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

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Abstract: ***To investigate the mechanism of alcohol induced liver injury in order to identify and develop therapeutic strategies to prevent disease progression. Albino rats were given herbal extract of Cassia auriculata to find its efficacy for hepatoprotective. Biochemical analysis of liver function test and histopathological study were done to prove the efficacy of plant extract Cassia auriculata.***

Keywords: Cassia auriculata, albino rats, ethanol, liver, hepatoproctective

1. Introduction

Alcohol has been a part of human culture for thousands of years. Alcoholism is now considered as a global health issue with a significant socio-economic burden in most societies. The term Alcoholic liver disease (ALD) comprises of a range of disorders including simple steatosis, steatohepatitis, cirrhosis, and end stage hepatocellular carcinoma. Alcohol abuse has a long history although it was not until the 20th century when it was studied with a scientific perspective. In 1965, pioneering work done by Lieber and colleagues identified that, the hepatotoxic effect of alcohol is a complex disease induced by alcohol abuse in broad spectrum.

Alcoholic liver disease (ALD) is a significant cause of morbidity and mortality globally (Gao and Bataller,2011; Beier*et al*.,2011; Szabo,2010) refers to a spectrum of hepatic pathologies resulting from acute or chronic alcohol exposure for which disease progression develops in a dose and time dependent manner . Alcohol abuse is a leading factor in mortality from liver disease and increase the risk for a wide range of adverse health effects. (Gao and Bataller, 2011; Beier*et al*., 2011) In the United states, the Centre for Disease control and prevention estimate that 50% of people aged 18 or older drink alcohol regularly and of these 5% are classified as heavy drinkers and 15% binge drinkers.

The toxic effect of alcohol are exerted on multiple organs; however, the liver as the primary site of alcohol metabolism, is a major target of injury (Lieber *et al*). Both chronic and acute drinking of alcohol deliver unique pathological consequences that affect liver (Gao and Bataller,2011). Understanding the pathology of ALD is impeded by the complexity of interactions including amount, duration, type consumed and different hepatic cell types. The effect of alcohol is further complicated by host genetics, variability in immunological and metabolic responses, nutritional status and the presence or absence of comorbid factors such as smoking and obesity (Szabo,2010; Tsukamoto *et al*.,).

Foccussing on the effects of alcohol on liver, rodent models of oral alcohol ingestion have been developed and extensively utilized. By engaging the rodents in voluntary drinking, these approaches largely replicate the overall process of human drinking habits as well as the general effects of alcohol on liver.

In rodents oral administration of ethanol induce acute hepatic injury. Using this approach ethanol is administered and exacerbated hepatic damage caused by endotoxin.

Traditional medicine is still the primary form of treating diseases of majority of people in developing countries including India; even among those to whom western medicine is available, the number of people using one form or another of complementary of alternative medicine is increasing worldwide. Increasing knowledge of metabolic process and the effects of plants on human physiology has enlarged the range of application of medicinal plants (Guruprasad C.Nille K .R.C.Reddy, 2015)

Cassia auriculata is one of the herbaceous plant that found throughout central and southern India, also cultivated in Punjab, Haryana, Uttar Pradesh and West Bengal. The shrub usually occurs on road sides, waste line and railway embankments. According to Ayurveda, *Cassia auriculata*contains Gunna (properties), Laghu (light), Ruksh (dry), Rasa (taste), Kashaya (astringent), Tickta (bitter), Virya (potency) and Sheet (cold). *Cassia auriculata* is well admired in the alternative medicines for its wide usage in Ayurveda, Naturopathy, and Herbal therapy. Cassia species are rich sources of Polyphenols, Anthraquinone derivatives, Flavanoids, Polysaccharides, Saponins, Tannins and Steroids. Cassia species are well known for their laxative and purgative constituents, and used for the treatment of skin diseases. *Cassia auriculata*is used in traditional medicine for diabetes, fever, constipation and urinary diseases. *Cassia auriculata* is used as the antifungal agent especially resistance to *Candida albicans* and Cryptococcus.

2. Methodology

Ethanol-induced hepatotoxicity

Albino rats (180 gm) were purchased. The rats were divided into 3 differentgroups**.** 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 sacrificed, their weight was measured accordingly. The administration of ethanol was performed through oral route.

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
Recieved normal feed and water daily for 20days	Recieved 5ml of Ethanol with normal feed and water daily	Recieved 50mg/body weight of *Cassia auriculata* extract after 20 days of ethanol exposure with normal feed and water daily

3.1 Biochemical Analysis of Serum

For the collection of blood sample to analyse the pathological biochemistry, the rats were first subjected to light anesthesia using chloroform and sacrificed. Blood samples were collected from abdominal aorta and by direct cardiac puncture using 2.5 ml syringe and transferred into 1.5 ml eppendorf tube and labelled appropriately (A,B,C). For coagulation, blood was kept about 10 minutes at room temperature. After centrifugation at 3000 r.p.m for 15 minutes at 4˚C using a thermo scientific centrifuge, serum was placed in a 1.5 ml eppendorf tube. The level of clinical biochemistry such as Aspartate Amino Transferase (AST), Alanine Transaminase (ALT), Alkaline phosphatise (ALP) and several other parameters was evaluated to determine the enzymatic activity of the liver of the control group and the experimental group (Rietman,S.,Frankel S.,1957) Serum AST and ALT are the enzyme bio markers to monitor the liver structural integrity, damage and aids in the clinical diagnosis of liver toxicity conditions (G.Kasarola, H.L.Tillmann.;2016) The level of serum Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) and several other parameters were determined by standard method based on the guidelines of International Federation of Clinical Chemistry (IFCC).

4. Results & Discussion

Table 1: Effect of Cassia auriculata on serum Biochemical parameters in ethanol-induced hepatic damage in albino rats

Parameters	Group (A) Control	Group (B) Ethanol exposure	Group (C) Treated with *Cassia auriculata*
AST (SGOT)	336 U/L	3374 U/L	1170 U/L
ALT (SGPT)	66 U/L	1729 U/L	255 U/L
ALP	98U/L	138 U//L	112 U/L
Bilirubin Total Serum	0.13 mg/dL	0.22 mg/dL	0.19 mg/Dl
Bilirubin Direct Serum	0.11 mg/dL	0.18 mg/dL	0.14 mg/dL
Bilirubin Indirect Serum	0.02 mg/dL	0.04 mg/dL	0.02 mg/Dl
Total Protein, Serum Biuret	5.25 g/dL	7.85 g/dL	5.98 g/Dl
Albumin, Serum BCP	2.32 g/dL	3.13 g/dL	2.51 g/Dl
Globulin Serum Calculated	2.62 g/dL	4.72 g/dL	2.89 g/Dl

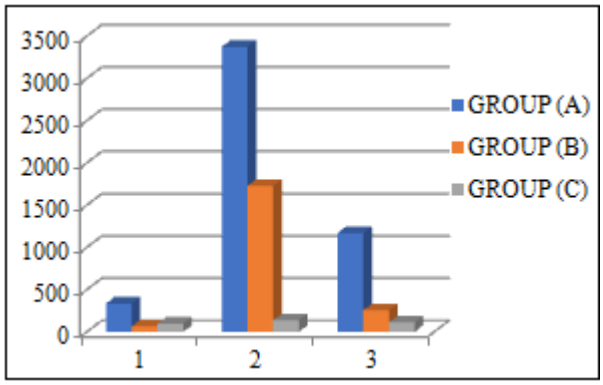

Figure 1: [Group (A) Control, Group (B) Oral Ethanol Administration and Group (C) Treated with *Cassia auriculata* extract]

The extract of *Cassia auriculata* was evaluated for its hepatoprotective potential in albino rats with ethanol-induced liver damage. Administration of ethanol to albino rats for 20 days causes elevation in serum Aspartate Transaminase (AST) (3734 U/L), Alanine Transaminase (ALT) (1729 U/L), Alkaline phosphatise (ALP) (138 U/L), Bilirubin Total serum (0.22 mg/dL), Bilirubin Direct serum (0.18 mg/dL), Bilirubin indirect serum (0.04 mg/dL), Total protein (7.85 g/dL) Albumin (3.13 g/dL), Globulin (4.72 g/dL)

Treatment of the ethanol intoxicated rats with *Cassia auriculata* at 50 mg/kg body weight reduced the levels of Aspartate Transaminase (AST) (1170 U/L), Alanine Transaminase (ALT) (255 U/L), Alkaline phoshatase (ALP) (112 U/L), Bilirubin Total serum (0.19 mg/dL), Bilirubin Direct serum (0.14 mg/dL), Bilirubin Indirect serum (0.02 mg/dL), Total protein (5.98 g/dL), Albumin (2.51 g/dL) and globulin (2.89 g/L) as shown in Table 1. Results of administration of *Cassia auriculata* extract were comparable to that of standard control group.

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 (Perumalsamy et al. 1999)

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 alcohic liver disesase 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 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 *Cassiaauriculata* 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. (Thapa &Walia, 2007).

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 at the biochemical analysis which provide evidence for the beneficial effect of *Cassia auriculata* extract in ethanol induced albino rats.

5. Conclusion

Hence, from the present study we conclude that the extract of *Cassia auriculata* showing hepatoprotective activity against ethanol induced hepatotoxicity.

6. Feature Scope

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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