

Histopathological Studies of Hepatoprotective Effect of *Cassia Auriculata* in Ethanol Induced Albino Rats

Aarthi BL¹, Sendhilvadivu M²

¹Department of Zoology Queen Mary's College, Chennai 600 004, India

²Assistant Professor, Department of Zoology Queen Mary's College, Chennai 600 004, India

Abstract: *Cassia auriculata* is used against liver toxicity. Present study is to investigate the efficacy of *Cassia auriculata* in ethanol induced albino rats to identify and develop therapeutic strategies to prevent disease progression. Histopathological study was done to prove the efficacy of plant extract *Cassia auriculata*.

Keywords: *Cassia auriculata*, histopathology, albino rats, ethanol, liver, hepatoprotective

1. Introduction

During early stages of ALD, alcohol consumption diverts metabolic pathways toward hepatic triglyceride accumulation^[1]. Lipid accumulation leads to increased dearrangement of metabolic function and increases hepatic sensitivity to toxins [6]. Metabolic disturbances also contribute to impaired nutrient absorption and distribution, and the effects that are dependent on both amount of alcohol consumed and patterns of alcohol consumption.

Chronic long term alcohol consumption and metabolism is associated with metabolic rearrangements and changes in nicotinamide adenine dinucleotide (NAD/NADH) ratios favouring accumulation of reducing equivalents in the liver NADH. Changes in NAD/NADH ratio contribute to hepatic accumulation of triglycerides and depress the citric acid cycle. Alcohol also affects mitochondrial membrane function, metabolic demand, and generation of reactive oxygen species (ROS) a factor exacerbated by alcohol-dependent induction of cytochrome. The toxic responses and injury associated with acute/binge drinking are mediated by amount and rate of alcohol consumption. During acute alcohol consumption, the majority of alcohol is metabolized by successive oxidation reaction, first via alcohol dehydrogenase (ADH) to acetaldehyde, which is in turn oxidized by acetaldehyde dehydrogenase (ALDH) to acetate and water^{[1] [2] [3] [4] [5]}. Acetaldehyde is a toxic intermediate that is usually processed efficiently to prevent accumulation. However chronic long term alcohol use leads to CYP2E1 enzyme induction, which also generates acetaldehyde a first metabolite during alcohol oxidation^{[1] [7] [8] [9]}. In addition CYP2E1 is associated with increased alcohol tolerance observed in individuals that chronically abuse alcohol and plays a significant role in activation of pro carcinogens to carcinogens^{[7] [8] [9]}.

In addition to changes in hepatic parenchymal cells (hepatocytes) physiology and function, human ALD is characterized by a state of chronic hepatic inflammation. Alcohol leads to alteration in the gastrointestinal mucosa by disrupting epithelial tight junctions allowing for bacterial endotoxin translocation into the liver via the portal vein^[10].

Hepatic response to gut derived endotoxin is the critical step in the development of ALD. Presence of LPS in the liver activates innate immune responses primarily via Kupffer cell sensitization^{[10] [11]} leading to intrahepatic inflammation and the production of Tumour necrosis factor a (TNFa) and other pro inflammatory cytokines^{[10] [12]}. Additionally innate immune response signalling is a mediator of tissue and organ homeostasis regulating proliferation and apoptosis of intestinal epithelial cells and modulating liver regeneration after loss of liver mass^[13]. Abberent regulation of immune system signaling may trigger harmful inflammatory responses that contributes to tissue and organ injuries, fibrosis and carcinogenesis. Collectively these cytokine cascades combine to orchestrate liver injury, largely through neutrophil recruitment, although other inflammatory cell types natural killer T lymphocytes and dendritic cells also become activated, each serving unique roles in liver injury, repair and remodeling^[12]. Finally prolonged alcohol induced changes to liver function, hepatic circulation and immune responses leads to hepatic cell activation from a quiescent lipid or vitamin A storing phenotype, to a pro mitogenic collagen producing state. Initially collagen deposition is localized to perivenular and pericellular regions, resulting in portal tract septal fibrosis that surrounds apoptotic or necrotic hepatocytes and the eventual formation of fibrous septae and scar tissue that encompasses regenerative hepatocytes.

2. Methodology

2.1 Ethanol-induced hepatotoxicity

Albino rats (180 gm) were purchased. The rats were divided into 3 different groups. The animals were grouped into Control (Group A) and experimental groups (B and C) consisting of two albino rats each. A pre weight measurement of the albino rats was taken during the period of acclimatization and fed with laboratory pellet feed and water ad libitum. They were housed in well ventilated labelled cages at the site of the experiment.

The weight of the rats was measured before the administration of alcohol (ethanol) and before they were

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sacrificed, their weight was measured accordingly. The administration of ethanol was performed through oral route.

2.2 Procurement of Herbal extract

The dried-powdered flower extract of *Cassia auriculata* was procured commercially from KCB drug store, Triplicane, Chennai.



3. Experimental Design

Group A (Control)	Group B	Group C
Received normal feed and water daily for 20 days	Received 5ml of Ethanol with normal feed and water daily	Received 50mg/body weight of <i>Cassia auriculata</i> extract after 20 days of ethanol exposure with normal feed and water daily

3.1 Sample Collection

For the collection of blood sample to analyse the pathological biochemistry, the rats were first subjected to light anesthesia using chloroform and sacrificed.

3.2 Histopathological Analysis

A portion of the liver was cut into fine pieces of approximately 6mm size and fixed in phosphate-buffered 10% formaldehyde solution. After embedding in paraffin wax, thin sections of 5mm thickness were cut and stained with haematoxylin-eosin dye^[14]. The thin sections of liver were made into permanent slides and examined under high-resolution microscope with photographic facility and photomicrographs were taken.

Microscopic analysis of cells and tissues requires the preparation of very thin, high quality sections mounted on glass slides and appropriately stained to demonstrate normal and abnormal conditions. Most fresh tissue is very delicate and easily distorted and damaged, and it thus impossible to prepare thin sections from it unless it is chemically preserved or fixed and supported in some way whilst it is being cut. Broadly there are two strategies that can be employed to provide this support.

- 1) We can freeze the tissue and keep it frozen while we cut our section. These sections are called frozen sections.
- 2) Alternatively we can infiltrate our tissue specimen with a liquid agent that can subsequently be converted into a solid that has appropriate physical properties which will

allow thin sections to be cut from it. Paraffin wax is such an agent. This produces paraffin sections.

After that it undergoes Fixation, Dehydration, Clearing, Wax infiltration, Embedding

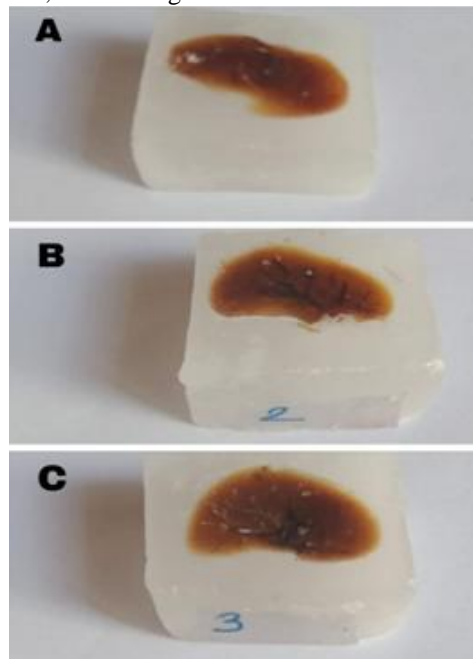


Plate 1: Histological Section of liver embedded in paraffin wax

[Control (A), Alcohol Induced Rat (B), Treated With *Cassia Auriculata* (C)]

4. Results and Discussion

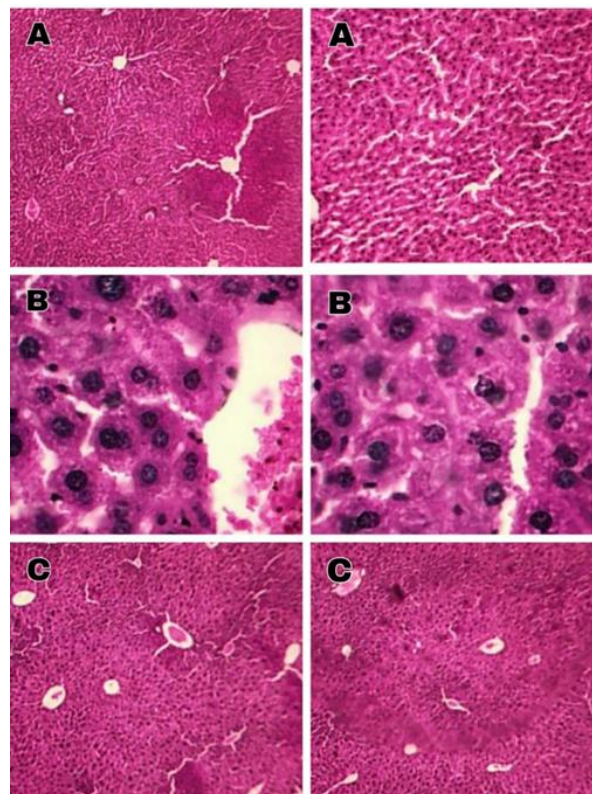


Plate: Effect of *Cassia auriculata* on liver histopathology [Control (A), Alcohol Induced Rat Group (B), Treated With *C. Auriculata* group (C)]

As shown in Plate, Results of histopathological examination reveals that Livers from animals of the control group showed a uniform pattern of the polyhedral hepatocytes with prominent nucleus and nucleolus with no evidence of inflammation, necrosis and central vein dilatation. As compared to normal control group, liver of ethanol experimental group showed extensive liver injuries characterized by cytoarchitectural changes.

After 20 days of ethanol administration, many histopathological changes were observed in the liver sections such as changes of cytoplasmic vacuolization, dilated and congested blood vessels with hemorrhage, ballooning hepatocyte, infiltration with inflammatory cells, nuclear pyknosis, karyorrhexis and sometimes karyolysis indicated liver damage^{[15] [16] [17] [18] [19] [20] [21] [22] [23]}. The liver sections of the albino rats treated with 50 mg/kg leaves extract of *Cassia auriculata* for 20 days showed little histological changes when compared to animals of experimental group.

Administration of *Cassia auriculata* at 50mg/kg dose levels reduced the severity of hepatic damage as indicated by minimal single cell necrosis, central vein dilatation. Hepatocytes also showed regenerative activity at some places.

Results were comparable to the standard control group, which showed almost normal hepatic architecture. The results were in good agreement in histopathological analysis and biochemical analysis.

Hepatocellular damage can be triggered by alcohol and can affect the normal metabolism. Hepatotoxicity implies chemical driven liver damage. The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Certain medical agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the organ. Other chemical agents, such as those used in laboratories and industries, natural chemicals and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins.

Medicinal components from plants play an important role in conventional as well as western medicine. One hundred and nineteen secondary metabolites derived from plants are globally used as drugs. 15% of all angiosperms have been investigated chemically and of that 74% pharmacologically active components have been discovered. The increasing medicinal interests highlight the importance of proper conservation of the biodiversity and cultural diversity of the ecosystem in order to safe guard and perpetuate our interdependence of plants as a source of medicine^[24].

Ethnopharmacology and traditional knowledge inspired approaches have been useful in drug discovery and development. Over this background, the present exploratory study was undertaken on *Cassia auriculata* extract to check its potency against alcoholic liver disease caused by ethanol induction in albino rats. Because of the strategic placement of liver in human body, it is predisposed to numerous disorders owing to continuous interaction with chemicals,

drugs and xenobiotics Currently available allopathic treatment for liver diseases is only symptomatic and does not treat the root cause of the disease. Their side effects further limit their use Thus, an actual curative therapeutic agent has not yet been found and management of liver disease is still a challenge to the modern health care system. Scientific studies available on medicinal plants indicate that promising phytochemicals can be developed for many health problems. A number of medicinal preparations have been advocated especially in Ayurvedic system of Indian medicine for the treatment of liver disorders. Ethanol-induced hepatotoxicity in rodents are commonly used screening models for evaluation of new hepatoprotective drugs.

Alcohol has varied effects on many systems of the body and is toxic at higher dose level or at moderate dose level when used for long periods. The major toxic metabolites of ethanol are acetaldehyde and free radicals. Long term alcohol consumption clearly plays major role in the development of alcohol related liver damage. More than half of heavy drinkers develops alcoholic hepatitis or cirrhosis.

In a developing country like India, the number of alcohol addicts is increasing day by day. Hence, we decided to evaluate hepatoprotective potential of *Cassia auriculata* against ethanol-induced hepatotoxicity in rats and to determine the possible mechanism of action for hepatoprotection. Consumption of ethanol leads to the formation of acetaldehyde via metabolism through alcohol dehydrogenase enzyme. Acetaldehyde can form adduct with substrates and these adducts act on proteins or small molecules such as cysteine, which mediate lipid peroxidation and free radical generation in mitochondria. This in turn leads to cell damage, necrosis and steatosis This study was undertaken to investigate the effect of *Cassia auriculata* extract on ethanol induced in experimental hepatotoxicity. Administering ethanol to rats for 20 days resulted in significantly elevated levels of Serum Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP), Serum Bilirubin, total protein, Albumin and globulin levels.

ALT and AST are important metabolic enzymes in liver cells and are usually at low level in the plasma. When the structural integrity of hepatic cells and even organelles such as mitochondria were damaged from xenobiotics, soluble enzymes such as ALT and AST compartmented will be released into the blood. Therefore the serum Transaminases (ALT and AST) usually are regarded as the optimum markers to diagnose liver injury In this model, we administered ethanol at the selected dose level to generate hepatotoxicity and was followed by treatment with *Cassia auriculata*. Our results confirmed the liver toxicity caused by ethanol at the selected dose level as manifested by increased serum levels of AST, ALT, ALP, serum bilirubin, albumin and globulin and total proteins as compared to normal rats. The treatment with *Cassia auriculata* shows depletion in AST, ALT, ALP, serum Bilirubin, total protein, albumin and globulin level suggest that *Cassia auriculata* may have a role in the improvement of hepatocellular damage caused by ethanol^[25].

The main characteristics of the acute alcoholic liver injury results in the histological damage which are accompanied by the destruction of cell structure as well as the changes in the concentration and vitality of serum indicators related to liver function such as ALT and AST which are very sensitive index employed in the diagnosis of liver diseases^[26]. In our study, the index of ALT, AST, ALP, Serum bilirubin, total protein, Albumin and globulin were increased by alcohol. Elevated ALT, AST, ALP, Serum Bilirubin, Total protein, Albumin and globulin activity may be associated with liver cell membrane injury, as these enzymes are usually localized in the cytoplasm and released into the blood after cell injury happened. It is evident that, the treatment with *Cassia auriculata* extract significantly decreased those damages, regulated liver cells, kept liver structure intact and relieved liver damage caused by alcohol.

Microscopy and photomicrography

Microscopic slides of liver was examined carefully under a compound light microscope. Slides from the different treated groups were evaluated for any toxic insult compared to slides from the control group. The histopathological studies were performed to find out fatty changes, normal hepatic architecture, hepatocellular necrosis and lymphocytic infiltration. Normal group showed no change, whereas rats treated with ethanol showed moderate to marked fatty changes microvesicular to vacuolar. Herbal formulation treated group produced a marked degree of protection against ethanol-induced alterations similar to those from normal rats.

From results obtained in this study following the administration of alcohol, it can be concluded that ethanol induced hepatotoxicity leads to the increase in liver enzyme markers such as Alanine Amino Transferase, Aspartate Amino Transferase and Alkaline Phosphatase indicating that the liver function is impaired. The Histopathological studies reveals the cytoarchitectural changes. Our data indicate that supplementation with *Cassia auriculata* flower extract can offer protection against ethanol experimental hepatotoxicity. In addition, histopathological studies of the liver confirmed the beneficial role of *Cassia auriculata* extract.

The treatment with *Cassia auriculata* shows a protective effect against ethanol-induced liver injury through histological work which provide evidence for the beneficial effect of *Cassia auriculata* extract in ethanol induced albino rats.

5. Conclusion

From the above study we conclude that the extract of *Cassia auriculata* showing hepatoprotective activity against ethanol induced hepatotoxicity. However, further studies are obligatory for the identification and separation of hepatoprotective components from the extract, to reveal the exact mechanism of action for the observed hepatoprotection.

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