Combination of Loperamide and Niacin modify DENA induced altered Liver enzymes

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ABSTRACT

Hepatocellular Carcinoma (HCC) is among the most lethal cancers which makes it the third most frequent cause of cancer related deaths. Diethylnitrosamine (DENA) is a potent initiator and hepatocarcinogen in rats. DENA induced Hepatocellular damage clearly demonstrates by the elevated levels of liver enzymes serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), and α-feto protein (AFP). This work is an attempt to test the hypothesis that Loperamide (5mg/kg) and Niacin in combination restores the DENA (160mg/kg) induced altered enzymes after single i.p administration in Wistar rats. The ability to alter the enzymes was measured by comparing biochemical serum markers and AFP. The results have confirmed the significant elevation of these parameters in DENA control group compared to normal control and the therapeutic groups. Therapeutic group significantly reveals that Loperamide and Niacin restores the altered hepatic enzymes towards the Normal. Key messages: Our data reveals and confirms that this remarkable combination possess the potential for the treatment of hepatocellular carcinomas in rats exposed to DENA. Administration of Loperamide + Niacin relatively improved the biochemical parameters to values approximating those of the normal controls.

Introduction

Hepatic injury in men is the fifth most frequently diagnosed cancer worldwide and the second most frequent cause of cancer death. In women, it is the seventh most commonly diagnosed condition and the sixth leading cause of cancer death. The distribution of liver injury is heterogeneous with a high prevalence seen in Asia and eighty percent of the burden is borne by countries in Asia and sub-Saharan Africa. The highest liver injury rate in the world is in China, according to the cancer registry reporting. Due to potential side effects and progressively increasing economic burden of currently available drugs and due to the strong resistance against standard chemopreventive drugs in treatment showed by hepatoma cells.

Diethylnitrosamine (DENA) an N-nitroso alkyl compound, is well established hepatic carcinogen and mutagen. It is very well used as a model to cause tumors in the GI tract, liver, skin and other organs. DENA causes cancer by inducing oxidative stress and generating free radicals. It activates microsomal enzymes such as the cytochrome P450 enzymes and causes mutagenesis in liver cells. Due to its mechanism of generating reactive oxygen species the use of some antioxidants, such as Niacin, may be benefit in reducing its toxicity caused by DENA. Loperamide an opiate agonists is same like morphine, buprenorphine, dynorphin A, and Etorphine, has been explored for the ability to produce apoptosis through the activation of specific membrane-bound opioid receptors. Loperamide has high affinity for the u subtype of the opioid receptor and one of the most used antidiarrheal drugs. Its effect on intestinal motility is well established. The apoptosis-inducing activity of Loperamide has also been reported by the researchers may be by promoting caspase 3 activity.

In this hypothesis we are trying to evaluate the liver function profile altered or restored by the synergistic activity by Loperamide and Niacin.

Material and methods

Drugs and chemicals

Loperamide and Niacin was provided as a gift sample from Dr. Firoz Anwar, Dean & Principal, Siddhartha Institute of Pharmacy, Dehradun; DENA was procured from Sigma–Aldrich Chemicals Co., St. Louis, USA and Chloroform and Diethyl ether from S.D. Fine Chem. Ltd., Mumbai, all the chemicals were of analytical grade.

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**Animals**
Adult, healthy, male Wistar albino rats weighing 100–125 g were procured in polypropylene cages in the animal house facility of Siddhartha Institute of Pharmacy for the present protocol under controlled conditions of temperature (22± 3°C) and light (14:10 h light and dark cycle) and provided with balanced pallet diet. The protocol was approved by the Institutional Animal Ethics Committee (IAEC) as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA); Ministry of Social Justice and Empowerment, Government of India.

**Experimental Design**
The rats (Wistar albino) were acclimatized and randomly divided into eight groups each having 6 rats for a 12 week study. Group-I rats served as normal control and were treated with saline orally. Group-II rats were administered a single dose of DENA, Group-III rats served as Loperamide control, Group-IV as Niacin control, Group-V as Loperamide and Niacin control, After 7th day of DENA (160 mg/kg) administration HCC was induced, Group-VI served as DENA and Loperamide control, Group-VII as DENA and Niacin control, Group-VIII served as a therapeutic group DENA + Loperamide + Niacin. The dose of Loperamide was selected as per the used dose in various researches in liver cancers and the treatment was started as soon as liver cancer was developed.

**Estimation of biochemical parameters**
Blood samples were collected on the termination day of the experiment from the retro-orbital plexus under light ether anesthesia without any anticoagulant and were allowed to stand for 30 min at room temperature, centrifuged at 2500 rpm for 10 min to separate the serum. Estimation of serum SGOT, SGPT, ALP, TC, TG, HDL and BIL was performed using standard kits (Nicholas India Pvt. Ltd.) with semi-auto analyzer (photometer 5010, Nicholas Pvt. Ltd.). Serum alpha-feto protein (AFP) was estimated by the method described by Premalatha and Sachdanandam (Julian et al., 2011).

**Statistical analysis**
Statistical analysis was carried out using Graph pad prism 5.0 (Graph pad software, San Diego, CA, USA). The results were expressed as Mean ± S.E.M. Statistical significance between more than two groups was tested using one-way ANOVA followed by Tukey’s multiple comparison tests. Values of p < 0.05 were regarded as significant.

**Results**

**Liver profile study**

**Serum glutamate pyruvate transaminase (SGPT/ALT)**
In DENA control group the SGPT levels were elevated significantly (p<0.001) as compare to Normal control group, DENA control group when compared to DENA + Loperamide control group reduced the elevated SGPT significantly (p<0.01) when compared to DENA + Niacin control which found to be slightly significant (p<0.05). Treatment with Loperamide in combination with Niacin decreased significantly (p<0.001) the elevated levels as compared to DENA control group (Table 1).

**Serum glutamate oxaloacetate transaminase (SGOT/AST)**
In DENA control group the SGOT levels were found to be increased significantly (p<0.001) as compare to Normal control animals. DENA control group when compared to DENA + Loperamide control group reduced the elevated SGOT significantly (p<0.01) and when compared to DENA + Niacin control it was slightly significant (p<0.05). Treatment with Loperamide in combination with Niacin decreased significantly (p<0.001) the elevated levels as compared to DENA control group (Table 1).

**Alkaline Phosphatase (ALP)**
ALP levels were significantly (p<0.001) increased in DENA control as compare to Normal control group. DENA control group when compared to DENA + Loperamide control group reduced the elevated ALP significantly (p<0.01) and when compared to DENA + Niacin control, the result obtained was not significant. Treatment with Loperamide in combination with Niacin decreased significantly (p<0.005) the elevated levels as compared to DENA control group (Table 1).

**High Density Lipoprotein (HDL)**
In DENA control group the HDL levels were elevated significantly (p<0.001) as compare to Normal control group, DENA control group when compared to DENA + Loperamide control group reduced the elevated HDL significantly (p<0.01) and when compared to DENA + Niacin control it was not significant. Treatment with Loperamide in combination with Niacin decreased significantly (p<0.001) the elevated levels as compared to DENA control group (Table 2).

**Bilirubin (BIL)**
In DENA control group the BIL levels were elevated significantly (p<0.001) as compare to Normal control group, DENA control group when compared to DENA + Loperamide control group reduced the elevated BIL significantly (p<0.001) and when compared to DENA + Niacin control it was not significant. Treatment with Loperamide in combination with Niacin decreased significantly (p<0.001) the elevated levels as compared to DENA control group (Table 2).

**Alfa Feto Protein (AFP)**
In DENA control group the AFP levels were elevated significantly (p<0.001) as compare to Normal control group, DENA control group when compared to DENA + Loperamide control group reduced the elevated HDL significantly (p<0.001) and when compared to DENA + Niacin control it was slightly significant (p<0.05). Treatment with Loperamide in combination with Niacin decreased significantly (p<0.001) the elevated levels as compared to DENA control group (Table 2).
Table no.1: Effect of Loperamide in combination with niacin on serum SGOT SGPT, ALP and TC level of animals

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>SGOT (mg/dl)</th>
<th>SGPT (mg/dl)</th>
<th>ALP (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>136.8±7.19</td>
<td>133±4.17</td>
<td>158±6.67</td>
</tr>
<tr>
<td>2</td>
<td>DENA control (imp)</td>
<td>270.4±9.92***</td>
<td>311.8±15.19***</td>
<td>306.4±7.40***</td>
</tr>
<tr>
<td>3</td>
<td>Loperamide control (imp)</td>
<td>210±9.92***</td>
<td>262±4.2***</td>
<td>175.6±5.53***</td>
</tr>
<tr>
<td>4</td>
<td>Niacin (nia) control</td>
<td>155±6.20***</td>
<td>145.0±12.91***</td>
<td>156.6±9.08***</td>
</tr>
<tr>
<td>5</td>
<td>Loperamide +nia control</td>
<td>224±7.2***</td>
<td>237.4±14.19*</td>
<td>157.0±6.17***</td>
</tr>
<tr>
<td>6</td>
<td>Loperamide + imp control</td>
<td>301±4.07***</td>
<td>269±17.01ns</td>
<td>224.0±19.21***</td>
</tr>
<tr>
<td>7</td>
<td>DENA+loperamide control</td>
<td>224±7.2***</td>
<td>237.4±14.19*</td>
<td>157.0±6.17***</td>
</tr>
<tr>
<td>8</td>
<td>Loperamide + imp control</td>
<td>301±4.07***</td>
<td>269±17.01ns</td>
<td>224.0±19.21***</td>
</tr>
</tbody>
</table>

Data showing comparison of serum SGOT SGPT, ALP and TC level of animals in normal control (NC), disease control (DC), and treated group.

Values are expressed in mean ± SEM. n=5 (#) Groups compared to normal control; (*) Groups compared to DENA control. ns – not significant; * (P<0.05); **(P<0.01); *** (P<0.001).

Table no.2: Effect of Loperamide in combination with niacin on serum BIL, HDL, and AFP level of animals

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>BIL (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>AFP (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>63.02±3.43</td>
<td>66.40±3.8</td>
<td>0.18±0.03</td>
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<tr>
<td>2</td>
<td>DENA control</td>
<td>136.3±3.72***</td>
<td>34.13±2.5</td>
<td>0.52±0.07***</td>
</tr>
<tr>
<td>3</td>
<td>Loperamide control (imp)</td>
<td>124.8±2.7***</td>
<td>53.60±2.9***</td>
<td>0.22±0.03***</td>
</tr>
<tr>
<td>4</td>
<td>Niacin (nia) control</td>
<td>69.4±3.72***</td>
<td>61.00±5.07***</td>
<td>0.10±0.03***</td>
</tr>
<tr>
<td>5</td>
<td>Loperamide +nia control</td>
<td>128.0±3.42***</td>
<td>60.80±2.26***</td>
<td>0.10±0.03***</td>
</tr>
<tr>
<td>6</td>
<td>Loperamide + imp control</td>
<td>125.6±5.06ns</td>
<td>37.71±10.1*</td>
<td>0.38±0.03ns</td>
</tr>
<tr>
<td>7</td>
<td>DENA+loperamide control</td>
<td>93.0±2.8***</td>
<td>36.20±2.28*</td>
<td>0.46±0.11ns</td>
</tr>
<tr>
<td>8</td>
<td>Loperamide + imp control</td>
<td>105.8±2.3***</td>
<td>44.44±2.82*</td>
<td>0.30±0.07*</td>
</tr>
</tbody>
</table>

Data showing comparison of serum TG, HDL, TB and AFP level of animals in normal control (NC), disease control (DC), and treated group.

Values are expressed in mean ± SEM. n=5 (#) Groups compared to normal control; (*) Groups compared to DENA control. ns – not significant; * (P<0.05); **(P<0.01); *** (P<0.001).

Discussion

Present study has been initiated to investigate loperamide and Niacin in combination play an important role to restore the DENA induced altered liver enzymes. DENA induced Hepatocellular damage clearly demonstrated that DENA significant (p<0.001) elevation in the levels of liver enzymes i.e. SGPT, SGOT, ALP and bilirubin and caused severe histopathological lesions in liver tissues. The elevated level of the liver enzymes may be due to sneaking from injured hepatocytes. [19, 19] SGOT also known as AST i.e. Aspartate Aminotransferase and SGPT also addressed as Alanine aminotransferase are the commonly used markers for the evaluation of any toxicity that has been induced to liver. These enzyme generally elevate and squeeze out of the liver cells during necrosis or hepatitis whether chronic hepatitis or cholestatis hepatitis. It has also been observed and established by the researchers that SGPT, SGOT, serum bilirubin level elevates significantly after DENA exposure in the experimental animals [19, 17]. In present study serum SGPT and SGOT levels elevated significantly (p<0.001) in all groups exposed to DENA as compared to the NC group. While the Therapeutic group loperamide with niacin SGOT and serum bilirubin levels brought towards the normal levels. These results confirms the role of loperamide with niacin as a successfull agent to counter DENA induced Hepatocellular carcinoma. ALP i.e. Alkaline Phosphotase is abundantly stored in the canaliculi of bile that is palace on the sinusoidal surface of liver cells. [20, 24] It is an enzyme which is located at the surface level...
closely bound to the membranes. Any toxicity to the liver cells causes the alteration of this enzyme by dissociating it from the membrane and its alteration extremely influence the permeability of membrane and likely to cause disarrangement in the expatriation of metabolites into the cells [21] while its increases level in serum to some extent mostly indicates some type of liver injury [17]. In present study serum ALP elevated significantly (p<0.001) in all groups exposed to DENA as compared to the NC group. While the Therapeutic group loperamide with niacin APL and brought towards the normal levels.

Previously the researchers has established that Plasma lipid metabolism are associated with hepatocellular carcinomas [20] alterations in lipid metabolism, affects cellular function and growth, further development of hepatocyte nodules in rat liver has been found with changes in lipid parameters and oxidative status [21]. Alteration in plasma lipid profile in malignant tissue are of important due to the effect on membrane integrity, fluidity and regulation of cellular process related to growth and cell survival. [22,23]. The present research concluded that therapeutic group (Loperamide + Niacin) maintained the lipid profile, hence it can be suggested that they may play the role in inhibition of carcinoma progression.

From the outcomes of the present research done on the experimental animals it was observed and concluded that Loperamide and Niacin restores the altered hepatic enzymes may be due to its antioxidant potential and apoptotic activity together.

Conclusion

With our results obtained, it is clearly observed that Loperamide and Niacin in combination act synergistically to alter the toxic effects of DENA in Wistar animals. Chemical parameters like SGPT, SGOT, AFP, ALP have been significantly restored to normal control. With the obtained result the hypothesis confirms that Loperamide (5 mg/kg) and niacin suppress the tumour lesions and decrease the biochemical markers which were elevated in liver injury induced by DENA.

Further, more comprehensive exploration of the combination is required to be done in terms of establishing the molecular mechanism and long term toxicity and adverse effect.

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Conflict of interest: We declare that we have no conflict of interest.

References
