A PROTECTIVE EFFECT OF CLOVE EXTRACTS ON DNA DAMAGE ASSESSMENT IN AN ETHANOL-TREATED RATS

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ABSTRACT:

Ethanol is a type of alcohol that consumes alcohol and is consumed by quite a few people and has many uses in different fields. Therefore, the aim of the research was to evaluate the effects of the genotoxicity of rats that were treated with ethanol alcohol by dosing them with this substance and for a certain period, using the alkaline comet assay based on measure the DNA damage that occurred in white blood cells (WBCs). The study was conducted on 40 Westerrats, and these rats were classified into four groups to study each group separately, the rats' ages ranged between (75_100) days, and their weights ranged between (140-180) gram per body weight. 10 rats treated with alcohol and (10) health controls were used in this study. DNA damage was significantly higher in ethanol-treated rats than in controls according to comet criteria. Thus, significantly higher levels of DNA damage were observed in rats treated with either ethanol (tail length 69.171 ±5.82, % Head DNA 30.829 ±5.82 and tail area1051 ±238.434) while lower in those treated with ethanol with cloves (tail length 26.8±38.13; Head DNA% 73.17± 8.13 and tail area450.51±22.39) compared to those treated with cloves only (tail length 8.780±0.720; Head DNA 91.057 ±0.727 % and tail area201.540±29.978) while the study was recorded as (tail length 8.943 ±0.727; Head DNA 90.104± 0.628 % and tail area207.667 ±30.989) for the control group. The present study concludes that exposure to ethanol is related to a high level of DNA damage as well as the results indicated that there could have been changes in the level of the comet test such as trying to detect DNA damage in rats treated with ethanol.

Keywords: Comet assay, Ethanol, Cloves, DNA damage

1. INTRODUCTION:

As pay more and more attention to the role of oxidative stress and the generation of reactive free radicals in ethanol-induced liver damage, so have studied various mechanisms by which ethanol can affect the formation of free radicals. Several studies are working on the production of reactive oxygen species (ROS) through ethanol-induced protein cytochrome P450 2E1 (CYP 2E1). Microsomal CYP 2E1 derived from ethanol-treated rats or human liver cancer-derived cell lines (HepG2) has a high ability to produce reactive oxygen intermediates such as superoxide radicals (O2.–) and hydrogen peroxide (H2O2)(1). Through the reactions of Fenton or Haber-Weiss s these reactive oxygen compounds subsequently lead to the formation of 1-hydroxyethyl (CH3CHOH) s radicals, followed by the formation of the hydroxyl radical catalyzed with iron (OH.)(4). Recent studies have shown that ethanol may exert its cytotoxicity through DNA damage, possibly due to the ROS generated by microsomal NADPH-dependent electron transfer and the oxidation of ethanol metabolite acetaldehyde (5). After acute use of ethanol, the number of single-strand breaks in rat brain cell DNA in vivo increased significantly (6); the combination of ethanol and acetaldehyde induced DNA lysis in rat hepatocytes (7) and ethanol-induced thymocytes were also observed DNA fragmentation and cell death (8). However,acetaldehyde induced both single- and double-strand breaks,whileethanol alone, did not induce in vitro DNA strand breaks in human lymphocytes, (9) indicating that ethanol can induce DNA strand breaks across its main metabolites. The destructive effect of acetaldehyde may be mediated by the formation of DNA cross-links (10) and the formation of acetaldehyde-protein adducts (11). The removal and repair of DNA cross-links may lead to DNA strand breaks. The aim of study was tracked the effects of acute and chronic doses of ethanol on the production of free radicals, and it was further tried to use Commet assay to correlate the production of free radicals with the evolution of DNA strand breaks.
II. MATERIALS AND METHODS:
The study was conducted on 40 Westerrats, and these rats were classified into four groups to study each group separately, the rats' ages ranged between (75_100) days, and their weights ranged between (140-180) gram per body weight, the experiment lasted about 30 days during the rats were administration. As the following groups, the first group, which include the control group, 10 rats, theses rats was given only distilled water without any other additives, while the second group also contained 10 rats that were orally administration with clove extract. The third group, which also consisted of 10 rats, were orally administration with 75% ethanol alcohol, and the remainder of the 10 rats fourth group, they were given orally 75% ethanol alcohol and then orally administration with clove extract 0.1 mg figure 1.

Blood samples were collected from all rats, and samples were coded to avoid the possibility of bias. 1 ml of s blood was drawn once from the heart of animals by heparin injection. Samples were immediately transferred on ice to the central scientific laboratory in the veterinary medicine college (12).

ethical approval sheet was performed from veterinary medicine college in university of Kerbala.

DNA damage analysis for all animal in this study (experimental and control) was done using the comet assay according to (13). Comet slides were prepared in duplicate per subject. Ten microliter of tissue grinding cells were mixed with 75 μl of 0.5% lowmelting point agarose. The mixture was cast into previously coated frosted slide with 0.5% normal melting point agarose and allowed to gel to be solidify. Then it were placed in cold lysis buffer (2.5 M NaCl, 10 mMTris-base, 100 mM disodium-EDTA, 1% Na-sarcocinat, 10% DMSO, 1% Triton X-100, pH 8) for 2 hour at 4°C before DNA was treated with alkaline solution (0.3 M mNaOH, 1 mM EDTA-NA2,pH 13.2) for 20 minutes to allow for unwinding of the DNA strands. Following, alkaline electrophoresis was run for 30 min at 300 mA and 24 volts, the DNA strands migrate toward the anode according to size.

The extent of migration depends on the number of strand breaks in the nucleoid. The electrophoresis slides were neutralized by washing twice for 5 min in neutralizing buffer (0.4 M Tris, pH 7.5) and once in water before dehydration in 100% ethanol for 20 min, and then it dried in 50°C for 30 min to be stored until use. For examination, stored slides rehydrated with chilled water for 30 min and it stained with 85 μl of ethidium bromide for 5 min and the slides washed to remove the excess of stain, covered with coverslip. The migration of damaged
DNA was visualized under a fluorescence microscope at 400x magnification, with a 450-490 nm emission filter and 515 excitation filter. The comet parameters (tail length, head DNA%, tail area) are then evaluated by a software. In this study total of 40 experimental rats were screened per subject (50 cells / each slide). Undamaged cells resemble an intact nucleus without a tail, while damaged cell has the appearance of a comet, its parameters resemble estimation the degree of DNA damage (14).

III. RESULTS AND DISCUSSION:

Alcohol, especially ethanol, is known to be a carcinogen and has many health risks, in the case of increased oxidative capacity [15], in this study the toxic effects on the DNA of ethanol were evaluated in rats administrated with doses of ethanol. The investigation was carried out by implementing a comet test. The level of DNA damage was determined as the percentage of cells with comets.

The results of comet assay of four groups were listed in table 1. Forty mice were studied, ten of which were orally administration with alcohol, ten of them with clove extract, ten of them with alcohol and then cloves, and 10 rats were given distilled water. In this study, a significant increase in comet assay in mice that DNA damage (Comet Area) was observed in rats given ethanol it was found significant increase of ethanol groups (1555.667 ± 88.895) rather than other groups as (1528 ± 135.011, 1523 ± 133.08 and 1543.51 ± 109.12) for Control, Cloves, and ethanol plus cloves, respectively. On the other hand it was found significant decrease of DNA in head in ethanol groups (30.829 ± 5.82) rather than other groups as (90.104 ± 0.628, 91.057 ± 0.727 and 73.17 ± 8.13) for Control, clove, and ethanol plus cloves, respectively. Regarding the last position (tail area), the results were significant differences our result was found to be more effect on the final portion of target DNA which was determine DNA damage, it was found 69.171 ± 5.82 in the ethanol group rather than other groups as (8.943 ± 0.727, 8.780 ± 0.720 and 26.8 ± 38.13 ), for control group, cloves, ethanol plus cloves, respectively, this result was agreement with (16) who was found an increased the DNA damage in vivo comet assay of acrylonitrile, 9-aminoacridine hydrochloride monohydrate and ethanol in rats.

The rats that were administration with ethanol had a significant effect in the comet test, as it statistically showed (P <0.05) an increase in DNA damage compared to the control group table 1. While the rats treated within the control group showed a significant increase (P <0.05) in the treatments compared to control group, which indicates DNA damage in rat have orally administration ethanol (17). The study was aimed the side effects of ethanol on the production of free radicals, and the comet assay was indicated that a correlate the production of free radicals by ethanol administration with the evolution of DNA strand breaks.

Table (1): Mean and Standard deviation in vitro assessment of DNA damage among rats groups.

<table>
<thead>
<tr>
<th>Treatment parameter</th>
<th>Control group</th>
<th>Cloves group</th>
<th>Ethanol</th>
<th>Ethanol plus cloves</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comet Area (px)</td>
<td>1528 ±135.01</td>
<td>1523±133.08</td>
<td>1555.667 ±88.895</td>
<td>1543.51± 109.12</td>
<td>0.047</td>
</tr>
<tr>
<td>%DNA in Head (%)</td>
<td>90.104±0.628</td>
<td>91.057±0.727</td>
<td>30.829±5.82</td>
<td>73.17± 8.13</td>
<td>0.036</td>
</tr>
<tr>
<td>Tail Area (px)</td>
<td>207.667±30.989</td>
<td>201.54±29.978</td>
<td>1051±238.43</td>
<td>450.51±22.39</td>
<td>0.038</td>
</tr>
<tr>
<td>%DNA in Tail (%)</td>
<td>8.943±0.727</td>
<td>8.780±0.720</td>
<td>69.171±5.82</td>
<td>26.8±38.13</td>
<td>0.027</td>
</tr>
</tbody>
</table>

IV. REFERENCES: