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Hepatoprotective effect of *Cassia auriculata* extract against ethanol-induced oxidative stress in Wistar albino rats

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Abstract

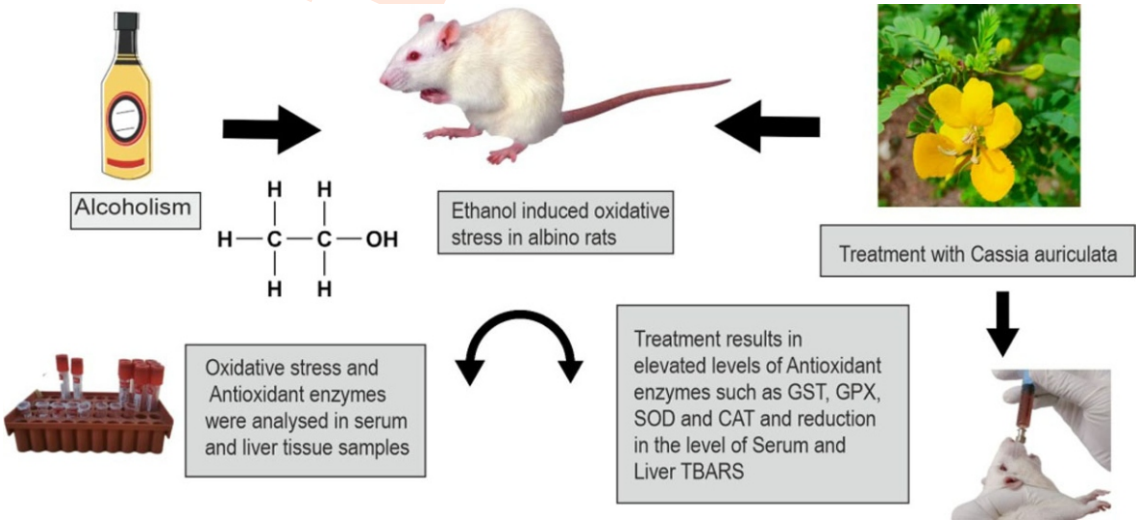
Aim: To investigate the potential hepato-protective effect of *Cassia auriculata* leaf extract against ethanol-induced oxidative stress in albino rats, by exploring the antioxidant properties of *Cassia auriculata* and its ability to mitigate oxidative damage caused by ethanol consumption, and the underlying mechanisms involved.

Methodology: In the present study, Group – I served as Control, Group – II (Ethanol induced group), where 50% ethanol was orally administered to the rats, Group – III (treatment group), where 250 mg kg^{-1} of *Cassia auriculata* leaf extract was orally administered to the hepato-toxic albino rats and in Group – IV, rats were treated with standard reference drug Silymarin (100 mg kg^{-1}) followed by estimating the parameters related to oxidative stress such as TBARS and antioxidants enzyme levels such as GST, GPx, SOD and CAT in the blood and liver tissue samples. Phytochemical analysis of *Cassia auriculata* leaf powder was conducted to determine the active phytoconstituents.

Results: Treatment with *Cassia auriculata* extract significantly ($p<0.001$) reduced the damage caused by oxidative stress by decreasing the levels of TBARS and significantly ($p<0.001$) elevated the levels of antioxidant enzymes such as GST, GPx, SOD and CAT in the serum and liver samples.

Interpretation: The present study provides an opportunity to explore the potential therapeutic properties of *Cassia auriculata* in combating ethanol-induced oxidative stress.

Key words: Albino rats, *Cassia auriculata,* Ethanol, Oxidative stress



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Introduction

Alcoholism in India is indeed a significant concern and threat with far-reaching consequences. Alcohol is one of the leading causes of death and disability globally and in India. According to recent data published by the World Health Organization (WHO), approximately 3.3 million deaths every year occurs due to alcohol consumption and 5.1% of the global burden of disease is attributable to alcohol consumption (Eashwar *et al*., 2020). Excessive alcohol consumption for a prolonged time may lead to variety of sociomedical and public issues (Nowak and Relja, 2020). Ethanol is a primary constituent present in most of the alcoholic beverages. It is primarily metabolized in the liver by the action of cytosolic alcohol dehydrogenase enzyme which has multiple isoenzymes and genetic polymorphisms. The important oxidative pathways include inducible CYP2E1 system which oxidizes alcohol and produces the toxic by product acetaldehyde (Jiang *et al.,* 2020). Antioxidant defense system involves the antioxidant enzymes such as glutathione-S-transferase, glutathione peroxidase, super oxide dismutase and catalase. These enzymes play an important role in eliminating reactive oxygen species and detoxify free radicals generated under normal physiological conditions (Ighodaro and Akinloye, 2018).

Alcohol mediated ROS causes the apoptosis of enterocytes and ubiquitin – dependent proteolytic degradation of junctional complex proteins (Cho *et al.,* 2018). The nicotinamide adenine dinucleotide phosphate (NADPH) and oxidase (NOX) systems are the main enzymatic reactions that generate ROS in the gastrointestinal tract and are associated with inflammatory and oxidative stress responses (Aviello and Knaus, 2018). Reactive oxygen species play an important role in cellular proliferation, differentiation, migration, apoptosis and necrosis. Low to intermediate levels of ROS and RNS are necessary for the maintenance of many important physiological functions, redox homeostasis and the regulation of key transcription factors. Excessive formation of ROS is responsible for disrupted redox homeostasis which in turn leads to oxidative stress and ROS mediated damage to all important biomolecules including DNA, proteins and cellular membranes (Liguori *et al.,* 2018).

The level of oxidative stress is estimated by the oxidative stress markers and it has been found elevated in the people with unhealthy lifestyle choices, diet, smoking, alcohol consumption and lack of physical exercise. Cells have evolved a complex antioxidant system, formed by enzymatic antioxidants such as superoxide dismutases (SODs), catalases (CAT) and glutathione peroxidases (GPXs), thioredoxin (Trx) as well as molecular antioxidants, play an important role in balancing the level of oxidative stress (Halliwell, 2022). In order to protect the body against the consequences of oxidative stress, an efficacious approach has been adopted by increasing the intake of antioxidants available from natural resources (Karthick Raja Namasivayam *et al.,* 2019). Medicinal plants are significant sources of hepatoprotective drugs. More than 700 mono and polyherbal preparations in the form of decoction, tincture and tablets have been used in various liver disorders. The 21st century has seen a paradigm shift toward therapeutic evaluation of herbal products in liver disease models by carefully synergizing the strength of the traditional system of medicine with that of the modern concept of evidence-based therapeutical screening, authentication, and randomized placebo-controlled clinical trials to support clinical efficacy (Ilyas *et al.,* 2016).

Senna auriculata (L.) Roxb.syn. *Cassia auriculata* L. belongs to Family –Fabaceae is a traditional medicinal plant, widely used for the treatment of various ailments in Ayurveda and Siddha system of medicine in India. Almost all the parts of the plant, such as flowers, leaves, seeds, barks, and roots have been reported for their medicinal uses. Traditionally, it has been used in the treatment of diabetes, asthma, rheumatism, dysentery, skin disease, and metabolic disorders. The principle phytochemicals present in *Cassia auriculata* are alkaloids, anthraquinone, flavone glycosides, sugar, saponins, phenols, terpenoids, flavonoids, tannins and steroids. The extract collected from different plant parts and isolated compounds possess a wide range of pharmacological activities such as antidiabetic, antioxidant, anti-inflammatory, antihyperlipidemic, hepatoprotective, nephroprotective, cardioprotective, anti-atherosclerotic, anticancer, antimutagenic, antimicrobial, antiulcerative, antipyretic, anthelmintic, immunomodulatory, antifertility, anti-venom anti-melanogenesis (Nille *et al.,* 2021).

Silymarin, commonly known as milk thistle, is a flavonolignan extracted from *Silybum marianum* (L.), one of the oldest plant, utilized for the treatment of liver diseases (Masood *et al.,* 2021). It has been reported to possess various pharmacological properties such as hepatoprotective, antioxidant, anti-inflammatory, anti-cancer and cardioprotective. It shows hepatoprotection via various underlying mechanisms of which most common are modulation of enzymatic and non-enzymatic liver biochemical markers (Venmathi Maran *et al.,* 2022). In the present study, Silymarin was used as a standard reference drug to compare the efficacy of proposed plant extract. Some other promising hepatoprotective agents from natural sources include Glycyrrhizin, a triterpenoid glycoside isolated from the root of *Glycyrrhiza glabra* L. commonly known as liquorice root. It shows hepatoprotective effect by increasing antioxidant defence in hepatic cell and reduces oxidative stress in animal models with hepatic injury (Orazizadeh *et al.,* 2014).

The findings of Chigurupati *et al.* (2016) suggests that consumption of proprietary glycyrrhizin during alcohol consumption may support improved liver health. Andrographolide and neoandrographolide are active chemical constituents present in the plant Andrographis paniculata Nees. commonly called as “king of bitterness” which is well known for curing liver diseases. Andrographolide inhibits inflammation, angiogenesis and fibrosis in chemically induced liver injury in animal models via antioxidant and anti-inflammatory mechanisms (Chen *et al.,* 2014). Nagalekshmi *et al.* (2011) revealed that administration of *Andrographis* extract after paracetamol intake restored the levels

of antioxidant enzymes in liver tissue, thus offered protection against hepatotoxicity induced by paracetamol. The most profound hepatoprotective mechanisms of the herbal plants are through free radical scavenging effect and anti-inflammatory pathway. Hepatic injury results in oxidation of fatty acid present in hepatocyte membrane causing distortion of cells and their organelles (Ezhilarasan, 2018). In the present investigation, the potential hepatoprotective properties of *Cassia auriculata* leaf extract against ethanol-induced oxidative stress in albino rats was explored. While existing literature acknowledges and highlights the anti-diabetic role of *Cassia auriculata*, the specific application of *Cassia auriculata* in mitigating the adverse effects of ethanol metabolism in alcoholic liver disease remains largely unexplored. Hence, the present study aimed to investigate the hepatoprotective potential of *Cassia auriculata* leaf extract in mitigating oxidative stress against ethanol induced albino rats.

Materials and Methods

Collection of plant material and Preparation of plant extract: The leaves of Cassia auriculata were collected from the premesis of Queen Mary's College, Chennai and were authenticated by a taxonomist. The collected leaves were washed with tap water and later with distilled water to remove any impurities and dust, thereafter shade dried for about 10 days and grounded into a fine powder. For preparing the plant extract, desired amount of powder was suspended in 100 ml of distilled water, boiled in water bath until it reduced to one fourth of its original volume, following the method suggested by Abubakar and Haque (2020). The concentrated extract was further filtered and 2 ml of the obtained extract was orally administered to Group – III rats.

Experimental animals: Adult male Wistar albino rats were purchased from Mass Biotech Chengalpet, Tamil Nadu, India, and were maintained under standard laboratory conditions at 22 ± 4 °C, relative humidity 30 to 70% with a 12: 12 hr dark: light cycle. Animals were fed with standard laboratory rat pellet diet (20% proteins and 5 % lipids) and water *ad libitum.* The experimental protocol approved by the Institutional Animal Ethics Committee (2084/PO/RcBt/S/19/CPCSEA) was followed for experimentation.

Acute toxicity study: Acute toxicity study was carried out according to the OECD Guidelines, Rule No. 423, Adult male Wistar albino rats were divided into four classes of three animals each. All the animals were kept on fasting overnight prior to the experiment and were orally administered 2 ml of *Cassia auriculata* leaf extract @ 50, 100, 200, 2000 mg kg^{-1} and the animals were monitored 14 days for any allergic and toxic symptoms.

Ethanol induced hepatotoxicity and Treatment protocol: Male albino rats 6-8 weeks old, weighing 150-200 g each were divided into three groups of 6 rats each. Group I served as Control were free access to standard laboratory rat pellet diet and water *ad libitum,* while Group II (Ethanol induced group) were exposed to 50 % ethanol at a dose of 2 ml kg^{-1} for 3 weeks orally to induce oxidative stress. The dosage of ethanol was finalized after testing the animals with different concentration (30%, 50%,70%) of ethanol. It was determined that the dose of 50% was appropriate to induce hepatotoxicity and this conclusion is supported by liver function test analysis. Further, to support the dose selection, the dosage of ethanol used in this study has been previously reported to induce tissue toxicity and oxidative damage (Han *et al.,* 2015). The rats in the treatment Group (Group – III) were orally administered with 50% ethanol for 3 weeks further treated with *Cassia auriculata* leaf extract (250 mg kg^{-1}) for 30 consecutive days. The dosage of *Cassia auriculata* was fixed after conducting acute toxicity studies on rats, and the dosage used was further supported by the existing literature.

The ethanol intoxicated Group–IV rats were orally administered with standard reference drug Silymarin (100 mg kg^{-1}) for 30 days. Silymarin has been extensively studied, and existing literature supports its use as a reference drug due to its hepatoprotective effects. Silymarin is generally considered safe, making it a suitable reference drug for preclinical and experimental studies. Silymarin is available in different forms such as capsules and tablets, with different dosages. Capsulated form of Silymarin was used in the present study. Based on the commercial formulation used, the recommended daily dosageranged between 420–600 mg. Majority of clinical trials have been conducted with a dosage of 140 mg three times a day (Gillessen and Schmidt, 2020). Hence, the dose of silymarin used in this study was fixed as 100 mg kg^{-1}. Behavioral changes, food and water intake were monitored daily throughout the study. Body weight of the animals were monitored weekly and blood samples were collected under isoflurane anesthesia by Retro orbital plexus puncture and used for biochemical analyses. At the end of the experiment, animals were euthanized and liver of each animal was dissected and washed with isotonic solution, and their wet weight was recorded. The liver tissue homogenate was prepared using phosphate buffer solution for oxidative stress analysis.

Phytochemical Analysis: A preliminary phyto-chemical analysis of *Cassia auriculata* leaf powder was performed to determine the presence of active phytoconstituents. A 2.5 g of sample were weighed and soaked in 25 ml ethanol. The extract was allowed to stand for 72hr and filtered using sterile filter paper. The filtrate was used for qualitative analysis of phytochemicals.

Analysis of oxidative stress: Oxidative damage was analyzed by estimating the levels of antioxidant enzymes present in the serum and liver tissue samples of albino rats following standard protocols.

Estimation of Serum and Liver TBARS: Thiobarbituric acid reactive substances (TBARS) were assessed to estimate the lipid peroxidation index using Malondialdehyde as a standard following the method of Ohkawa *et al.* (1979).

Estimation of Antioxidant enzymes: Glutathione S Transferase (GST) in serum and liver tissue homogenate was estimated

according to the method of Habig *et al.* (1974). The level of Glutathione peroxidase (GPx) was determined according to the method of Rotruck *et al.* (1973). Super oxide dismutase (SOD) was analyzed according to the method of Beauchamp and Fridovich (1971) whereas the Catalase activity (CAT) was determined by the method of Maehly and Chance (1954).

Statistical analysis: The results are expressed as Mean±S.D. Differences in antioxidant enzyme parameters and the level of TBARS were determined by One-way ANOVA. Individual groups were compared using Tukey's test. Differences with $P < 0.001$ were considered statistically significant.

Results and Discussion

Plant and plant derived compounds possess enormous biological activities and have been aiding in the curation of diseases for many decades (Newmann and Cragg, 2016). Plants have always been considered as a source of food and medicinal compounds. More than 200 species of plants have medicinal value and 25 % of the medicines have originated from plants. Metabolites derived from plant biomass mainly medicinal plants exhibit a wide spectrum of biological activities with high efficacy and biocompatibility (Snega *et al.,* 2023). Phytochemicals are a group of compounds that belong to secondary metabolites derived from plant sources, which includes a wide range of chemical entities such as polyphenols, flavonoids, steroids, saponins, organosulphur compounds and vitamins. Most of the phytochemicals and herbal products are often reported in literature as "nutraceuticals" emphasizing their health promoting properties and are useful for the treating cancer, cardiovascular diseases, diabetes and neural disorders (Forni *et al.,* 2019).

In the present study, the phytochemical screening on Cassia auriculata leaf extract confirmed the presence of Alkaloids, Flavonoids, Tannins, Saponins and Proteins (Table1). Flavonoids exhibit strong antioxidant potential and act as a safeguard against cell degeneration. Alkaloids play an important role in scavenging and lipid peroxidation inhibition (Abbas *et al.,* 2017). During acute toxicity study period, rats were given graduated doses of aqueous *Cassia auriculata* leaf extract (50, 100, 200, 2000 mg kg^{-1}) which produced no significant changes in breathing, behavior, sensory and tactile responses and no signs of toxicity such as irritability, choking, abnormal body posture, abnormal vocalization, increased or decreased urination or defecation were found. No mortality and signs of abnormality were observed up to a dose range of 2000 mg kg^{-1} after 24 hr of extract administration in the acute toxicity sample. Findings on acute toxicity conducted by Kalaivani *et al.* (2008) also revealed that the administration of graduated doses of *Cassia auriculata* leaves and flower extracts up to a dosage of 1000 mg kg^{-1} per day for 30 days produced no effect on the general behavior or appearance of the animals and all the rats survived the test period (Guruprasad and Reddy, 2015). Ethanol – induced liver disease remains a world-wide health concern without effective therapies. Since oxidative stress is a well – established factor in the progression of liver damage due to alcohol intake, using antioxidants to ameliorate oxidative stress and alleviate ethanol hepatotoxicity is a logical approach (Luo *et al.,* 2014). Oxidative stress induced cell death is associated with an increase in the production of ROS, such as hydrogen peroxide, nitric oxide, super oxide anion, hydroxyl radicals and singlet oxygen (Shen *et al.,* 2013).

The accumulation of ROS in the liver causes dysfunction of cellular membrane systems, protein and DNA oxidation and eventually hepatocyte injury. This damage if left unrepaired cause cells to undergo apoptosis (Prietsch *et al.,* 2014). Ethanol mediated oxidative stress is caused due to increase in the production of ROS and by the depletion of oxidative defense in the cell. Thus, removal of excess ROS or suppression of their generation by antioxidants may be effective in preventing oxidative stress induced by ethanol (Luo *et al.*, 2014). Alcohol mediated oxidative stress and the decline of scavenging activity of free radical is one of the main causes of hepatic damage, which is widely evidenced in rodents and humans. Simultaneously, the involvement of free radical mechanisms in the pathogenesis of alcoholic liver disease is demonstrated by detecting lipid peroxidation markers in the liver and serum of patients with alcoholism (Albano, 2006). Previous studies have confirmed that

Table 1: Phytochemical analysis of *Cassia auriculata* leaf extract

Phytochemicals	*Cassia auriculata*
Tannins	+
Saponin	+
Flavonoids	+
Alkaloids	+
Proteins	+
Steroid	-
Terpenoid	+
Quinone	+
Cardiac Glycosides	+
Phenol	+

+: Presence;- : Absence

Table 2: Effect of *Cassia auriculata* on the status of antioxidant enzymes GST, GPx, SOD and CAT in serum of ethanol induced experimental animals

Enzymes	Control	Ethanol*	*Cassia auriculata***	Silymarin**
GST	0.73 ± 0.14	0.05 ± 0.04	0.71 ± 0.12	0.66 ± 0.10
Gpx	1.27 ± 0.32	0.04 ± 0.03	1.10 ± 0.24	1.02 ± 0.26
SOD	0.36 ± 0.06	0.05 ± 0.02	0.32 ± 0.08	0.32 ±0.05
CAT	0.78 ± 0.13	0.07 ± 0.04	0.82 ± 0.11	0.78 ± 0.11

Table 3: Effect of *C. auriculata* on the status of antioxidant enzymes GST, GPx, SOD and CAT in liver tissues of ethanol induced experimental animals

Parameters	Control	Ethanol *	*Cassia auriculata***	Silymarin **
GST	0.21 ± 0.07	0.03 ± 0.01	0.23 ± 0.08	0.20 ± 0.07
Gpx	0.30 ± 0.06	0.04 ± 0.02	0.29 ± 0.05	0.27 ± 0.05
SOD	0.26 ± 0.03	0.09 ± 0.17	0.26 ± 0.03	0.24 ± 0.03
CAT	0.80 ± 0.07	0.12 ± 0.04	0.73 ± 0.09	0.71 ± 0.08

Table 4: Effect of *Cassia auriculata* on serum and liver tissue TBARS level of ethanol induced experimental animals

Parameters	Control	Ethanol*	*Cassia auriculata***	Silymarin**
TBARS - Serum	0.13 ± 0.03	0.37 ± 0.12	0.13 ± 0.03	0.10 ± 0.01
TBARS - Liver	0.05 ± 0.02	0.26 ± 0.10	0.05 ± 0.02	0.05 ± 0.02

Results are represented as mean ± SD (n = 6). The experimental rats were administered with 50% ethanol for 21 days. Treatment groups (Ethanol + CA) (*Cassia auriculata* 250 mg kg^{-1} for 30 days), (Ethanol + Silymarin) (Silymarin 100 mg kg^{-1} for 30 days). * represents the group compared with control and ** represents the group compared with ethanol. Values are statistically significant at $p < 0.001$.

[GST-Glutathione S Transferase, GPx- Glutathione peroxidase, SOD- Super oxide dismutase, CAT-Catalase] Units : GST – min / mg of protein, GPx – µg of reduced glutathione utilized mg^{-1} of protein min^{-1}, SOD – Units mg^{-1} of protein, CAT – µ mole of H_2O_2 consumed mg^{-1} of protein. [TBARS – Thiobarbituric acid reactive substance] Unit: µ moles mg^{-1} protein

the administration of antioxidants affords a marked protection against liver damage caused by hepatotoxins such as CCl_4 (Khan *et al.,* 2012) and paracetamol (Mahmood *et al.,* 2014).

Antioxidant enzymes such as GST, GSH, GPx, SOD and CAT play an important role in combating oxidative stress by involving in the catalytic transformation of ROS and their toxic byproducts into stable nontoxic molecules. GST protects the cells or tissues against oxidative stress and damage by detoxifying various toxic substrates derived from cellular oxidative processes (Sharma *et al.,* 2004). GPx is an important cellular antioxidant enzyme found in the cytoplasm and mitochondria of mammalian cells. As an antioxidant enzyme, GPx modulates the balance between necessary and harmful levels of reactive oxygen species (Handy *et al.,* 2022). SOD forms the first line of defense against reactive oxygen species mediated injury (Kangralkar *et al.,* 2010). Catalase is a key enzyme which uses hydrogen peroxide, a non-radical ROS, as its substrate. It is responsible for neutralization through decomposition of hydrogen per oxide, there by maintaining the cell signalling processes (Nandi *et al.,* 2019). In our findings, Ethanol intoxication for 21 days significantly ($P < 0.001$) reduced the levels of antioxidant enzymes such as Glutathione S Transferase (GST) (0.05 ± 0.04) Glutathione peroxidase (GPx) (0.04 ± 0.03), Super oxide dismutase (SOD) (0.05 ± 0.02) and Catalase (CAT) (0.07 ± 0.04) in serum and GST (0.03 ± 0.01), GPx (0.04 ± 0.02), SOD (0.09 ± 0.17) and CAT (0.12 ± 0.04) in liver tissue samples compared with the normal control group, GST (0.73 ± 0.14), GPx (1.27 ± 0.32), SOD (0.36 ± 0.06) and CAT (0.78 ± 0.13) in serum and GST (0.21 ± 0.07), GPx (0.30 ± 0.06), SOD (0.26 ± 0.03) and CAT (0.80 ± 0.07) in liver tissue samples. Ethanol intoxication hinders the production of antioxidant enzymes, thereby leading to decreased levels in both serum and liver tissues (Table 2). Treatment of hepatotoxic rats with 250 mg kg^{-1} of *Cassia auriculata* for 30 days significantly ($P < 0.001$) increased the production of antioxidant enzymes GST (0.71 ± 0.12), GPx (1.10 ± 0.24), SOD (0.32 ± 0.08) and CAT (0.82 ± 0.11) in serum and GST (0.23 ± 0.08), GPx (0.29 ± 0.05), SOD (0.26 ± 0.03) and CAT (0.73 ± 0.09) in liver tissue samples to counteract the oxidative stress when compared with the ethanol intoxicated rats (Table 3). When compared to the reference drug Silymarin, *Cassia auriculata* gave better results by improving the production of antioxidant enzymes.

The percentage changes in the activity levels of antioxidant enzymes in blood (Fig. 1) and liver tissue (Fig. 2) samples showed a significant decrease in the levels of

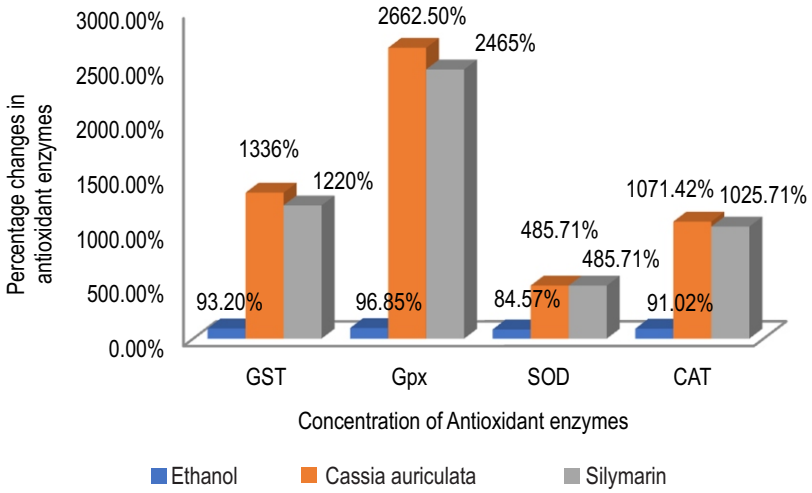

Fig. 1: Percentage changes in activity levels of serum antioxidant enzymes viz. GST, GPx, SOD and CAT in control compared with Ethanol, Treatment groups – *Cassia auriculata* and Silymarin compared with Ethanol group.

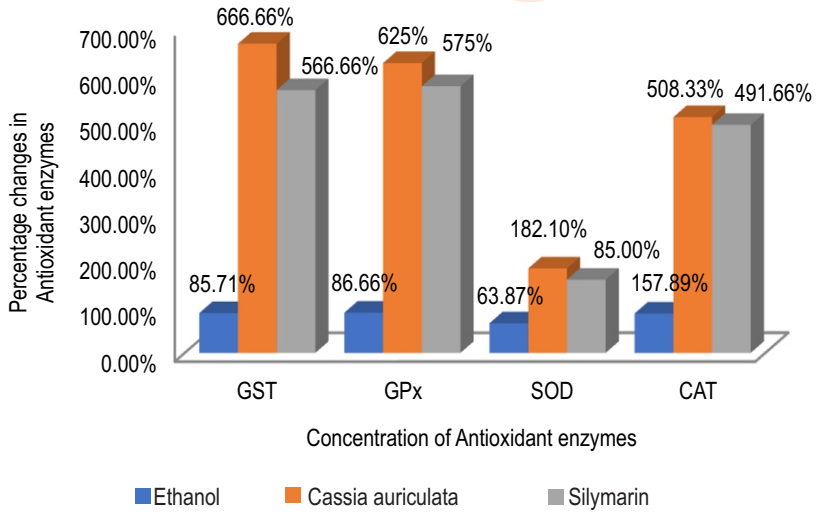

Fig. 2: Percentage changes in activity levels of liver antioxidant enzymes viz. GST, GPx, SOD and CAT in control compared with ethanol, Treatment groups – *Cassia auriculata* and *Silymarin compared* with Ethanol group.

antioxidant enzymes in ethanol group when compared with control group. However, the treatment groups (*Cassia auriculata* and Silymarin) showed a significant increase in the production of antioxidant enzymes when compared with the ethanol intoxicated group.

A study conducted by Senthil *et al.* (2003) revealed that the supplementation with Cassia auriculata leaf extract can offer protection against free radical mediated oxidative stress in experimental hepatotoxicity. Histopathological examination of *Cassia auriculata* also confirmed the beneficial role of Cassia auriculata. Jaydeokar *et al.* (2014) revealed that root extract of *Cassia auriculata* possess potent hepatoprotective activity against ethanol and antitubercular drug induced hepatotoxicity in albino rats, which could be due to inhibition of hepatic metabolizing enzymes and antioxidant activity. Recently, Ajay Guru *et al.* (2023) investigated the effect of IW13 peptide on inhibiting lipid accumulation and regulating the antioxidant mechanism to normalize the lipid metabolism in HFD induced zebra fish larvae and reported that co-treatment with IW13

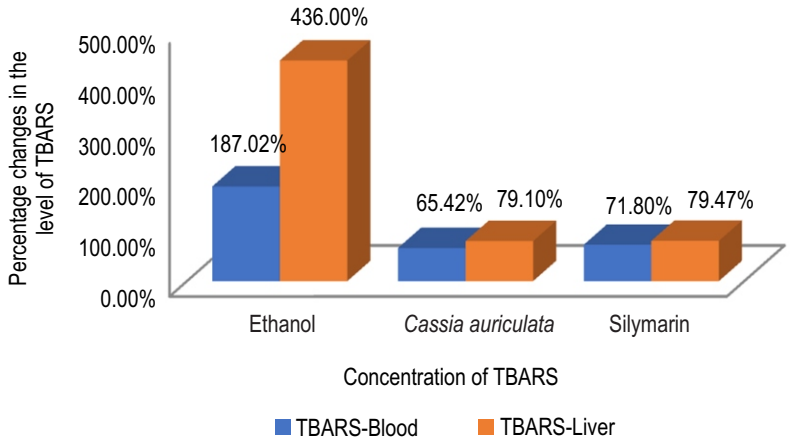

Fig. 3: Percentage changes in the levels of Thiobarbituric acid reactive substances (TBARS) in Control compared with Ethanol induced group and *Cassia auriculata* and Silymarin compared with Ethanol group.

peptide showed a protective effect in HFD zebra fish larvae by increasing the survival rate. However, IW13 peptide co-treatment reduced triglycerides and cholesterol levels while also restoring the SOD and CAT antioxidant enzymes. In addition, IW13 co-treatment inhibited the formation of lipid peroxidation and superoxide anion by regulating the glutathione level.

In-silico analysis of *Cassia auriculata* showed that, it possess top 20 enriched targets predicted for involving in insulin signalling pathway, e.g., PTPN1, PCK – α, AKT2, P13K – γ. Cassia auriculata may enhance glucose uptake and glucose transporter expressions via IRS signalling pathway. In-vivo analysis showed significant increase in the expression of IRS, AKT, GLUT2 and GLUT4. *Cassia auriculata* may also be involved in the gluconeogenesis and glycogenolysis in the liver based on the downregulation of G6PC and PCK-1 genes. *Cassia auriculata* treated groups also showed decreased levels of cholesterol, triglyceride, glucose, CRP and Hb1Ac levels and increased insulin and C – peptide levels (Mohd Fauzi *et al.,* 2017). The findings of this study confirmed that the treatment with *Cassia auriculata* extract significantly increased the production of antioxidant enzymes, providing protection against ethanol–induced hepatotoxicity. Exposure to chemical or physical agents trigger membrane free radical reactions in living cells, which accelerates lipid peroxidation (Gokila *et al.,* 2013). A large number of toxic byproducts are formed during lipid peroxidation such as MDA, 4- hydroxynonenal, conjugated-dienes, lipid hydroperoxides and isoprostanes (De *et al.,* 2007). Lipid peroxidation is a major consequence of oxidative stress, administration of hepatotoxins increases the level of TBARS in the blood. The concentration of MDA in cells or tissue lysates is considered to be a major lipid peroxidation marker (Senthil *et al.,* 2012) and it is clearly evident from our findings that, when compared with the normal control group the administration of 50 % ethanol significantly increased the level of TBARS in the blood (0.37 ± 0.12) and liver tissues (0.26 ± 0.10) of albino rats which acts as an indicator for oxidative damage. Subsequent treatment with *Cassia auriculata* (250 mg kg^{-1}) leaf extracts significantly ($P < 0.001$) reduced the TBARS level in blood (0.13 ± 0.03) and liver tissue samples (0.05 ± 0.02) when compared to the ethanol group, thereby recovered the liver from oxidative damage (Table 4). The percentage of changes in the activity levels of TBARS in blood and liver of ethanol and treatment groups showed a significant increment in the levels of TBARS in ethanol intoxicated group when compared with the normal control and treatment groups. The treatment groups showed reduction in the level of TBARS which is a clear indication for recovery (Fig. 3). Silymarin, a polyphenolic plant flavonoid derived from *Silybum marianum* produces anti-inflammatory, hepatoprotective and carcinogenic effects. It shows hepatoprotection via various underlying mechanisms of which most common are modulation of enzymatic and non-enzymatic liver biochemical markers (Venmathi Maran *et al.,* 2022).

Milk thistle is traditionally used as a medicine to treat hepatic conditions such as chronic hepatitis, and liver disease associated with alcohol consumption (Clichici *et al.,* 2016). Silymarin has beneficial effects on human hepatocytes in non-alcoholic steatohepatitis, non-alcoholic fatty liver disease, and fibrosis patients (Marin *et al.,* 2017). Silybin- an active component present in silymarin helps in regulating oxidative stress, hepatic fat storage and insulin in the blood (Federico *et al.,* 2017). Treatment with Silymarin significantly ($P < 0.001$) improved the production of antioxidant enzymes such as GST (0.66 ± 0.10), GPx (1.02 ± 0.26), SOD (0.32 ± 0.05) and CAT (0.78 ± 0.11) in serum and GST (0.20 ± 0.07), GPx (0.27 ± 0.05), SOD (0.24 ± 0.03) and CAT (0.71 ± 0.08) in liver tissues of albino rats. Silymarin is used as a reference drug in various studies. However, in our findings *Cassia auriculata* showed more progression than Silymarin by increasing the production of antioxidant enzymes.

The protective role of phytochemicals may be associated with their antioxidant activity, since overproduction of oxidants such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) in human body is involved in the pathogenesis of many chronic diseases (Zhang *et al.,* 2015). So far, about 10,000 phytochemicals have been identified and still a large percentage remains unknown. The identified phytochemicals include tannins, flavonoids, triterpenoids, steroids, saponins and alkaloids (Barbosa *et al.,* 2013). Free radicals are considered to be involved in the multistage carcinogenic process and can independently cause mutations in the DNA, which is crucial for the initiation of carcinogenic process. Antioxidants present in the phytochemical compounds modulates the initiation of carcinogenic process by giving protection against DNA damage. Studies have shown that green tea extract, grape seeds, cucurmin and silymarin possess the ability to protect against adverse effect of UV radiation influenced inflammation, oxidative stress, DNA damage and suppression of immune responses (Zhang *et al.,* 2015).

The study conducted by Shika and Soni (2023) on hepatoprotective activity of *Adina cordifolia* leaves against hepatotoxicity in rats suggests that the plant extract protects the hepatic cells from ethanol-induced hepatocellular injury and used Silymarin to compare the efficacy of their plant extract. Alhejaily *et al.* (2023) investigated the potential gastro-protective effect of Qaysum (*Achillea fragrantissima*) against ethanol-induced gastric ulcer in wistar albino rats in which a significant increase in the levels of antioxidant enzymes were found. Similar to the previous findings on various plant extracts, *Cassia auriculata* leaf extract significantly reduces the oxidative damage and acts as a hepatoprotectant by elevating the levels of antioxidant enzymes.

In conclusion, our findings regarding the efficacy of Cassia auriculata leaf extract revealed its effectiveness against oxidative stress caused by ethanol. The observed hepatoprotective effect was possibly due to the presence of active phytoconstituents such as alkaloids and flavonoids. However, further studies are essential to reveal the exact mechanism of action for the observed hepatoprotection.

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