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#### ABSTRACT

Garcinol (GC), a natural extract obtained from Garcinia species mostly from Garcinia indica has been known for its various biological effects like anti-cancer, anti-oxidant, anti-inflammatory effect. The objective of this study was to evaluate hepatoprotectant effect of GC in various doses in acute paracetamol induced liver injury and chronic alcohol induced liver injury. GC showed significant effect in the pracetamol induced cytokines, Transforming growth factor beta 1, Tumour Necrosis Factor-alpha and Intercellular Adhesion Molecule 1 and also inhibited the alcohol induced increase in Interleukin-12, Interleukin-1beta and Tumour Necrosis Factor-alpha. Both alcohol and paracetamol increased the serum animotransferases levels, GC after oral administration significantly reduced the elevated liver enzymes. Also the drug induced oxidative stress was reversed by GC in dose dependent manner. In conclusion, our study suggests that GC exerted the significant hepatoprotective effect in experimental rodents.

Keywords: Garcinol, GC, Paracetamol, APAP, Ethyl Alcohol, liver injury, hepatoprotection.

#### LIST OF ABBREVIATIONS

ALP- Alkaline Phosphotase ALT- Alanine Transaminase ANOVA- Analysis of Variance APAP – Acetaminophen (paracetamol) AST- Aspartate Transaminase CPSEA- Committee for the Purpose of Control and Supervision of Experiments on Animals CTL- Cytotoxic T Lymphocyte DMN- Dimethylnitrosamine ECM- Extracellular Matrix ELISA- Enzyme Linked Immune-sorbent Assay GC- Garcinol GSH-Glutathione ICAM- Intercellular Adhesion Molecule

Liver disease progresses slowly and usually remain asymptomatic for long duration as hepatic cells reserves high self regeneration and reparative activity. The major function of liver is to metabolize toxins including medications and natural products. Also liver produces bile, IL- Interleukin JNK - c-Jun N-terminal kinases LDH- Lactate dehydrogenase LP- Lipid Peroxidation LPS- Lipopolysaccharide NAPQI- N-acetyl-p-benzoquinone imine NK- Natural Killer ROS- Reactive Oxygen Species SD- Standard Deviation TGF - Transforming Growth Factor Th- T helper cell TLR- Toll-like Receptor TNF- Tumor Necrosis Factor

proteins, clotting factors, synthesises glycogen and manufactures triglycerides and cholesterol and small damage in liver can affect the entire human body. [1]

Paracetamol- Acetaminophen (APAP) is the most widely used analgesic-antipyretic that is safe up to the dose of 4g/day in divided doses,

when dose exceeds the toxic metabolites are formed by cytochrome p-450 which further causes paracetamol to induce hepatotoxicity in absence of or decreased glutathione and cytochrome production.[2,3,4]

Nowadays, excessive intake of alcohol and use of non-steroidal anti-inflammatory drugs and analgesics is the major cause of toxicants induced hepatotoxicity with higher prevalence rate. Main reason for liver related mortality throughout the world is alcoholic liver disease which is induced due to portal concentration of alcohol and its metabolic consequences. [5,6] In average 90% alcoholics who drinks more than 16g/day is affected by steatosis and other forms of alcoholic liver diseases are steatohepatitis, fibrosis and even cirrhosis.[6,7] Usually these damages are noticeable at the later stage and becomes difficult to control and are irreversible. [5]

Natural products are always explored due to fewer side effects which produce the long lasting effect with its natural way of healing most importantly in liver disease through inhibition of tumour growth factor and fibrogenesis, elimination of pathogens and defending the oxidative stress. [8] Garcinol (GC) is one of the natural ingredients found mostly in Garcinia species. It is primarily extracted from Garcinia indica which has been used for many disease ailments since ages. GC is demonstrated as antioxidant, anti-inflammatory, and anticancer agent in numerous studies. [9,10] Comparatively higher level of antioxidant activity of this fruit extract has been reported. It exhibited the 1,1 diphenyl-2-picrylhydrazyl free radical scavenging activity by three times greater by weight in aqueous ethanol solution than the DL- $\alpha$ tocopherol.[9] We investigated GC as the natural hepatoprotectant with varying doses in two different models using paracetamol (chemical) and alcohol(toxins) to induce hepatic damage.

Expression of the pro-inflammatory cytokine tumour necrosis factor (TNF)- $\alpha$  occurs in many acute and chronic liver diseases, as well as following exposure to hepato-toxic chemicals. It helps influence the damage processes that occur following these insults by regulating additional mediators. [11]

Secretion of interleukin (IL)-1 $\beta$  and TNF- $\alpha$  by hepatic macrophages, which are accumulated in the liver during chronic hepatic inflammation, has been reported. [12] Increased hepatic and systemic injury is related with a high production

of pro-inflammatory cytokines such as  $TNF-\alpha$ . Interleukin-2, Interleukin-4, as well as growth factors viz. transforming growth factor (TGF)-β and intercellular adhesion molecule (ICAM) 1 Toll-like Receptor (TLR)-4 signalling in hepatic stellate cells participate to the development of fibrosis by enhancing alcoholic TGF-β signalling. During liver disease progression, factors/cytokines and both growth the Extracellular matrix (ECM) alter the TGF-B1 signals which stimulates the increase in ECM accumulation leading to fibrosis. [13]

Interleukin (IL)-12, a proinflammatory cytokine produced by antigen-presenting cells upon stimulation by various stimuli enhances cytotoxic activity of NK cells and CTL and also plays a role of growth factor for NK, T and NKT cells promoting the development of Th1 cells. [14] IL-12 levels have been demonstrated to increase in chronic alcoholism. Recently its relationship with the different stages of alcoholic liver disease along with the status of alcoholism is reported. [15]

Paracetamol absorption prolongs with the overdose and further increases the saturation of glucuronidation pathway of metabolism which leads to increased production of reactive metabolites. This toxic metabolite mediates hepatotoxic effect of paracetamol leading to cell injury.[16] Liver damage results in elevated serum enzymes levels due to cellular leakage and loss of functional integrity. [2, 3, 4]

The alcohol-induced liver disease comprises increased gut-derived lipopolysaccharide (LPS) entry into the liver. LPS/TLR-4 activates Kupffer cells and produce TNF- $\alpha$  and various other cytokines and growth factors, acts on alcohol exposed hepatocytes and induce apoptosis. [6,12]

### MATERIALS AND METHODS

#### Garcinol

Garcinol (GC) ( $C_{38}H_{50}O_6$ ), polyisoprenylated benzophenone derivative was extracted from *Garcinia indica* fruit rind.[9] It has the molecular weight of 602.39252 and melting point of 122°. As indicated by molecular formula and absorption spectral data GC is probably related to the isomeric Xanthochymol and as per optical rotation to the Cambogin. The existence of a saturated carbonyl group (1727 cm<sup>-1</sup>) and two  $\alpha$ ,  $\beta$ -unsaturated carbonyl groups (1668 and 1642 cm<sup>-1</sup>) are shown by Infrared (IR) spectrum of the trimethyl ether, accountable for all the oxygen atoms.[17]

As mentioned in our previous paper dried Garcinia indica fruit rind was extracted with hexane and fractionated bv column chromatography. Hexane extract was completely crystallized in ethanol and vacuum dried. Thus, obtained residue of GC was dissolved in cold hexane and re-crystallized at room temperature to obtain as pale yellow needle like crystals. The acquired GC from the above process was standardized with microcrystalline cellulose powder. [18]

#### Chemicals

The entire chemicals used were of analytical grade. The chemicals and reagents used were purchased from Sigma Aldrich (St. Louis, MO, USA). We used an enzyme-linked immunoassay kit for cytokines from liver and blood of rats (R & D Systems Inc., Minneapolis, Minnesota, USA).

#### Animals

Male Wistar Rats of 180-200gms, housed maximum of 6 per cage in a polypropylene cage with maintained room temperature of 20.2-23.5 °C, relative humidity of 30 - 70% with 12h fluorescent light and 12h dark cycle were used in the studies. Animals were fed with rodent feed and purified water that was provided *ad libitum*. Animals were kept in sterilized rice husk beddings changed along with the cage twice a week during acclimatization and entire experimental study period.

#### **Ethics**

The hepatoprotectant effect of GC studies were conducted accordance in with the recommendation of the committee for the purpose of control and supervision of experiments on animals (CPCSEA) guidelines for laboratory animal facility published in the gazette of India, December 15th 1998. The protocols of the studies were also approved by Institutional Animal Ethics Committee with the approval number of ACP/IAEC/03/2009.

#### **EXPERIMENTAL DESIGN**

#### Acute Paracetamol Induced Hepatotoxicity

The individual freshly prepared dose of test item, standard and toxicant were calculated as per the body weight which was measured on day 1 of the study. The graded doses of GC (1.25, 2.50, 5.00 and 10 mg/kg, p.o), Silymarin (50 mg/kg, p.o)) were administered. Experimental animals orally received paracetamol (400 mg/kg body weight) for seven days. Six rats each in seven groups were used in this study. Out of remaining two groups one was given the same dose of paracetamol which served as toxin control group and to the other the proportionate volume of vehicle was given which served as vehicle control. Blood and liver samples were collected in humanly manner, 2h after the last treatment for different estimations.

#### **Chronic Alcohol Induced Hepatotoxicity**

Seven groups with six rats each were used in this study. The graded doses of GC (1.25, 2.50, 5.00 and 10 mg/kg, p.o), Silymarin (50 mg/kg, p.o)) were administered. Experimental animals orally received alcohol (400 mg/kg body weight) for seven days. Out of remaining two groups one was given the same dose of alcohol which served as toxin control group and to the other the proportionate volume of vehicle was given which served as vehicle control. The dose of the test item, standard and toxicants were estimated according to the individual body weight of rats measured at day 1 of the study. Blood and liver samples were collected 2h after the last treatment for different estimations in chronic alcohol induced liver toxicity model.

#### **Blood Biochemistry**

Blood samples were collected in glass tubes from orbital sinus to obtain haemalysis free clear serum for the analysis of Alanine transaminase (ALT) & Aspartate Transaminase (AST) [19], Alkaline Phosphotase (ALP) [20], and bilirubin [21] by standard methods in both the studies.

#### Measurement of Oxidative Stress Markers from Liver Homogenate

All the animals were sacrificed and livers were quickly excised freed from any adhering tissues, washed and perfused with chilled Normal saline, minced and homogenized in ice bath using homogenizer (1100 rpm for 2 minutes) in chilled 10mM Tris-HCl buffer (pH 7.4) to obtain 10% liver homogenate for the estimation glutathione(GSH) [22] and lipid of peroxidation(LP) [23], by using standard methods in both acute paracetamol induced hepatotoxicity and chronic alcohol induced liver toxicity model studies.

# Measurement of Cytokines from Serum and Liver Homogenate

All the cytokines (TGF- $\beta$ 1, ICAM-1, TNF- $\alpha$ , IL-12, IL-1 $\beta$ ) from Liver homogenate and serum were analysed using Enzyme linked Immunosorbent Assay (ELISA) adhering to the user manual of the manufacturers.

#### **STATISTICAL ANALYSIS**

Raw data was analysed using the statistical Graphpad software. The data of each group were analyzed by analysis of variance (ANOVA) and expressed as mean  $\pm$  standard deviation (SD). *T*-test was used to compare the difference between the treated and control groups and treated with standard group. The statistical significance of the differences between groups was determined by one-way ANOVA. All analyses and comparisons were evaluated at a probability level of 10% ( $p \leq 0.01$ ). Percentage change was assessed for categorical data.

#### **RESULTS**

## TGF-β1 expression in liver homogenate of Paracetamol treated rats

TGF- $\beta$ 1 in the liver homogenate was measured to evaluate the effect of GC in paracetamol treated rats comparative to Silymarin (**figure1**). There was significantly high expression of TGF- $\beta$ 1 in paracetamol alone treated group as compared to control as well as other groups treated with GC or Silymarin. GC at the dose of 5 & 10mg/kg/day showed the better effect than 1.25, & 2.5mg/kg/day dose groups which was comparative to Silymarin treated group.



**Figure 1.** Effect of different doses of GC on TGF- $\beta$ 1 expression in liver homogenate of Paracetamol treated rats

ICAM-1expression in liver homogenate of Paracetamol treated rats

Increased ICAM-1 in liver homogenate due to paracetamol induced liver damage was

suppressed by various doses of GC (**figure2**). 5 & 10 mg/kg/day showed higher effect than lower doses of GC but were slightly lower than Silymarin.



Figure2. Effect of different doses of GC on ICAM-1expression in liver homogenate of Paracetamol treated rats

Effect of GC on TNF- $\alpha$  in paracetamol treated rats.

GC in various doses inhibited the serum TNF- $\alpha$  expression in the paracetamol induced hepatic

damage (**figure3**). Effect of GC was significantly increased according to dose increment but 5mg/kg p.o dose group showed better effect than 10 mg/kg p.o dose which was comparative to the Silymarin treated group.



**Figure3.** Flowcytometry histogram plot showing one representative GC effect on TNF- $\alpha$  of paracetamol treated rats.

Effect of GC on biochemical parameters of paracetamol treated rats.

Serum biochemical parameters of paracetamol treated rats are as shown in (**table 1**). The activity of Aspartate Aminotransferase, Alanine Aminotransferase and Alkaline phosphotase was significantly increased in paracetamol only

treated group as compared to control. Further, GC from the dose of 2.5mg/kg showed the significant effect increased activity of AST, ALT and ALP. There was lower percentage change in ALP but was significant in the doses of 5mg/kg p.o. and 10mg/kg p.o GC treated group.

**Table1.** Effect of GC (GC) against oxidative stress parameters of Paracetamol (APAP) induced liver damage in rats

Hepatic parameters						
Treatments	Dose mg/kg	GSH µmoleGSH/g	LP µmoleMDA/g liv.	Average Protection%		
Vehicle	-	9.54±0.12	$40.88 \pm 2.48$	-		
Vehicle + APAP	-	5.24±0.46	72.20±2.40	-		
GC+APAP	1.25	5.96±0.56 (13.74)	66.50±4.20 (7.89)	10.81		
GC+APAP	2.5	6.32±0.26* (20.61)	60.24±2.40 (16.56)	18.58		
GC+APAP	5.0	6.88±0.42** (31.29)	52.24±2.60 (27.64)	29.46		
GC+APAP	10.0	7.04±0.36** (34.35)	50.48±1.60** (30.08)	32.21		
Sily + APAP	50	7.45±0.28** (42.17)	48.86±1.60** (35.09)	38.63		

Sily: Silymarin; (Values as Mean  $\pm$  SE, n = 6); Percent change in parenthesis;

P value \*: < 0.01; \*\*: <0.001. (LP) Lipid peroxidation; (GSH) Glutathione reductase

## Effect of GC on Oxidative parameters of paracetamol treated rats

Reduction in glutathione reductase due to paracetamol treatment was thus improved by

GC (**table 2**). The percentage protection was dose dependent; higher the dose better te protection. Lipid peroxidation increased by paracetamol was suppressed by GC significantly in higher dose.

Table2. Effect of GC (GC) against Paracetamol (APAP) induced biochemical changes in rats

Treatments	Dose	ALT µmole/min/lt AST µmole/min		ALP	Average
	mg/kg			µmolePNP/min/lt	Protection%
Vehicle	-	98.32±5.20	117.49±8.26	21.42±2.04	-

Vehicle + APAP	-	1102.10±46.98	839.79±59.17	40.54±3.61	-
GC+APAP	1.25	862.97±53.38	761.61±43.96 (9.30)	37.21±3.44 (8.21)	
		(21.69)			13.06
GC+APAP	2.5	808.64±38.55*	650.88±35.84*	34.28±1.98 (15.44)	
		(26.62)	(22.49)		21.51
GC+APAP	5.0	661.67±51.40**	463.41±38.56**	30.74±2.05** (24.17)	
		(39.96)	(44.81)		36.31
GC+APAP	10.0	590.22±50.98**	536.39±44.13**	33.60±1.93* (17.11)	
		(46.44)	(36.12)		33.22
Sily + APAP	50	377.04±14.44**	379.64±23.22**	26.90±2.24** (33.64)	
-		(65.78)	(54.79)		51.40

*Values as Mean*  $\pm$  *SE*, n = 6*); Percent change in parenthesis; P value* \*: < 0.01*;* \*\*: < 0.001*. (ALT) Alanine transaminase; (AST) Aspartate aminotransferase; (ALP) Alkaline phosphatase.* 

Effect of GC on expression of TNF- $\alpha$  and IL-1 $\beta$  in serum of animals from Ethyl alcohol induced liver damage

There was significant up regulation of proinflammatory cytokine (IL- $1\beta$ ) and

inflammatory cytokine (TNF- $\alpha$ ) in the serum of ethyl alcohol treated rats (**figure 4**). GC showed its significant effect to decrease the serum level of IL-1 $\beta$  and TNF- $\alpha$ . The effect of GC in the dose of 5mg/kg p.o expressed higher effect in comparison to Silymarin treated group.



(Values as Mean ± SE, n = 6); P value \* : <0.01., \*\* : <0.001 GC : Garcinol;)

**Figure4.** Effect of GC on expression of TNF- $\alpha$  and IL-1 $\beta$  in serum of animals from Ethyl alcohol induced liver damage

# Effect of GC on expression of IL-12 in serum of animals from Ethyl alcohol induced liver damage

Ethyl alcohol subsequently increased the level

of IL-12, GC in various doses significantly reduced the level of IL-12 (**figure 5**). GC in dose group 5mg/kg showed better reduction than Silymarin treated group.



**Figure5.** *Effect of GC on expression of IL-12 in serum of animals from Ethyl alcohol induced liver damage* **Effect of GC in serum biochemical parameters in Ethyl Alcohol induced liver damage** 

Alcohol induced elevation of biochemical parameters were reduced with GC treatment in different doses comparatively similar to Silymarin (table 3).

Treatments	Dose	ALT µmole/min /	AST	ALP µmole PNP	Bilirubin	Average
	mg/kg	ltr	µmole/min/lt	/min/lt	mg%	Protection%
Vehicle	-	118.10±10.20	106.40±16.60	32.30±0.52	$0.44 \pm 0.08$	-
Vehicle+ ETOH	-	$1820.08 \pm 42.80$	1076.32±48.22	104.22±8.60	$2.08 \pm 0.02$	-
GC + ETOH	1.25	1320.40±52.20	858.80±32.64	88.68±8.58	$1.94 \pm 0.02$	17.32
		(27.45)*	(20.20)	14.91)	(6.73)	
GC + ETOH	2.5	1080.50±48.56**	726.78±46.50*	76.12±4.88**	$1.68 \pm 0.04*$	29.82
		(40.63)	(32.47)	(26.96)	(19.23)	
GC + ETOH	5.0	876.58±76.80**	650.26±56.20**	68.32±5.60**	1.42±0.06**	39.39
		(51.83)	(39.58)	(34.44)	(31.73)	
GC + ETOH	10.0	870.80±48.40**	632.48±44.56**	69.00±4.82**	1.38±0.04**	40.20
		(52.15)	(41.23)	(33.79)	(33.65)	
Sily + ETOH	50	854.88±38.46	662.70±38.40	66.54±6.30**	1.34±0.02**	40.79
		(53.03)	(38.44)	(36.15)	(35.57)	

Table 3. Effect of GC (GC) in serum biochemical parameters in Ethyl Alcohol induced liver damage

*Ethyl alcohol (Value Mean*  $\pm$  *SE, n* = 6); *Percent change in parenthesis; P value* \*: < 0.01; \*\*: < 0.001. (*ALT*) *Alanine transaminase; (AST) Aspartate aminotransferase; (ALP) Alkaline phosphatise.* 

## Effect of GC against the alcohol induced oxidative stress parameters

Glutathione reductase and lipid peroxidation was measured in liver homogenate of ethyl

alcohol induced liver damaged rats (**table 4**). GC increased the Glutathione reductase level which was significantly decreased by ethyl alcohol and reduced the lipid peroxidation rate.

 Table4. Effect of GC (GC) against Ethyl Alcohol induced liver damage in rats

Treatments	Dose mg/kg	GSH µmoleGSH/g	LP µmoleMDA/g liv.	Average
				Protection%
Vehicle	-	9.62±0.16	44.00±1.60	-
Vehicle + ETOH	-	4.24±0.12	94.60±4.20	-
GC + ETOH	1.25	4.92±0.22 (16.03)	83.50±6.20 (11.73)	13.88
GC + ETOH	2.5	5.42±0.2* (27.78)	73.64±4.00* (22.15)	24.96
GC + ETOH	5.0	6.04±0.20** (42.45)	62.44±4.60** (33.99)	38.22
GC + ETOH	10.0	6.40±0.24** (50.94)	62.42±3.80** (34.01)	42.47
Sily + ETOH	50	6.95±0.20** (63.91)	58.88±1.40** (37.75)	50.83

Sily: Silymarin; EtOH : Ethyl alcohol (Values as Mean  $\pm$  SE, n = 6); Percent change in parenthesis; P value \*: < 0.01; \*\* : <0.001. (LP) Lipid peroxidation; (GSH) Glutathione reductase.

#### DISCUSSION

Garcinol is a polyisoprenylated benzophenone derivative extracted from Garcinia indica fruit rind. Its use in traditional and folk medicine is apparent since ancient time and recently its various clinical benefit including anticancer, antioxidant and anti-inflammatory activities has been studied.[9] It is a potent hepatoprotectant protection effect in dimethy and its Initrosamine (DMN) induced liver injury was recently explored. [24,25] Paracetamol is the most commonly used antipyretic and analgesic with hepatoxic effect when used exessively. Similarly extreme use of alcohol triggers the hepatic injury. So, we used paracetamol and alcohol as the toxicants and studied the hepatoprotectant effect of GC in experimental rats.

In this study GC was found to decrease the elevated biomarkers in both acute paracetamol induced hepatotoxicity and chronic alcohol

induced hepatotoxicity. Drug induced hepatitis was monitored by various serum tests and cytokines test in liver homogenate.

Paracetamol is assumed to be most commonly used medication around the globe due its availability over the counter in various dosage forms.[26] Davidson and Eastham reported that paracetamol in overdose produces hepatotoxic effect for the first time in the year 1996.[27] Paracetamol in normal dose is metabolized into N-acetyl-p-benzoquinone imine (NAPQI) and glutathione further detoxifies to the non-toxic metabolite (APAP-GSH) but when dose exceeds the therapeutic level, triggers the hepatic injury due to covalent bond between NAPQI and hepatic cells and reactions with hepatic proteins. [28,29]

TGF- $\beta$  is the major profibrogenic cytokine which enhances the damage to epithelial cells inducing oxidative stress and myofibroblast activation.[30] In our study Silymarin and GC

inhibited the TGF- $\beta$ 1 restoring a regenerative reaction in acute hepatitis.

ICAM-1, a marker tissue damage is expressed after chronic paracetamol use and controls the enhanced migration of lymphocytes into the sites of inflammation binding to the b2 integrins lymphocyte function associated with antigen -1.[31] GC significantly reduced the increased ICAM-1 level in dose dependent manner in Paracetamol treated rats.

TNF- $\alpha$ , one of the apoptosis-inducible factors and has possible mechanism in progression of liver injury.[32,33] It is evidenced that TNFalpha-TNF-Rp55 axis plays major role in pathogenesis of paracetamol induced liver damage. [34] GC significantly inhibited the TNF- $\alpha$  released post paracetamol ingestion in a dose dependent manner.

Serum aminotransferases i;e AST, ALT, total bilirubin and ALP concentration elevates as a result of liver injury proportional to the severity both in acute and chronic condition.[35,36] Silymarin reduced the elevated serum enzymes by almost 42% and there was comparable reduction up to 35% in GC treated groups probably as a result of repair and regeneration of hepatic cells.

As a result of NAPQI protein binding mitochondrial dysfunction occurs resulting in the glutathione depletion and oxidative stress.[37] GC reversed the oxidative stress parameters, thereby, suppressing the lipid peroxidation and elevating levels of Glutathione reductase in paracetamol induced liver disease.

Alcohol induced liver disease is highly accountable for the alcohol related mortalities through hepatocellular injury, fat accumulation and inflammation and even the liver cirrhosis and carcinoma.[38] The systemic manifestations of alcohol induced liver inflammation are characterized by cytokine production.[39] Kupffer cells generates ROS which activates the JNK and enhanced secretion of chemokines and cytokine including TNF- $\alpha$ .[40] GC in various doses suppressed the expression of TNF- $\alpha$ induced by alcohol intake in rats. The progression of several types of chronic inflammatory diseases is triggered through. IL- $1\beta$  an inflammatory cytokine that signals through IL-1 receptor 1 (IL-1R1) leading to an cascade.[41,42,43] inflammatory Alcohol increased the IL- $\beta$  which was significantly reduced by GC and Silymarin in the experimental rats.

IL-12 is produced by Kupffer cells in endotoxemia developed due to chronic alcohol intake further increases the production of antiinflammatory cytokines. It also induces the expression of interferon  $\gamma$  in T cells and natural killer cells, which leads to macrophage activation, impairment of microcirculation and inflammation.[44,45,46] increased GC significantly reduced the IL-12 produced by alcohol ingestion possibly by repairing the microcirculation further decreasing the inflammation.

As the part of liver function test, commonly used biomarkers for liver injury are ALT, AST, ALP and bilirubin and specifically ALT in serum indicates loss of hepatocyte membrane integrity.[47,48] Serum level of liver enzymes ALT, AST, ALP and bilirubin was evident post alcohol administration in rats which was reduced by GC. The oxidative stress and lipid peroxidation markers increased after alcohol intake was subsequently reduced by GC in similar pattern to the standard Silymarin.

#### CONCLUSION

GC inhibited the TGF- $\beta$ 1, ICAM-1 and TNF- $\alpha$ elevated in paracetamol induced liver injury. Also inhibited increased levels of TNF- $\alpha$ . IL- 1 $\beta$ and IL-12 induced by TLR-4 activation of kupffer cells by LPS of gram negative bacteria in the gut the activation and excessive growth of which is due to ethyl alcohol intake. GC reduced the hepatitis induced increased levels of AST, ALT, ALP and Bilirubin. GC reversed the paracetamol and ethyl alcohol induced oxidative stress. thereby, suppressing the Lipid peroxidation and elevating levels of Glutathione reductase. We can conclude from the above studies GC is potent natural hepatoprotectant which can further be explored through targeted studies for better understanding and its exact mechanism involved in hepatoprotection.

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