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Original Research Article

ASSESSMENT OF HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITY OF SALVADORA PERSICA L. AGAINST ALACTOSAMINE INDUCE OXIDATIVE STRESS IN RATS

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ABSTRACT

Two essential changes bring about irreversible cell injury in necrosis is cell digestion by lytic enzymes and denaturation of proteins. These processes are morphologically identified by characteristic cytoplasmic and nuclear changes in necrotic cell. The cytoplasm appears homogeneous and intensely eosinophilic. Occasionally, it may show vacuolation or dystrophic calcification. Hepatotoxicity can affect hundreds of millions of people worldwide. It is the common non-neo plastic cause of death among hepatobiliary and digestive disorders serious side effects, the cost of the modern medicine and improper channel of treatment and competitive efficacy of natural products made the person through the world to look for classical plant drugs for the treatment of hepatotoxicity. In view of the pharmacological and biological properties and chemical constituents of the plant Salvadora Persica, it was decided to an attempt is made to find out the extent of hepatoprotective and antioxidant activity of plant.

Keywords: Hepatoprotective, Antioxidant Activity, *Salvadora persica*, Phytoconstituents.

INTRODUCTION

Plants have been utilised as a natural source of medicinal compounds since thousands of years. Human is using numerous plants and plant derived products to cures and relief from various physical and mental illness. These plants are used in traditional Chinese, Ayurveda, Siddha, Unani and Tibetan medicines. Ancient literature such as Rigveda, Yajurveda, Atharvaveda, CharakSamhita and SushrutSamhita also describes the use of plants for the treatment of various health problems. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. In last five decades, these plants have been extensively studied by advanced scientific techniques and reported for various medicinal properties viz, anticancer activity, antibacterial activity, antifungal activity, antidiabetic activity, antioxidant activity, hepatoprotective activity, haemolytic activity, larvicidal activity and anti-inflammatory activity etc (Bamola *et al*., 2018; Marrelli, 2021). The liver is a vital organ of involved in the metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites and detoxification of the exogenous and endogenous challenges like xenobiotics, drugs, viral infections andchronic alcoholism. A liver disease is a worldwide problem; conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Herbal drugs

have gained importance and popularity in recent years is numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practice as well as traditional system of medicine in India. Necrosis is defined as focal death along with degradation of tissue by hydrolytic enzymes liberated by cells. Various agents such as hypoxia, chemical, physical agents, microbial agents and immunological injury can cause necrosis. Two essential changes bring about irreversible cell injury in necrosis is cell digestion by lytic enzymes and denaturation of proteins. These processes are morphologically identified by characteristic cytoplasmic and nuclear changes in necrotic cell. The cytoplasm appears homogeneous and intensely eosinophilic. Occasionally, it may show vacuolation or dystrophic calcification. The nuclear changes include condensation of nuclear chromatin which may either undergo dissolution or fragmentation into many granular clumps. Centrilobular necrosis frequently exhibits a zonal distribution. The most obvious necrosis of hepatocytes immediately around the terminal hepatic vein, an injury that is characteristic of ischemic injury occurred for a number of drug and toxic reactions. A variable mixture of hepatocellular death and inflammation is encountered. The hepatocyte necrosis may be limited to scattered cells within hepatic lobules. Bridging necrosis is more severe inflammatory injury; necrosis of continuous hepatocytes may span adjacent lobules in a portal to portal, portal to central and central to central fashion. Sub massive necrosis of entire lobules or most of the liver is usually accompanied by hepatic failure. With disseminated candidal or bacterial infection, macroscopic abscesses may occur (Tennant, 1997; Corless and Middleton, 1983).

Hepatotoxicity can affect hundreds of millions of people worldwide. It is the common non- neo plastic cause of death among hepatobiliary and digestive disorders serious side effects, the cost of the modern medicine and improper channel of treatment and competitive efficacy of natural products made the person through the world to look for classical plant drugs for the treatment of hepatotoxicity (Ozer *et al.*, 2008) . In view of the pharmacological and biological properties and chemical constituents of the plant Salvadora Persica, it was decided to an attempt is made to find out the extent of hepatoprotective and antioxidant activity of plant (Halawany, 2012).

MATERIALS & METHODS

Solvent extraction (Hot percolation method)

Preparation of petroleum ether, chloroform and ethanolic extracts of *Salvadora persica*.

Method

The *Salvadora persica* L. plant was collected and identified. The leaf was cut down into small pieces, shade dried and powdered to get moderately coarse powder, which is sieved under mesh. About 500gm of dry powder was extracted with petroleum ether, chloroform and ethanol at 60-70^{o}c by hot continuous percolation using soxhlet apparatus. The extraction was continued for 72hrs. the petroleum ether, chloroform and ethanolic extract was filtered and concentrated to a dry mass by using vaccum distillation the petroleum ether extract (4gms) was obtained as dark green residue. The chloroform extract

(5gms) was obtained as dark brown residue. The ethanolic extract (7.2gms) was obtained as dark brown residue (Jones and Kinghorn, 2005).

Experimental pharmacological studies in animal liver

To investigate and evaluate hepatoprotective substance, it is customary to subject animals to a range of toxic agents. These hepatotoxicants include carbon tetrachloride, D- galactosamine, thioacetamide, ethanol, aflatoxin B1, alpha amanitine, phalloidin, cadmium, paracetamol, hydrazine, halothane, isoniazid etc that causes damage of rat liver, resulting in biochemical and histopathological changes. Different toxicants used for experimental liver damage with dose range, route, vehicle and detailed schedule of treatment.

Induced by ethanol

The basic mechanism in the induction of hepatotoxic by ethanol is principally metabolized to acetaldehyde in the liver and seldom in other tissue by alcohol dehydrogenase as well as CAT(catalase). Acetaldehyde is further oxidized into acetate by acetaldehyde dehydrogenase oxidase.,leading to the generation of ROS/free radical. Ethanol is also oxidised by a microsomal Ethanol oxidising system($CYP2E_1$) to acetaldehyde and 1- hydroxyethyl radical especially following chronic ethanol consumption by which $CYP2E_1$ is induced. Excessive alcohol intake results. and iron overload which further enhance oxidative stress by catalyzing the formation of more noxious hydroxyl free radical**.** Hence induction of $CYP2E_1$and iron overload by ethanol are critical path way by which ethanol generates a state of oxidative stress in hepatocytes (Kanbak *et al*., 2021).

Induced by paracetamol

The mechanism by which over dosage with paracetamol leads to hapatocellular injury and death involves its conversion to the toxic NAPQ1(N- acetyl – Para benzoquinone imines) metabolite. The glucoronide sulfa conjugation pathways become saturated and increasing amount undergo CYP-mediated N - hydroxylation to form NAPQI. This is eliminated rapidly by conjugation with GSH and then further metabolized to a mercapturic acid and excreted into urine. In the setting of paracetamol overdose, hepatocellular level of GSH become depleted. The highly reactive NAPQ1 metabolite binds covalently to cell macromolecules leading to dysfunction of enzymatic system and structural and metabolic disarray further more depletion of intracellular GSH renders the hepatocytes highly susceptible to oxidative stress and apoptosis (Kurtovic and Riordan, 2003).

Induced by CCl_4

CCl_4 induce liver damage by producing free radical intermediates. CCL_4 is converted to trichloromethyl radical (CCl_3) by the P-450 system. Which in turn is converted to Peroxy radical (CCl_3O_2) which causes the damage (Gilani and Janbaz, 1995).

Induced by D- galactosamine

Galactosamine is a hexosamine derived from galactose. It causes liver injury via the generation of free radicals and depletion of UTP nucleotides. Galactosamine produces the hepatotoxic effect by selectively reducing the

uridine pool in hepatocytes. This intern inhibits mRNA and protein synthesis, alters the composition of cellular membranes and finally leads to cellular damage as a result of lipid per oxidation. The hepatocyte death is represented as apoptosis and subsequently necrosis. Other mechanism of galactosamine hepatotoxicity stated that galactosamine increases intestinal permeability and subsequently facilitates bacterial translocation to the liver. Lipo polysaccharides activate kupffer cells to secrete tumor necrosis factor-α ,which raises expression of intercellular adhesion molecule 1 in endothelial cells and this promotes the adhesion of polymorphonuclear cells to vascular and hepatic endothelial cells , leading to polymorphonuclear infiltration and hepatocyte damage. Galactosamine induces rise in SGOT, SGPT and total bilirubin where as decrease in total protein. Galactosamine shows pathological changes like moderate degeneration and necrosis of hepatocyte (Aristatile *et al*., 2009)**.**

Methodology

On day 22 after 24 hrs of Galactosamine administration animals in all the groups were humanely sacrificed using Ketamine HCl and 4ml of blood was withdrawn by cardiac puncture and allowed to clot for 30mins at room temperature. The serum was separated by using cooling centrifuge and used for the assay of marker enzymes viz AST, ALT, ALP, TP, TB,GGPT and total albumin. The livers were dissected out immediately, washed with ice- cold saline and 10% homogenates in phosphate buffer solution (PH 7.4) were prepared Liver homogenate was used for the assay of Lipid peroxidation (Lpo) while some fraction of homogenates were centrifuged at 7000rpm for 10 min at 4^0 C using refrigerated centrifuge, and the supernatants were used for the assay of Superoxide dismutase (SOD), catalase(CAT), Glutathione peroxidase (GPx). Some portion of liver from each group was aseptically excused and stored in 10% formalin for histopathological studies .

Statistical analysis

The Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by NewmannKeul's multiple range tests. The values are represented as Mean $\pm$ SEM. Probability value at $P < 0.01$ was considered as statistically significant.

RESULTS AND DISCUSSION

Biochemical observations

Significant increase in ($P< 0 .01$) Serum Aspartate Transaminase (AST), Alanine Transaminase (ALT) , Alkaline phosphatase (ALP) , Total bilirubin (TB) and Gamma-glutamyl transpeptidase(GGTP) and significant decrease in ($P< 0.01$) Total protein(TP) and Total albumin(TA) levels were observed in animals treated with galactosamine 25mg/kg (Group II) as compared to normal control group(Group I).

Pretreatment with Ethanolic extract of *Salvadora Persica L.* (EECP) at a dose 200 mg and 400mg /kg ,orally for 21days decreased the levels of above indices like AST, ALT, ALP, TB, GGTP and increased levels of TP and TA significantly($P <0.01$)in group IV and V.

Vitamin-E pretreatment produced significant decrease in ($P< 0.01$) serum AST, ALT, ALP, TB, GGTP and significant increase in TP and TA at ($P< 0.01$) in group III.

Biochemical observation in liver homogenate tissue

In liver homogenate, there was significant decrease in SOD, CAT and GPx levels and increase in LPO levels were observed in animals treated with galactosamine 25mg/kg (group II) as compared to normal control group (Group I).

Pretreatment with Ethanolic extract of Salvadora Persica L. (EECP) at a dose of 200mg/kg and 400mg/kg orally for 21 days increase the levels of above indices like SOD,CAT and GPx levels and decrease levels of LPO significantly ($P<0.01$) in group IV and V.

Vitamin-E pretreatment produced significant increase in ($P< 0.01$) Liver homogenate enzyme such as SOD, CAT, GPx levels and decrease the levels of LPO significantly ($P<0.01$) in group III.

Table no shows the levels of non-enzymatic antioxidants such as reduced glutathione, Vitamin C and Vitamin E in the tissues (liver) of D-galactosamine hepatotoxic and control rats. The levels of non-enzymatic antioxidants in D-galactosamine hepatotoxic rats

significantly decreased. EECP both doses administered rats showed significantly increased levels of these non-enzymic antioxidants as compared with untreated hepatotoxic rats.

Histopathological observations

Histology of liver sections of normal control animals (Group I) showed normal liver architecture with were brought out central vein, were preserved cytoplasm and prominent nucleus and nucleolus. The liver sections of galactosamine treated animals (Group II) showed hepatic cells with serum toxicity characterized by inflammatory cell collection, scattered inflammation across liver parenchyma, focal necrosis and swelling up of vascular endothelial cells.

Vitamin-E (Group-III) exhibited protection from galactosamine induced changes in the liver.

Ethanolic extract of *Salvadora persica* L. (EECP) pretreatment at a dose of 200mg and 400mg/kg (group IV and V) appeared to significantly prevent the galactosamine toxicity as revealed by the hepatic cells with were preserved cytoplasms. EECP pretreatment also caused marked decrease in inflammatory cells.

Table No. 1: Effect of *Salvadora persica* L. and *Vitamin E* pre-treatment on biochemical parameters of the rats intoxicated with D-Galactosamine

Group. No.	Treatment dose (mg/Kg)	AST (IU/mL)	ALT (IU/mL)	ALP (IU/mL)	TP (gm/dl)	TB (mg/dl)	GGTP (mg/dl)	Total Albumin(g/dl)
I	Normal control 10ml/kg normal saline	44.40± 1.52	30.09± 1.49	23.68± 1.30	5.15± 0.08	1.92± 0.08	96.90± 2.75	3.80± 0.16
II	Toxic control 25mg/kg D-galactosamine	*a 105.90 ± 2.40	*a 94.49± 1.05	*a 144.10± 2.35	*a 3.16± 0.22	*a 4.40± 0.26	*a 173.42± 2.90	*a 2.20± 0.07
III	Standard control Vitamin E 25mg/kg	*b 60.10± 1.20	*b 40.56± 1.06	*b 56.4± 1.70	*b 3.90± 0.08	*b 2.8± 0.15	*b 122.20± 1.95	*b 2.90± 0.05
IV	Treatment control EECP 200mg/kg	*b 68.65± 1.46	*b 54.82± 2.72	*b 65.86± 2.30	*b 4.60± 0.25	*b 3.30± 0.20	*b 136.30± 3.04	*b 2.54± 0.04
V	Treatment control EECP 400mg/kg	*b 62.45± 1.15	*b 47.94± 0.97	*b 58.50± 1.95	*b 4.05± 0.26	*b 2.95± 0.18	*b 130.94± 1.23	*b 2.30± 0.09

- Values are expressed as Mean ± SEM.
- Values are found out by using one way ANOVA followed by Newmannkeul's multiple range tests.
- *a – values are significantly different from Normal control at $P< 0.01$.
- *b – values are significantly different from Toxic control(G2) at $p< 0.01$.

Table No. 2: Effect of *Salvadora persica* L. and Vitamin E pre-treatment on biochemical liver parameter in D-Galactosamine induced hepatotoxicity

Group. No.	Treatment dose (mg/Kg)	SOD (U/mg) Protein	CATALASE (U/mg) Protein	GPX (U/mg) Protein	MOA (U/mg) Protein
I	Normal control 10ml/kg Normal saline	132.25± 2.40	290.40± 2.40	1.10± 0.05	3.90± 0.17
II	Toxic control 25mg/kg D-galactosamine	*a 68.20± 1.65	*a 190.75± 2.70	*a 0.40± 0.02	*a 7.40± 0.12
III	Standard control Vitamin E 25mg/kg	*b 118.05± 2.80	*b 260.45± 1.92	*b 0.85± 0.02	*b 4.50± 0.14
IV	Treatment control 200mg/kg EEPL	*b 96.50± 1.60	*b 230.05± 1.80	*b 0.55± 0.02	*b 5.60± 0.28
V	Treatment control 400mg/kg EEPL	*b 105.65± 2.62	*b 240.75± 2.65	*b 0.74± 0.02	*b 4.80± 0.08

- Values are expressed as Mean ± SEM.
- Values are finding out by using one way ANOVA followed by Newmannkeul's multiple range tests.
- *a – values are significantly different from Normal control at $P< 0.01$.
- *b – values are significantly different from Toxic control (G2) at $p< 0.01$.

Table No. 3: Effect of EECP on the levels of non enzymatic antioxidants in the liver tissue of d-galactosamine –hepatotoxic and control rats

Groups	Glutathione MG/100G TISSUE	Vitamin-C MG/100G TISSUE	Vitamin-E MG/100G TISSUE
Normal control 10ml/kg normal saline	132.60±3.45	0.82±0.08	5.92±0.60
Toxic control 25mg/kg D-galactosamine	73.55±1.70*a	0.30±0.02*a	2.40±0.30*a
Standard control Vitamin E 25mg/kg	110.32±2.70*b	0.74±0.07*b	5.60±0.55*b
Treatment control EECP 200mg/kg	98.05±2.16*b	0.60±0.04*b	4.92±0.50*b
Treatment control EECP 400mg/kg	91.90±1.95*b	0.69±0.06*b	5.02±0.48*b

- Values are expressed as Mean ± SEM.
- Values are found out by using one way ANOVA followed by Newmannkeul's multiple range tests.
- *a – values are significantly different from Normal control at P< 0.01.
- *b – values are significantly different from Toxic control (G2) at p< 0.01.

Histopathological studies of liver tissue

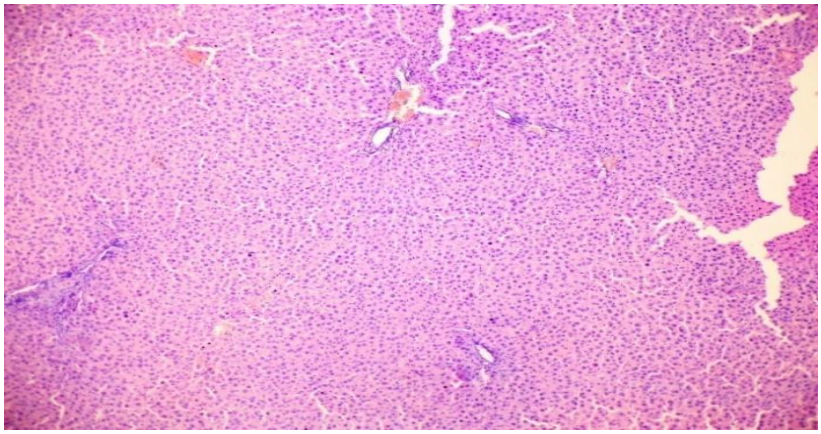

Fig. no. 1: (Liver section of GP_1 (Normal control)

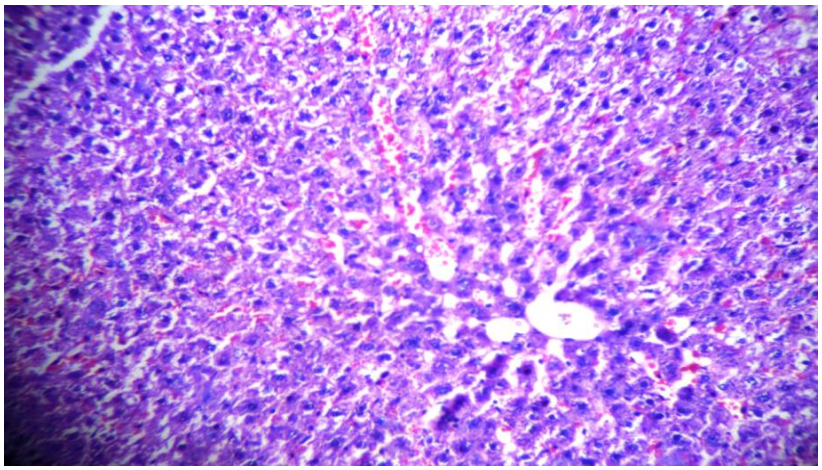

Fig. no. 2: Liver section of GP_2 (toxic control)

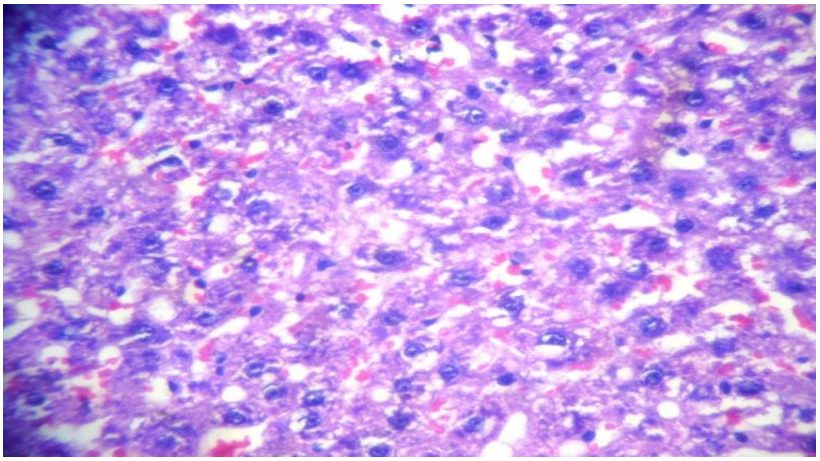

Fig. no. 3: (Liver section of GP_4 (Salvadora Persica L.200 mg/kg/rat)

CONCLUSION

D-galactosamine is a well-established hepatotoxicant that induces a diffuse type of liver injury closely resembling human viral hepatitis. Liver damage induced by D-galactosamine, reflects disturbances of liver cell metabolism, which lead to characteristic changes in the serum enzyme activities. Elevated serum enzymes are indicative of cellular leakage and loss of functional integrity of the hepatocyte.When the liver cell plasma membrane is damaged, a variety of enzymes such as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase ,total bilurubin and gamma- glutamyl transpeptidase are released into the blood stream. Their estimation in the serum is useful as a quantitative marker of the extent and type of hepatocellular damage.

In D-galactosamine induced toxicity, increased activities of aspartate aminotransferase, alanine aminotransferase,alkaline phosphatase,total biluribin and gamma- glutamyl transpeptidase and decrease activities of total protein and total albumin were observed in serum. EECP seems to preserve the structural integrity of the hepatocyte membrane as evidenced from the significant reduction in the activities of these enzymes. The 400mg/kg dose had a better effect than the low dose of EECP (200mg/kg). The higher concentration might have resulted in the production of more by products that would have interfered with the activity. Treatment with EECP significantly decreased these enzyme activities, indicating that EECP has a hepatoprotective effect against a D-galactosamine- induced liver injury.

D-galactosamine-induced oxidative damage is generally attributed to the formation of the highly reactive hydroxyl radical (OH·), the stimulator of lipid peroxidation and the source of destruction and damage to the cell membrane. D-galactosamine toxicity enhanced lipid peroxidation and reduced antioxidants were reported in the kidney. The previous studies show that D-galactosamine-induced rats significantly increased thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes in liver and kidney. In the present study, we observed an increase in the levels of thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes in the tissues of D-galactosamine-hepatotoxic rats. Increased lipidperoxidation in various tissues has long been known to cause functional degradation; thus, the degradation of vital tissue leading to complications may be indirectly due to increased oxidative stress.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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