Assessment of the pharmacological effect of silymarin on ethanol-induced DNA damage by single-cell gel electrophoresis

Consumption of alcohol is part of the human lifestyle almost worldwide. Ethanol oxidation gives rise to the generation of free radicals, both in vitro and in vivo, and such events are associated with the induction of DNA damage. For example, the DNA strand breaks and modification of DNA bases occurs. Oxidative damage to DNA is of great importance because of the growing recognition that such damage can both initiate and promote carcinogenesis. Epidemiological studies show that the consumption of alcoholic beverage carries an associated increased risk of several cancers in humans, and this risk increases with increasing levels of alcohol consumption.

Silymarin is a standardized extract of flavonolignans (e.g., silybinin, isosilybinin, silydiianin and silychristin) and other minor compounds from *Silybum marianum* (L.) Gaertn. It is known to protect the liver against xenobiotic injury and to have a positive impact on the course of liver diseases. These compounds are consumed in substantial quantities by humans daily and are popularly used to treat alcoholics. Despite extensive research on the mechanisms by which it exerts its protective effects, the effect of silymarin on alcohol genotoxicity is still not known. Therefore, this study was conducted to evaluate the effect of silymarin on ethanol-induced DNA damage in rat lymphocytes using single-cell gel electrophoresis (comet assay).

Male albino Wistar rats (140–160 g) were purchased and housed in the Central Animal House, Rajah Muthiah Medical College, Annamalai University. They had free access to tap water and standard laboratory rat chow (M/s. Pranav Agro Industries Ltd., Bangalore, India). The experimental and animal-handling procedures were approved by the institutional animal ethics committee (IAEC). They were randomly divided into three groups (n = 8) and treated as follows:

- **Group 1:** Normal control.
- **Group 2:** Rats received ethanol as a 20% aqueous solution, prepared using absolute alcohol, twice a day (2.5 mL/100 g, body weight) at 9.00 AM and 5.00 PM for 60 days by intragastric infusion.
- **Group 3:** Rats were treated as group 2 and in addition were coadministered with silymarin for the last 30 days at the dose of 10 mg/kg, b.w., twice a day (because this dosage has been reported to offer maximum protection against various hepatotoxins).

This experimental model was designed to mimic the lifestyle of alcoholics who come to hospital with alcohol-related problems and have medication without avoiding alcohol (because of addiction).

After 60 days, the rats were allowed to fast overnight, anaesthetized by the intramuscular injection of ketamine hydrochloride (24 mg/kg), and were sacrificed by cervical decapitation. Blood was collected in a heparinized tube and the lymphocytes were separated immediately by Ficol density gradient centrifugation using Histopaque-1077 (Sigma Chemical Company, St. Louis, MO, USA). Isolated lymphocytes were suspended in phosphate-buffered saline (PBS). Salt (present in PBS) maintains the integrity of DNA by the repulsive electrostatic interactions between the negatively charged phosphate groups. Salt also shields this charge interaction and therefore stabilizes the duplex structure. The cell viability was measured by the trypan blue-exclusion method, and only the samples containing at least 95% viable lymphocytes were used for the experiments.

Alkaline single-cell gel electrophoresis was used to measure DNA damage (DNA strand breaks and base modifications) in freshly isolated lymphocytes. In short, lymphocytes were embedded in agarose on a microscope slide, and lysed with triton X-100 and 2.5 M NaCl to produce nucleoids. During electrophoresis, in alkaline condition (0.3 M NaOH and 1 mM EDTA, pH 13), the DNA moves towards the anode, and this is more significant if breaks are present (as supercoils in the tightly packed nucleoids are relaxed under this condition), which gives it the appearance as a comet. After staining with SYBR gold, 25 cells from each slide were observed with a fluorescent microscope. The tail length of the comet and the tail moment (% of DNA in tail × tail length) were determined using the image-analysis software, Komet 5.0 (Kinetics Imaging Ltd., Liverpool, UK).

The results were expressed as mean±SD for eight rats in each group. Statistical analysis was performed by one-way ANOVA followed by Duncan’s multiple range test (P<0.05). In this study, the extent of DNA damage was quantified by measuring the displacement of the genetic material between the cell nucleus (comet head) and the resulting ‘tail’. When the alkaline single-cell gel electrophoresis was introduced, the tail length was used as an index of DNA damage. However, the introduction of computerized image analysis made it possible to calculate other features (such as tail moment) of the comet image as well, thereby providing better descriptions of the overall DNA damage. The significantly increased tail length and tail moment in alcohol-administered rats [Figure 1] clearly shows the relation between alcohol consumption and DNA damage. These results are in correlation with many human...
Figure 1. DNA damage in experimental rat lymphocytes expressed as tail length (µm) and tail moment (arbitrary units = % tail DNA x tail length).

Values not sharing a common superscript differ significantly at P<0.05.

and animal studies. But, some human studies reported that alcohol consumption had no effect on DNA damage. The reason for this discrepancy may be due to the difference in the amount or type of alcoholic beverage consumed and the nutritional status of the alcoholics. Coadministration of silymarin significantly decreased the DNA damage (group 3) when compared with alcoholic rats (group 2). The treatment reversed the DNA damage to near normal levels (group 3) despite continued alcohol administration. Cessation of alcohol administration during the treatment period and/or the expansion of treatment period might provide better protection. The protective effect of silymarin can be explained by the scavenging of free radicals before they cause damage to nuclear DNA. In this context, Yu and Anderson[8] have shown the ability of silymarin to protect the DNA against reactive oxygen species-induced damage.

On the basis of this study, we suggest that silymarin is useful to treat alcoholic DNA damage, in addition to alcoholic liver injury. This report is likely to advance/add to the existing knowledge in relation to the treatment of alcoholics. Further, more clinical investigations are needed in human volunteers.

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