
<td>4.34±0.06#</td>
<td>20.6±0.23#</td>
<td>163.5±5.2#</td>
</tr>
<tr>
<td>Group IV: CLI+NAC</td>
<td>109.9±5.9#@</td>
<td>4.1±0.34#</td>
<td>21.6±0.93#</td>
<td>185.3±4.6#@</td>
</tr>
<tr>
<td>Group V: CLI+Ghrelin+NAC</td>
<td>57.6±3.46#@</td>
<td>3.3±0.08#@</td>
<td>22.2±0.44#@</td>
<td>137.9±4.86#@</td>
</tr>
</tbody>
</table>

*: Significant difference (p<0.001) compared with normal control.
#: Significant difference (p<0.001) compared with CLI group.
@: Significant difference (p<0.001) compared with CLI+Ghrelin group.
&: Significant difference (p<0.001) compared with CLI+NAC.

Histopathology:

Light microscopic evaluation of the Control Group (I) (Fig. 1) revealed a central vein (CV) with radially arranged hepatocytes (h) that appear polyhydral in shape with acidophilic cytoplasm and large rounded nuclei. The hepatic blood sinusoids (S) are seen as narrow spaces in between the hepatic cords. There was no collagen fiber proliferation by Masson’s Trichome stain as demonstrated in (Fig. 2). However, in the CLI Group (II) H & E (Fig. 3) showed severe congested central vein (CCV), vacuolated hepatocytes (vh) and edema. Masson’s Trichome stain showed severe fibrosis (Fig. 4). The ghrelin treated CLI group (III) showed mild congestion in central vein (cv), with few vacuolated hepatocyte (Fig. 5). There were very few collagen fibers and fibroblastic proliferation demonstrated by Masson’s Trichome in (Fig. 6). In addition, the NAC treated CLI Group (IV) showed moderate congestion in central vein (ccv), with moderate vacuolated hepatocyte. (Fig. 7) and Masson’s Trichome staining showed moderate fibrosis (Fig. 8). Furthermore, the ghrelin and NAC treated CLI Group (IV) showed no congestion in central vein (cv), with no vacuolated hepatocyte (Fig. 9). There were no collagen fibers and fibroblastic proliferation demonstrated by Masson’s Trichome stain in (Fig. 10).

Fig. (1): A photomicrograph of a section in liver of control (g1) showing a central vein (cv) with radially arranged hepatocytes (h) that appear polyhydral in shape with acidophilic cytoplasm and large rounded nuclei. The hepatic blood sinusoids (s) are seen as narrow spaces in between the hepatic cords. (H&E, X 630).

Fig. (2): A photomicrograph of a section in liver of control group (l) showing a group of hepatocytes (h) arranged in cords radiating from a central vein (cv). The hepatic blood sinusoids (s) are seen as narrow spaces in between the hepatic cords. (Masson trichrome stain, X 630).

Fig. (3): A photomicrograph of a section in liver of CLI (group II) showing severe congested central vein (ccv), vacuolated hepatocytes (vh) and edema. (H&E, X 630).

Fig. (4): Liver of rat in CLI (group II) showing marked fibrosis (fi) Masson’s Trichome X 630.
**Discussion**

There are no effective antifibrotic therapies for patients with liver diseases. The pathogenesis of liver fibrosis resulting from CLI depends mainly on oxidative stress and inflammation, in the present study Ghrelin and NAC were used as many studies revealed that they have antioxidant and anti-inflammatory effects. Moreover many studies showed that serum level of Ghrelin decreases in patients with liver fibrosis [28,34]. So this experimental study was performed to investigate whether ghrelin, and NAC can modulate liver fibrogenesis and CLI or not.

Thioacetamide was selected for the present study because in rats it produces the liver cirrhosis with similar histological appearances as found in...
liver cirrhosis of human beings and the intraperitoneal administration of thioacetamide was proved successful in producing cirrhosis within 6 weeks as reported by Lukivskaya et al., [14]. Thioacetamide biotransformation resulted in the formation of hydrogen peroxide (H$_2$O$_2$) which leads to liver injury with oxidative destruction [15].

In the current study CLI induced by administration of TAA caused hepatic dysfunction as evidenced by a significant increase in ALT, AST and ALP levels with disturbance in secretory function of the liver as evidenced by significant increase in bilirubin level and disturbance in synthetic function of the liver as evidenced by significant decrease in albumin level. These findings are similar to the results of Kantah et al., [29], Shaker et al., [30] and Mu-En Wang et al., [31] as they showed that Chronic TAA treatment effectively induced liver injury and dysfunction and caused hepatic fibrosis without damage to other organs. TAA is known to exert its hepatotoxicity through the generation of free radicals and oxidative stress. Free oxygen radicals initiate lipid peroxidation by receiving a hydrogen atom from polyunsaturated fatty acids, and, ultimately, H$_2$O$_2$ are generated. As a result of these reactions, cell membranes lose their viscosity, and membrane integrity deteriorates, leading to release of cell fractions to internal environment, and resulting in cell death. In addition, these released subcellular structures promote inflammation and aggravate the injury [32]. In cirrhotic animals, iNOS shows increased expression due to liver damage and the inflammatory mediators released as a result of it [15].

In the current study treatment of CLI with ghrelin leads to significant decrease in liver enzymes and bilirubin with increase in albumin. This can be explained by its significant decrease in lipid peroxidation by decreasing MDA level and significant increase in the antioxidant enzyme GSH-Px level in hepatic tissue as well as significant decrease in TNF-$\alpha$ and nitric oxide metabolites. This coincided with the results of Iseri et al., [13]; Shadi et al., [33]; Obay et al., [7]; Montserrat et al., [34] and Nashwa et al., [15] as they explained the hepatoprotective effect of Ghrelin by its effect in decreasing oxidative stress and, thus, lipid peroxidation. When free oxygen radicals begin to accumulate after toxic injury to the liver, Ghrelin stimulates hepatic cells to create a defense mechanism by increasing expression or activity of antioxidant enzymes such as catalase, myeloperoxidase, glutathione peroxidase and glutathione reductase.

GSH-Px is a key enzyme in the antioxidant defense system, and it acts by catalyzing the transformation of H$_2$O$_2$ into water, being dependent on reduced glutathione. It is depleted in the cirrhotic group. The group treated with NAC, the activity levels of this enzyme and MDA were found to be similar to those of control animals. This may be accounted by the fact that NAC serves as a substrate for glutathione, thus restoring the hepatic levels of this enzyme [35]. NAC acts as an antioxidant which effectively reduces ROS. It presents potent ability to interact directly with oxidant agents, acting as a scavenger of free radicals, and it exerts an indirect effect on the antioxidant mechanism, since it contributes to restore glutathione [36]. So NAC improved the liver function by its antioxidant effect as showed in our study.

The present work showed that CLI induced by administration of TAA in group (II) caused significant increase in serum TNF-$\alpha$ compared to the Control Group. These results were reversed by the treatment with Ghrelin and NAC. Our findings were in agreement with; Atef [37]; Suzy et al., [3] and Nashwa et al., [15] as they stated that the pro-inflammatory cytokine TNF-$\alpha$ was elevated in the cirrhotic liver of rats indicating a high inflammatory state. Kupffer cells are the phagocytic macrophages of the liver, when activated by hepatic toxicity release numerous signaling molecules, including a number of inflammatory cytokines as TNF-$\alpha$ and interleukins [38]. Previous findings indicated that ghrelin treatment ameliorated cholestasis induced chronic oxidative hepatic injury and fibrosis by inhibiting the release of TNF-$\alpha$, and in part by scavenging ROS [13], and ameliorated oxidative gastric damage by decreasing serum TNF-$\alpha$ levels [39]. Ghrelin acts on receptors on lymphocytes to produce anti-inflammatory effects, including reduction of circulating cytokines [40]. Other study explained the anti-inflammatory effect of ghrelin in colitis by its pharmacologic inhibition of iNOS [41]. As well as Galicia-Moreno et al., [42] reported that NAC prevents oxidative damage by decreasing TNF-$\alpha$.

In the current study although there were no significant changes in body weights in the different experimental groups, higher liver weights as well as liver body weight ratios had been observed in TAA-treated rats compared to rats in control groups. The enlargement of livers in TAA-treated rats signified hepatic lesions and liver injury associated with the toxic effects of thioacetamide. These significant changes in the liver weights may be attributed to the accumulation of collagen in liver tissue evidenced by significant increase in hepatic hydroxyproline level. These findings are similar to results of [43-45] who explained that in chronic
liver injury, inflammatory lymphocytes infiltrate the hepatic parenchyma. Some hepatocytes undergo apoptosis, and Kupffer cells activate, releasing fibrogenic mediators as transforming growth factors. Hepatic stellate cells (HSCs) proliferate and undergo a dramatic phenotypical activation, secreting large amounts of extracellular matrix (ECM) proteins. Lipid peroxidation has also been shown to stimulate collagen production in fibroblasts and HSCs [46]. However, the liver enlargement and liver weight index was significantly reduced in rats treated with Ghrelin and NAC as they caused significant decrease in collagen content in liver tissue evidenced by significant decrease in hepatic hydroxyproline. These results coincide with the results of [15,33,47].

The influences of NO on hepatic injury are controversial, mainly derived from its diverse functions. NO may be protective or toxic at various concentrations. Intrinsic hepatic NO generation attenuates sinusoidal perfusion failure, improves liver tissue oxygenation, andameliorates apoptotic liver damage [48]. In contrast, excessive NO production can be detrimental since it may downregulate cytochrome P450, suppress liver protein and DNA synthesis, thus induce apoptosis, necrosis and fibrosis [49]. NO is generated from L-arginine by a reaction which is catalyzed by three different nitric oxide synthases (NOSs). The constitutive NO synthases are the endothelial NO synthase (eNOS) and the neuronal NO synthase. Both synthesize low amounts of NO and regulate physiological NO homeostasis and cellular signaling. In contrast, the inducible form of NO synthase (iNOS) produces high amounts of NO induced by cytokines, such as interferon-gamma (INF-γ), tumor necrosis factor-alpha (TNF-α) or interleukin 1-alpha (IL-1 α) [4]. In the current study CLI caused significant increase in nitric oxide metabolites in hepatic tissue, we can explain that by the increase in the (iNOS) produces high amounts of NO while treatment with Ghrelin and NAC significantly decreased nitric oxide metabolites. This probably due to decrease the level of iNOS that produces large amount of NO.

In contrast to our results Mandana et al., [50] showed that liver injury induced by sodium valproate significantly decreases NO metabolites level in the liver tissue but ghrelin elevated it to the normal level. This may be due to the use of sodium valproate once a day for four consecutive days to induced acute liver injury.

The antifibrotic effect of ghrelin was explained by the decrease in collagen deposition evidenced by decrease in hepatic hydroxyproline level, liver weight and liver index. As well as by the histopathological examinations of the rat liver tissues, as there was marked decrease in collagen fiber proliferation by ghrelin treatment as recorded in (Fig. 6). Our study revealed that NAC has antifibrotic effect but it is significantly less than the antifibrotic effect of Ghrelin as there was significant decrease in hydroxyproline level than by NAC. As well as Ghrelin has more protective effect than NAC in histopathological examination.

Conclusion:
- Different mechanisms may explain the hepatoprotective effects of Ghrelin and NAC in the injured liver. First, Ghrelin and NAC have anti-inflammatory effect by decreasing TNF-α. Second, they decrease the extent of oxidative stress in the liver, which is a major pathogenic event in the fibrosis of chronic liver injury evidenced by significant decrease in MDA and significant increase in GSH-Px. Third; they reduce collagen synthesis evidenced by significant decrease in hepatic hydroxyproline level. Finally, they reduce the nitric oxide metabolites meaning that they may reduce NO synthesis by reducing iNOS.
- Ghrelin is superior in the anti-fibrotic effect than NAC as it has more anti-inflammatory and more ability to decrease nitric oxide metabolites.
- Combination of Ghrelin and NAC reach the control level.

Recommendations:
Our results suggest that Ghrelin and NAC combination could be useful in patients with CLI and liver fibrosis. Moreover, due to the orexigenic properties of ghrelin, ghrelin receptor antagonists have been recently proposed for the treatment of diabetes and obesity [51]. Due to its protective effects, prolonged blockade of ghrelin receptors may cause adverse effects such as accelerated tissue fibrosis, which is commonly seen in the liver, the heart and the kidney of patients with metabolic syndrome.

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References


الملخص العربي

خلفية: الجريلين هو هرمون من الأمعاء له تأثيرات واقية على إصابة العديد من الأنسجة بينما دوره في إصابة الكبد المزمنة لا يزال غير واضح. فضلاً عن أن (إن - أسبيتيل سيستين) له فوائد صحية قوية مستمدة من قدرته على استعادة مستويات الخلايا من الجلوكوتيون، وهو أقوى مضاد للأكسدة في الجسم.

الهدف: تم تصميم هذه الدراسة للكشف عن إمكانات هرمون جريلين أو إن - أسبيتيل سيستين والجمع بينهما في إصابة الكبد المزمنة المستفيدة باليوستاميد في الفئران والألياف المتميزة المتغيرة.


وأظهرت النتائج أن هرمون جريلين أو إن - أسبيتيل سيستين أدلى إلى إنخفاض بشكل ملحوظ في أنزيمات الكبد، أسبيتيل النتيروك، ماليديالد، أنتيم، جلوتاثيون بيكسيديز، وعامل نخر الدم. مع زيادة مستويات إن - أسبيتيل سيستين من الجريلين وأين - أسبيتيل سيستين يؤدي إلى تحسين في جميع المعايير للوصول إلى المستويات في المجموعة الضابطة. وعلاوة على ذلك، أظهر فحص الأنسجة أن الجريلين لديها أكثر تأثير وقابل عن إصابة الكبد المزمنة من إن - أسبيتيل سيستين، في حين أظهرت الفحوصات المتغيرة أبستيل سيستين، فضلاً عن أن الجريلين لديها أكثر تأثير وقابل من إن - أسبيتيل سيستين في فحص الأنسجة.

الخلاصة: هرمون جريلين أو إن - أسبيتيل سيستين لديهما إمكانات واضحة ضد إصابة الكبد المزمنة عن طريق تأثيرهم كمضاد للأكسدة.