Applied Biological Research 24(3): 376-387; (2022)
DOI: 10.5958/0974-4517.2022.00046.5

HEPATOPROTECTIVE POTENTIAL OF MALABAR TAMARIND (*Garcinia gummi-gutta*) FRUIT-RIND EXTRACT IN ACETAMINOPHEN-INDUCED TOXICITY THROUGH MODULATION OF *CYP2E1* GENE EXPRESSION IN HEPATIC CELLS OF WISTAR RATS

A.V. Rehna, A K. Deepa, P.T.A. Usha, Preethy John, R. Uma[1], Maria Lukose and G. Anu

Department of Veterinary Pharmacology &Toxicology, [1]Department of Veterinary Biochemistry, College of Veterinary & Animal Sciences, Mannuthy, Thrissur - 680 651, Kerala (India)
*e-mail: *avrehna@gmail.com*

(Received 22 June, 2022; accepted 31 August, 2022)

ABSTRACT

The present study was aimed to assess the hepatoprotective effect of ethanolic extract of *Garcinia gummi-gutta* (GGE) fruit rind on acetaminophen-induced toxicity in a rat model and to explore the possible mechanism of action by assessing hepatic histopathology, serum biochemical parameters and expression of *CYP2E1* gene in hepatic cells. The group I and 11 animals served as normal control and hepatotoxic control group, respectively; while group III, IV and V were administrated with GGE fruit-rind extract @ 50, 100 & 200 mg kg^{-1} orally, respectively, for 10 days. The group VI (positive control) received drug silymarin @ 100 mg kg^{-1} day^{-1} orally for 10 days. Hepatotoxicity was induced by single oral administration of acetaminophen (2 g kg^{-1}) on 8^{th} day. The results revealed that the extract produced dose-dependent hepatoprotective effect against acetaminophen-induced hepatic damage by improving the serum biochemical profile towards the normal range. Histopathology of liver showed that pretreatment with fruit-rind extract attenuated the toxicant-induced hepatocellular necrosis and enhanced the regeneration of hepatic cells. The hepatic expression level of *CYP2E1* gene was found down-regulated in a dose dependent manner in extract-treated group as compared to the normal control group. The study revealed remarkable hepatoprotective effect of GGE in acetaminophen-induced toxicity in rats through regeneration of hepatic cells and by decreasing the formation of reactive metabolite, NAPQ1 by modulation of *CYP2E1* gene expression in liver.

Keywords: Acetaminophen, CYP2E1, *Garcinia gummi-gutta*, liver, Wistar rats

INTRODUCTION

Liver is the largest visceral organ in human body and serves as the chief site of metabolism and excretion. It plays a pivotal role in the regulation of numerous interrelated vital physiological processes and biochemical pathways of body responsible for growth, immunity, homeostasis, metabolism of carbohydrate, protein and fat, secretion of bile, blood coagulation and detoxification of exogenous and endogenous substances (Stanger, 2015). The maintenance of a healthy liver is a crucial for good health. Continuous exposure to xenobiotics, pollutants, toxic chemicals, food additives, heavy metals, alcohol, infections, prescribed and over-the-counter drugs can eventually lead to several liver pathophysiological problems like hepatitis, hepatic encephalopathy, abscess, cirrhosis, metabolic liver disease, carcinoma etc. (Brennan *et al.*, 2021; Thuluvath *et al.*, 2021).

Acetaminophen (N-acetyl-*p*-aminophenol, acetaminophen, APAP) is a widely used anti-pyretic and analgesic agent which is considered safe at therapeutic doses, but may cause fatal liver damage in human and animals due to over-dosage. Drug-induced intrinsic hepatotoxicity produced by acetaminophen is a persisting and challenging critical global health issue which accounts for >50% acute liver failure cases in the world (Katarey *et al.*, 2016). In conditions of acetaminophen overdosage, the toxicity is initiated by P450-mediated reactions, convert APAP to the reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI), causing depletion of hepatic glutathione antioxidant defense system and subsequent oxidative stress (Das *et al.,* 2010; Hinson *et al.,* 2010; Raskovic *et al.*, 2014). Cytochrome P450 2E1 (*CYP2E1*) that is highly expressed in liver has been reported to be the principal cytochrome P-450 gene involved in the conversion of acetaminophen to NAPQI (Manyike *et al.*, 2000; Jing *et al.*, 2015). The reactive metabolite binds covalently to cellular proteins, induce cellular and organelle oxidative stress (e.g. mitochondrial or endoplasmic reticulum stress), mitochondrial dysfunction, lipid peroxidation, formation of mitochondrial protein adducts, derangement of calcium homeostasis and either lead to lethal consequences like development of acute hepatic necrosis or activation of apoptosis (McGill *et al.*, 2012; Yuan *et al.*, 2013; Iorga *et al.*, 2017; Noureddin *et al.*, 2018). The consequent damage to hepatic cellular membrane and subsequent leakage of hepatocellular enzymes into blood results in increased serum levels of hepatic cellular enzymes alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and hyperbilirubinemia due to failure of excretory function of the liver and blockage of biliary tract. (Thomas *et al.,* 2012; Islam *et al.,* 2021).

The effective therapeutic interventions for liver disease are unduly scarce despite the momentous advances in modern medicine and molecular pathophysiology of hepatic diseases. Reports indicate that there are very few reliable liver protective safe drugs that stimulate liver function, protect it from damage and promote regeneration of hepatic cells. This shifted the focus to explore the plant-based traditional systems of medicine that were used for treatment of liver disease due to their safety, efficacy and cost effectiveness. The therapeutic potential of medicinal plants and their formulations in hepatoprotective activity can plausibly be attributed to the presence of free radical scavenging antioxidant phytomolecules notably the phenolic compounds (flavonoids and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids and amines), lignans, carotenoids and terpenes. Studies have shown phytomolecules are capable of mitigating the role of free radicals in oxidative processes and lipid peroxidation in hepatotoxicity by virtue of their intrinsic ability to scavenge reactive oxygen species and augment glutathione activity at cellular level (Ganesan *et al.,* 2017; Tungmunnithum *et al.,* 2018).

Garcinia gummi-gutta (L) Roxb. Syn. *Garcinia cambogia*, popularly known as Malabar tamarind/ Kudampuli and belonging to the family *Cluisaceae,* is a dicotyledonous medium-sized tree distributed widely in evergreen forest of southern and eastern India and Andaman Islands. A semi-domesticated crop in Kerala, the fruits of this tree are ovoid in shape with 6-8 seeds surrounded by a succulent aril. It flowers in summer season and the fruits ripen in rainy season (Raghavendra *et al.,* 2017). The fruits are used in Ayurvedic medicine for the clinical management of aliments like haemorrhoids, diarrhea, rheumatism, etc. and also used as a flavor condiment and preservative for fish (Samarajeewa and Shanmugapirabu, 1983). Studies have shown that *G. gummi-gutta* possesses hypolipidemic (Guillén-Enríquez *et al.,* 2018), antioxidant (Barathane e*t al.,* 2020), immunomodulatory (Mahendran *et al*., 2001), anticancer (Mazzio and Soliman, 2009), antifungal (Dhanya *et al.,* 2014), anti-inflammatory (Prasanth *et al.,* 2013), anthelminthic (Mathew *et al*., 2011) and antibacterial properties (Sumayya *et al.,* 2016). Even though *G. gummi-gutta* (GGE) has many medicinal properties, reports on *in vivo* studies on hepatoprotective potential of its fruit rind on acetaminophen induced hepatotoxicity are scanty (Semwal *et al.,* 2015). Considering the high incidence of acetaminophen-induced acute liver failure cases amid growing demand for novel hepatoprotective agents, the present study was aimed to evaluate the hepatoprotective of fruit rind extract of *G. gummi-gutta* on acetaminophen-induced toxicity in rats and elucidate the mechanism underlying the protective effect by assessing the expression level of *CYP2E1* gene in rats.

MATERIALS AND METHODS

Plant source and preparation of crude extract

The fresh fruits of *G. gummi-gutta* were procured locally from Thrissur, Kerala (India) and its identity authenticated by National Institute of Science Communication & Information Resources, New Delhi, India. The fruit rinds were cut into small pieces, shade-dried and pulverized using an electrical pulverizer. The powdered material was extracted using Soxhlet apparatus with 95% ethanol. The ethanolic extracts were concentrated in a rotary vacuum evaporator (Equitron, Germany) under reduced pressure and temperature (55°C); and stored under refrigeration till further use. The yield of extract was 44% on dry matter basis.

Chemicals, drugs and diagnostic kits

All the chemicals used were of analytical grade. The chemicals were procured from Thermo Scientific and Sigma-Aldrich, USA, Merck and HiMedia, India. The reference drug Silymarin, used in this study, was purchased from Micro Labs Ltd, India. Diagnostic kits procured from Agappe Diagnostics Ltd, Ernakulam, Kerala were used for the estimation of serum ALT, AST and ALP.

Acute toxicity studies

Acute oral toxicity study was performed as per the guidelines of OECD (Test No. 423) for testing of chemicals (OECD, 2001). The 90-day old adult female Wistar rats (n = 3 for each test dose) weighing 150-200 g, were selected by random sampling technique. The animals were fasted overnight prior to the experiment and maintained under standard laboratory conditions. Afterwards the ethanolic extract of *G. gummi-gutta* fruit rind extract was given at a dose level of 500, 1000 and 2000 mg kg^{-1} body weight (Ghosh, 1984; Babu *et al.*, 2011). The rats were observed for 1 h continuously and then hourly for 4 h and finally after every 24 h up to 14 days for any physical signs of toxicity (writhing, gasping, palpitation and decreased respiratory rate) and mortality.

In vivo evaluation of hepatoprotective activity

Test animals: Forty-eight adult Wistar male rats (150-200 g), procured from Small Animal Breeding Station, Kerala Veterinary & Animal Sciences University, Thrissur, Kerala (India), were used in the study. The animals were housed in clean polypropylene cages in a well-ventilated temperature-controlled room (22 ± 2°C) with relative humidity (44-55%) under 12 h light and dark cycles. An acclimatization period of 7 days was allowed before the commencement of experiment. The animals were provided with a standard rodent pellet diet and clean drinking water *ad libitum.* The animals were handled and used in accordance with the guidelines established by the Institutional Animal Ethical Committee constituted under the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment, Forest and Climate Change, Government of India. The experimental procedure was approved by the Institutional Animal Ethical Committee (IAEC) of College of Veterinary and Animal Sciences, Mannuthy constituted as per CPCSEA guidelines.

Experimental design: Animals were randomly divided into six groups of eight animals each. The group 1 served as normal control and received only the vehicle (distilled water at a dose rate of 10 mg kg^{-1}) orally throughout the duration of experiment. The group 2 served as hepatoxicity control and received distilled water for 7 days followed by a single dose of acetaminophen @ 2 g kg^{-1} on 8^{th} day. The group 3, 4 and 5 received 50, 100 and 200 mg kg^{-1} of ethanolic fruit rind extract of *G. gummi-gutta* (GGE) dissolved in distilled water once daily for 7 consecutive days followed by a single dose of acetaminophen (2 g kg^{-1}) on 8^{th} day. A modified experimental procedure of Khatib *et al.* (2010) and Folarin *et al.* (2014) were adopted for the present study. The group 6 received standard drug silymarin (Tab. Silybon - 70 mg) @ 100 mg kg^{-1} day^{-1} orally, dissolved in distilled water once daily for 7 consecutive days followed by a single dose of acetaminophen @ 2 g kg^{-1} on 8^{th} day (Keshari *et al.*, 2019; Sarkar *et al.*, 2020). The animals were sacrificed 24 h after

acetaminophen administration by cervical dislocation. Blood was collected from retro-orbital plexus under anesthesia in sterile centrifuge tubes without anticoagulant. The serum was separated for carrying out different biochemical assays. Liver sections were kept in 10% formalin for histo-pathological studies and in RNA *later*[@] (Sigma-Aldrich, USA) at -80°C for assessing the expression level of *CYP2E1* mRNA in hepatic cells by gene expression studies using real time (RT)-qPCR.

Measurement of serum liver function biomarkers

The collected blood was allowed to clot and serum was separated after centrifugation at 3200 rpm for 10 min. Serum was used for the estimation of biochemical parameters like serum total protein, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and serum alkaline phosphatase (ALP) in semiautomatic blood analyzer (Hospitex, Italy) using diagnostic kits obtained from Agappe Diagnostics Ltd., India according to the instructions of the manufacturer.

Histopathology

The liver tissue was dissected out and representative tissue samples were fixed in 10% neutral buffered formalin (NBF). It was then dehydrated in ethanol (50-100%), cleared in xylene and embedded in paraffin. The 4-5 µ sections were stained with haematoxylin and eosin (H& E) stains for photomicroscopic observations to study the histopathological changes.

Real-time quantitative polymerase chain reaction (RT-qPCR) for CYP2E1 expression

The expression of *CYP2E1* gene was evaluated using real time relative quantitative polymerase chain reaction (RT-qPCR). Total RNA was isolated from the liver tissue of animal by using GenElute mammalian total RNA kit (Sigma-Aldrich, USA). Extracted RNA was quantified spectrophotometrically at 260/280 nm (NanoDrop™ 1000 spectrophotometer, Thermo Scientific, USA) and integrity was checked using 1.5% agarose gel electrophoresis. Total RNA (500 ng) was reverse-transcribed into complementary DNA (cDNA) using a High Revert Aid first strand cDNA synthesis kit (Thermo scientific, USA) as per manufacturer's protocol.

Quantitative real-time PCR (RT-qPCR) was performed using the Power SYBR Green PCR Master Mix reagent (Applied Biosystems, USA) in a final reaction volume of 20 µL as per the manufacturer's instructions. The forward and reverse primer sequences for *GAPDH* (housekeeping gene) and *CYP2E1* were designed using online "Primer3" primer design software (Primer3, *http://bioinfo.ut.ee/primer3/*). The specificity was checked using Primer3 and BLAST (*http://www.ncbi.nlm.nih.gov/tools/primer-blast/*). The primers used for rat *CYP2E1* gene were sense 5'-TGAAAAAGCCAAGGAACACC-3' and antisense: 5'-TGTGCTGGTGGTCTCAGTTC-3' (187 bp) and for the rat *GAPDH* gene were sense 5'-GTATCGGACGCCTGGTTAC-3' and antisense :5'-CTTGCCGTGGGTAGAGTCAT-3' (128 bp). The reactions were performed using Step One Plus™ Real-Time PCR system (Applied Biosystems, USA) under the following conditions: for rat *CYP2E1* gene one cycle of initial incubation 95°C for 3 min followed by 40 cycles of amplification cycle with initial denaturation at 95°C for 3 sec, annealing at 58.4°C for 30 sec and extension at 72°C for 30 sec; and for rat *GAPDH* gene one cycle of initial incubation 95°C for 3 min followed by 40 cycles of amplification cycle with initial denaturation at 95°C for 3 sec, annealing at 56.6°C for 30 sec and extension at 72°C for 30 sec. Fluorescence signals were measured in each cycle. The specificity of the products obtained was confirmed by analysis of the dissociation curves of the amplified product. Melting curves analysis was performed for each PCR reaction to ensure the purity/specificity of amplification product. The relative change in expression of *CYP2E1* gene was analyzed by comparative C_T (cycle threshold) method (Livak and Schmittgen, 2001) was expressed as 'n' fold change up/down regulation of the transcripted gene in relation to the untreated control group which were defined as 1.0. All assays were performed in triplicate.

Data analysis

The values expressed as mean ± standard error (SE) (n = 8). The statistical analysis was performed by one way analysis of variance followed by Duncan's multiple range tests for comparison between the groups using IBM SPSS statistics, version 21.0 software program (SPSS Inc., USA).

RESULTS AND DISCUSSION

Drug-induced liver failure is considered an important health problem worldwide because its prevalence is increasing each year, so requiring the development of drugs that protects liver from adverse effects of drug over dosage (Kullak-Ublick *et al.,* 2017). Despite the tremendous advances in modern medicine, an effective hepatoprotective drug with sufficient antioxidant properties that stimulate liver function protects from liver damage or boost hepatic regeneration are meagre. The present study assessed the hepatoprotective effect of ethanolic extract of *G. gummi-gutta* at 3 doses against hepatotoxicity induced by acetaminophen in Wistar albino rats along with the comparison of expression level of *CYP2E1* gene in hepatocytes. In present study the ethanolic fruit rind extract of *G. gummi-gutta* fruit rind was subjected to acute toxicity test in Wistar albino rats and the animals were observed for 24 h. The fruit rind extract did not cause any mortality up to 2000 mg kg^{-1}, and hence three doses (50, 100 and 200 mg kg-1, p.o) were selected for the present study.

Effect of GGE on serum biochemical markers in acetaminophen intoxicated rats

The estimation of enzymes in serum is a useful quantitative marker of the extent and type of hepatocellular damage. The hepatoprotective effect of ethanolic fruit rind extract of G. *gummi-gutta* on serum biochemical parameters in acetaminophen liver damage are summarized in Table I. Toxic dose of acetaminophen (2 g kg^{-1}) in rats caused hepatic tissue damage and necrosis as evidenced by significant increase in serum liver enzymes (ALT, AST, ALP) and reduced level of tissue protein as compared to control animals (group 1). Conversely, the animals pretreated with GGE @ 50, 100 and 200 mg kg^{-1} (group III, IV and V) exhibited significant decrease in serum AST, ALT, ALP levels and increased total protein levels as compared to the toxic control group (group II). The reason for depression of serum enzymatic level by GGE appears to be the presence of phyto-

Table I: Effect of ethanolic extract of *G. gummi-gutta* fruit rind on serum biomarkers in the liver of acetaminophen intoxicated rats

Treatment groups	ALP (IU L^{-1})	AST (IU L^{-1})	ALT (IU L^{-1})
G_I : Healthy control	$94.88^{c} \pm 6.77$	$159.63^{d} \pm 4.28$	$55.50^{b} \pm 2.29$
G_{II} : Hepatotoxic control	$141.75^{a} \pm 2.99$	$232.00^{a} \pm 10.08$	$92.63^{a} \pm 1.87$
G_{III} : 50 mg kg^{-1} GGE	$108.37^{b} \pm 1.67$	$191.38^{b} \pm 2.21$	$62.38^{b} \pm 2.54$
G_{IV} : 100 mg kg^{-1} GGE	$97.25^{c} \pm 0.65$	$179.25^{bc} \pm 3.83$	$60.50^{b} \pm 2.68$
G_V : 200 mg kg^{-1} GGE	$93.63^{c} \pm 0.65$	$169.13^{cd} \pm 3.89$	$59.63^{b} \pm 2.90$
G_{VI} : Silymarin	$91.50^{c} \pm 1.30$	$167.13^{cd} \pm 4.56$	$60.13^{b} \pm 4.37$

Values are expressed as mean ± SD (n = 6), AST = Aspartate amino transferase, ALP = Alkaline phosphatase, ALT = Alanine amino transferase, Means bearing different superscripts in columns differ significantly ($P < 0.001$) as compared to the paracetamol group.

chemicals with antioxidant properties which on reacting with free radicals produced by acetaminophen toxic metabolism, moderate the depletion of glutathione, consequent hepatocyte membrane damage and ultimately protecting cells from free radical-induced damage (Abdel-Daim *et al.,* 2018; Rehna *et al.,* 2021). Pretreatment with the standard hepatoprotective drug-Silymarin (group VI) also decreased serum biochemical levels towards normalcy and the observed values were comparable to group V that was pretreated with fruit rind extract at a dose of 200 mg kg^{-1}.

The elevated serum levels of AST, ALT, and ALP are direct reflection of damage of liver, because they are released into circulation after hepatocellular damage owing to their cytoplasmic location (Sallie *et al.,* 1991). The reactive species (NAPQI) produced by paracetamol overdose harm the hepatic cells by lipid peroxidation and thereby damage the cellular permeability resulting in higher serum levels of ALT, AST and ALP (Islam *et al.,* 2021). In present study, acetaminophen treated group developed significant hepatic damage as observed through substantial increment in the concentration of liver serum markers (AST, ALT and ALP). Treatment with plant fruit rind extract reduced elevation in ALT, AST and ALP levels due to acetaminophen intoxication in group

III, IV and V rats in a significant manner (P<0.001) when compared with the normal control and silymarin treated groups in a dose-dependent manner. This suggested protection of hepatocellular architecture, cellular membrane and thus reduced leakage of marker enzymes into the circulation. The attenuation of acetaminophen mediated-increase in hepatocellular enzymes highlight the protective ability of GGE against acetaminophen-mediated toxicity and hepatocellular damage, attributed to the presence of antioxidant phytocomponents and its ability to scavenge free radicals (Abushouk *et al*., 2018., Engwa, 2018).

On day 10, the mean values of extract-treated groups were comparable to the normal control and silymarin treated groups which upholds the findings of Sarvankumar *et al*. (2014) who opined that silymarin and *Vitex negundo* bark extract significantly prevented the elevation of serum marker enzymes like AST, ALT and ALP with acetaminophen intoxication. In present study, AST and ALT activity in acetaminophen intoxicated groups was significantly high as compared to the other treated groups indicating the hepatic damage that correlates well with the previous scientific report of Abirami *et al.* (2015), Reddy *et al.* (2017) and Kokhdan *et al.* (2017).

Treatment with plant extract (GGE) at 200 mg kg^{-1} reduced serum AST and ALT levels that was significantly (P<0.001) similar to the mean values of normal control and standard drug groups. This finding is in agreement with Lim *et al.* (2021) who reported hepatoprotective effect of aqueous extract of *G. indica* against acetaminophen-induced liver damage in rats. The tendency of these enzymes to return to normality in the extract-administered group is a clear manifestation of hepato-protective effects of extract. Similar justifications of serum liver markers improvement by using different medicinal plants were previously reported (Singh *et al.*, 2015; Janghel *et al.*, 2019).

In liver, ALP is located mostly within the biliary canaliculi and epithelial cells and forms primarily biomarkers of hepatobiliary functions and cholestasis. In present study, the observed mean serum ALP levels significantly (P<0.001) increased in acetaminophen group at the end of the experiment. The GGE treatment significantly reduced serum ALP levels when compared to the acetaminophen intoxicated group in a dose dependent manner. These results corroborate the findings of Smith and Adanlawo (2015), Okokon *et al*. (2017), Subramanya *et al.* (2018) and Nili-Ahmadabadi *et al*. (2018) who opined that serum ALP levels were restored with the regeneration and improvement in the secretory mechanism of the hepatic cells.

Effect of GGE on histopathology in acetaminophen-intoxicated rats

Histopathological liver sections of rats from all the six experimental groups are shown in Fig. 1 which provide supportive evidence to the results of biochemical analysis. The liver section in normal control animals (Fig. 1A) demonstrated considerable dose-dependent protective and healing effect on liver cells after the extract treatments on the pathological changes induced by acetaminophen intoxication and showed the presence of binucleated regenerating hepatocytes. The results were comparable to the group pretreated with standard drug, silymarin (G) section in normal control animals (Fig. 1A) showed normal hepatic cellular architecture with distinct hepatic cells and central vein whereas rats (group II, Fig. 1B, C) treated with toxic dose of acetaminophen (2 g kg^{-1}) showed the total loss in liver architecture with the patterns of diffuse vacuolar degenerative changes, lymphocytic infiltration, centrilobular necrosis and sinusoidal congestion. The liver sections of the animals pretreated with GGE @ 50 mg kg^{-1} bw showed moderate hepatoprotection with severity of cellular damage being less in comparison to the toxic control group as evidenced by the presence of regenerating binucleated hepatocytes and reduced extent of centrilobular necrosis (Fig. 1D). However, there were mild hepatic lesions like mild portal congestion and inflammatory cell infiltration. Pretreatment with GGE extracts @ 100 and 200 mg kg^{-1} bw effectively ameliorated the acetaminophen induced liver damage (Fig. 1E, F) and the results were comparable to the group pretreated with standard drug silymarin (Fig. 1G). The liver sections showed lesser derangement of hepatocytes, indicating marked regeneration activity with an almost near normal liver architecture.

The rats pretreated with plant extract effectively ameliorated the liver impairment induced by

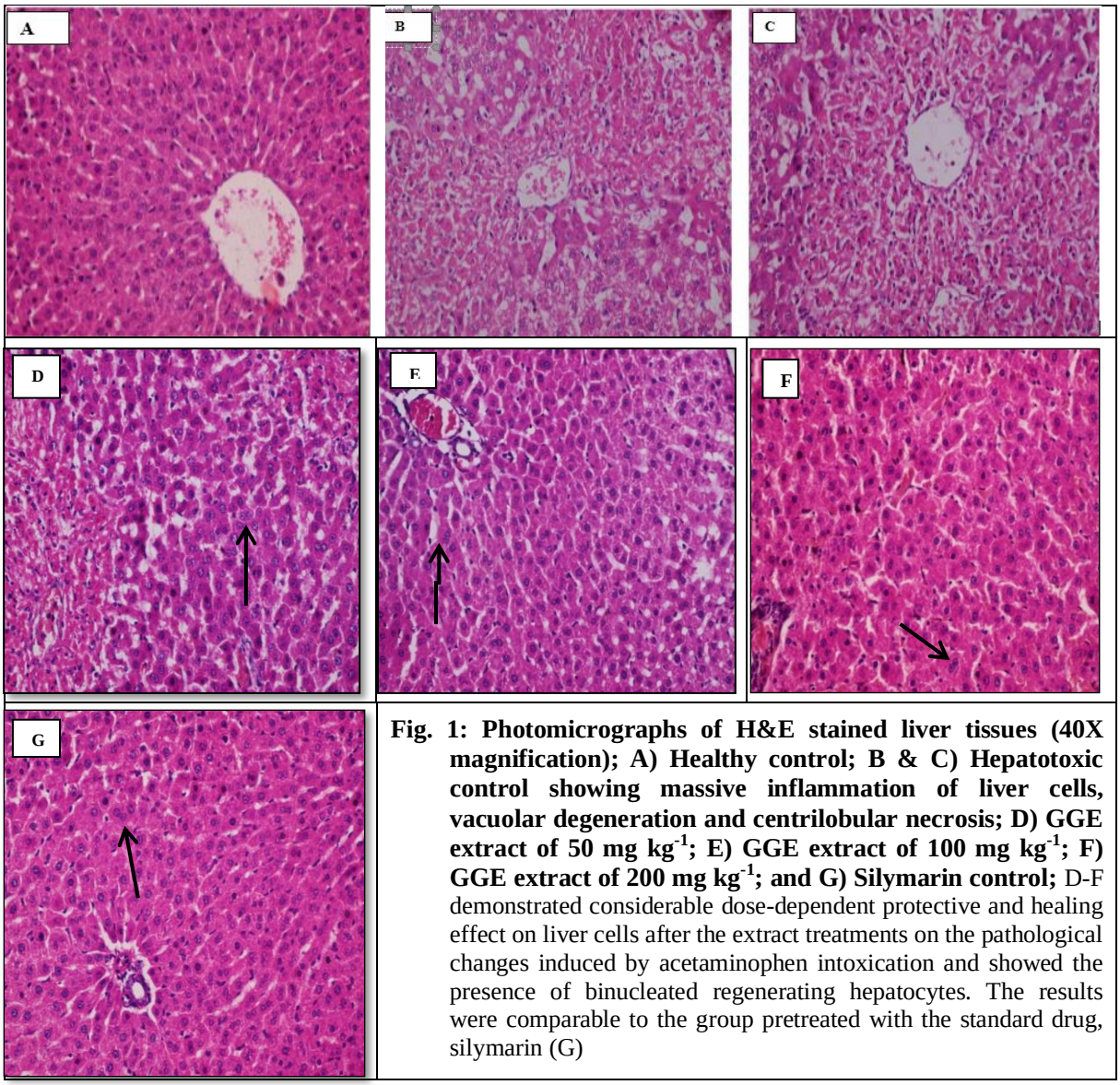

Fig. 1: Photomicrographs of H&E stained liver tissues (40X magnification); A) Healthy control; B & C) Hepatotoxic control showing massive inflammation of liver cells, vacuolar degeneration and centrilobular necrosis; D) GGE extract of 50 mg kg^{-1}; E) GGE extract of 100 mg kg^{-1}; F) GGE extract of 200 mg kg^{-1}; and G) Silymarin control; D-F demonstrated considerable dose-dependent protective and healing effect on liver cells after the extract treatments on the pathological changes induced by acetaminophen intoxication and showed the presence of binucleated regenerating hepatocytes. The results were comparable to the group pretreated with the standard drug, silymarin (G)

acetaminophen in a dose dependent manner. This revealed the potential of plant extract in protecting the structural integrity of hepatocellular membrane and cellular architecture of liver cells from acetaminophen intoxication. Histopathological studies of liver tissues of negative control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein; whereas the liver sections of acetaminophen-treated group showed extensive alterations in liver architecture with diffuse vacuolar degenerative changes, centrilobular necrosis, cellular infiltration of inflammatory cells and congestion. These observations are in agreement with Smith and Adanlawo (2015) who reported neutrophilic cellular infiltration, vacuolar degenerative changes, bile duct hyperplasia, and severe portal congestion in hepatic tissue of acetaminophen-intoxicated rats. Wang *et al.* (2017) and Ghobadi *et al.* (2019) also noticed severe centrilobular necrosis with nuclear disappearance in liver of acetaminophen-treated rats.

The histopathological examination of liver of group III rats revealed moderate cellular damage as compared to the group II that was confirmed by the presence of regenerating binucleated hepatocytes and reduced extent of centrilobular necrosis. However, there were mild hepatic lesions like mild portal congestion, and inflammatory cell infiltration. There were no histological alterations in liver of group IV and V that received the extract @ 100 and 200 mg kg^{-1} bw indicating

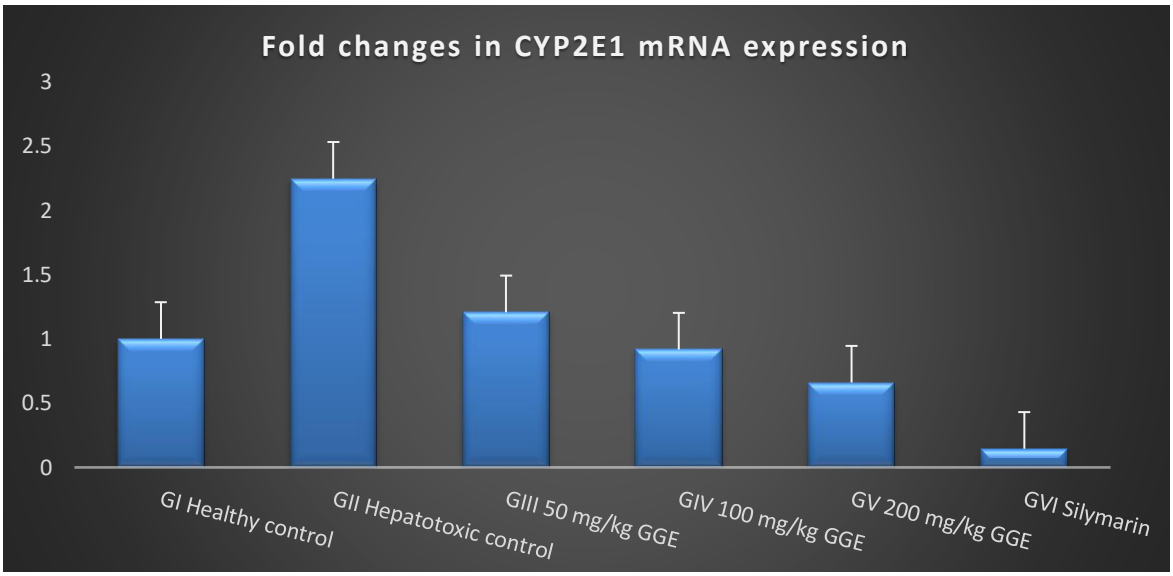

Fig. 2: Effect of ethanolic extract of *G. gummi-gutta* fruit rind on changes in CYP2E1 relative mRNA expression in liver in paracetamol induced hepatotoxicity

complete cellular protection against acetaminophen-induced hepatic injury. Group VI treated with reference drug silymarin showed almost normal histology of liver. This is in agreement with Mundugaru *et al.* (2014) who noticed complete hepatoprotection against the hepatic damage caused by acetaminophen in rats pretreated with fruit extract of *G. pedunculata*. Similar observations were reported by Deore *et al.* (2011) wherein aqueous and ethanolic extract of *G. indica* demonstrated protective activity against carbon tetrachloride induced liver damage by hepatic regeneration with almost complete normalization of architecture of hepatocytes. Histopathological evaluation of liver of rats pretreated with *G. kola* root extract showed marked hepatoprotection against acetaminophen intoxication as evident by the recovery of hepatocytes with mild diffuse vacuolar degeneration of hepatocytes (Smith and Adanlawo., 2015).

CYP2E1 gene expression study

CYP2E1 is the main cytochrome P-450 isoenzyme associated with the metabolic activation of acetaminophen to toxic metabolite NAPQI that, in turn, causes production of excessive reactive electrophiles and free radicals like reactive oxygen species. In present study the acetaminophen intoxication resulted in 2.2-fold increase in the expression of *CYP2E1* gene as compared to the normal control (Fig. 2). Gene expression of *CYP2E1* was significantly down-regulated in extract-treated group in a dose dependent manner as compared to the control group. The rats treated with standard drug silymarin showed least expression profile (0.0045 ± 0.001) for *CYP2E1* gene.

The three families of cytochrome P450 (CYP450) isoforms, mainly involved in the biotransformation of xenobiotics, were CYP1, CYP2, and CYP3. Several studies have depicted the imperative role of CYP2E1, CYP1A2 and intracellular GSH in acetaminophen-induced hepatotoxicity (Hinson *et al.*, 2010). The increased *CYP2E1* activity has a vital role in augmenting the oxidative stress. Studies with knockout mice model showed the involvement of CYP2E1 and CYP1A2 in the metabolism of acetaminophen (Gill *et al.*, 2017). Thus, the agents with inhibitory effect on *CYP2E1* activity show good therapeutic potential for alleviating the toxic effects of acetaminophen-induced liver impairment.

In present study a significant upsurge in relative gene expression of *CYP2E1* mRNA in acetaminophen intoxicated group was observed in comparison to the normal control group. These findings are in line with Yamaura *et al.* (2011), Chen *et al.* (2014) and Garcia *et al.* (2014) who observed elevation in CYP2E1 protein activity after the administration of toxic dose of acetaminophen. Ha *et al.* (2005) and Bohi *et al.* (2009) noticed remarkable increase in the

expression of *CYP2E1* mRNA in hepatic cells due to the toxicity induced by carbon tetrachloride. In present study, a dose dependent down-regulation of CYP2E1 gene was noticed in extract treated groups in comparison to normal control animals. There is lack of information on the modulating effect of *Garcinia* species on the expression pattern of hepatic *CYP2E1* gene in rats challenged with acetaminophen. The results also imply that hepatoprotective action by the ethanolic extract of *G. gummi-gutta* fruit rind against acetaminophen induced hepatotoxicity was due to the down regulation of *CYP2E1* that might have resulted in reduced formation of toxic metabolite**.**

Conclusion: The present study on the evaluation of hepatoprotective potential of ethanolic fruit rind extract of Malabar tamarind (*G. gummi-gutta*) in rats intoxicated with acetaminophen revealed that the ethanolic extract produced hepatoprotection in a dose dependent manner. A significant reduction in serum profile was also noted. Gene expression of *CYP2E1* was significantly down-regulated in plant extract supplemented group in a dose-dependent manner. Histopathological studies showed the occurrence of numerous binucleated hepatocytes indicative of hepatic regeneration. The study revealed that the dried fruit rind extract of *G. gummi-gutta* has remarkable hepatoprotective effect, as pretreatment of extract protected the liver against acetaminophen-induced oxidative impairment and damage, alterations in serum profile and histopathology by down regulation of *CYP2E1* expression. However, further research is needed to decipher the mechanisms of hepatoprotective effect of fruit rind of *G. gummi-gutta* by adopting methods for isolation and characterization of pharmacologically active bio-constituents from the crude extract, metabolomic research studies and molecular docking studies.

Acknowledgments: Authors are thankful to the Department of Veterinary Pharmacology and Toxicology, Department of Veterinary Pathology, Central Instruments laboratory (CIL) of College of Veterinary and Animal Science, Mannuthy, Kerala for rendering all kind of guidance and facilities for carrying out the research work.

Funding: This work was supported by Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala.

Declaration of conflict of interest: All authors declare there is no conflict of interest.

REFERENCES

Abushouk, A.I., Reggi, R., Yarla, N.S., Palmery, M. and Peluso, I. 2018. Association of antioxidant nutraceuticals and acetaminophen (paracetamol): Friend or foe? *Journal of Food and Drug Analysis*, **26**(2): S78–S87.

Abirami, A., Nagarani, G. and Siddhuraju, P. 2015. Hepatoprotective effect of leaf extracts from *Citrus hystrix* and *C. maxima* against paracetamol induced liver injury in rats. *Food Science Human Wellness*, **4**(1): 35-41.

Babu, P.S., Krishna, V., Maruthi, K., Shankarmurthy, K. and Babu, R. 2011. Evaluation of acute toxicity and hepatoprotective activity of the methanolic extract of *Dichrostachys cinerea* (Wight and Arn.) leaves. *Pharmacognosy Research*, **3**(1): 40. [*doi:10.4103/0974-8490.79114*].

Barathane, D., Manimekalai, K. and Salwe, K.J. 2020. Hypolipidemic and antioxidant effect of *Garcinia gummi-gutta* Barkson atherogenic diet-induced hyperlipidemic rats. *Biomedical and Pharmacology Journal*, **13**(4): 1903-1912.

Brennan, P.N., Cartlidge, P., Manship, T. and Dillon, J.F. 2021. EASL clinical practice guidelines: Drug-induced liver injury (DILI). *Frontline Gastroenterology*, **13**(4): 332-336.

Chen, W., Yang, C. and Yin, M. 2014. Protective effects from *Houttuynia cordata* aqueous extract against acetaminophen-induced liver injury. *Biomedicine (Taipei)*. **4**(1): 5. [*10.7603/s40681-014-0005-2*].

Das, J., Ghosh, J., Manna, P. and Sil, P.C. 2010. Acetaminophen induced acute liver failure via oxidative stress and JNK activation: Protective role of taurine by the suppression of cytochrome P450 2E1. *Free Radical Research,* **44**(3): 340-355.

Deore, A.B., Sapakal, V.D. and Naikwade, N.S. 2011. Antioxidant and hepatoprotective activity of *Garcinia in*dica Linn fruit rind. *Pharmacie Globale*, **6**: 1-5.

Dhanya, P. and Benny, P.J. 2014. Larvicidal action of *Garcinia gummi-gutta.* Robs. var. *gummi-gutta* on dengue victim *Aedes aegypti. Indian Journal of Applied Research,* **4**(1): 21-22.

Engwa, G.A. 2018. Free radicals and the role of plant phytochemicals as antioxidants against oxidative stress related diseases. *Phytochemicals – Source of Antioxidants and Role in Disease Prevention.* [*doi:5572/intechopen.76719*].

Kokhdan, E.P., Ahmadi, K., Sadeghi, H., Sadeghi, H., Dadgary, F., Danaei, N. and Aghamaali, M.R. 2017. Hepatoprotective effect of *Stachys pilifera* ethanol extract in carbon tetrachloride-induce hepatotoxicity in rats. *Pharmaceutical Biology*, **55**(1): 1389-1393.

Gill, P., Bhattacharyya, S., McCullough, S., Letzig, L., Mishra, P.J., Luo, C., Dweep, H., Omirinde, J.O., Bejide, R., Isola, T.O., Usende, L.I. and Basiru, A. 2014. Comparative hepatoprotective activity of ethanolic extracts of *Cuscuta australis* against acetaminophen intoxication in Wistar rats. *International Scholarly Research Notices*, 2014, pp.1–6. [d*oi:10.1155/2014/730516*].

James, L. 2017. MicroRNA regulation of CYP 1A2, CYP3A4 and CYP2E1 expression in acetaminophen toxicity. *Scientific Reports*, **7**(1): 12331 [*doi:10.1038/s41598-017-11811-y*].

Guillén-Enríquez, C., López-Teros, V., Martín-Orozco, U., López-Díaz, J., del Hierro-Ochoa, J., Ramos-Jiménez, A., Astiazarán-García, H., Martínez-Ruiz, N. and Wall-Medrano, A. 2018. Selected physiological effects of a *Garcinia gummi-gutta* extract in rats fed with different hypercaloric diets. *Nutrients*, **10**(5): 565. [*doi:10.3390/nu10050565*].

Ganesan, K., Jayachandran, M. and Xu, B. 2017. A critical review on hepatoprotective effects of bioactive food components. *Critical Reviews in Food Science and Nutrition*, **58**(7): 1165-1229.

García, N.P., Lores, O.F., Fuentes, D.P, Martínez, D.T. and Hernandez, J.B. 2014. Reduction of hepatotoxicity induced by acetaminophen overdoses in a mouse model of inflammation induced by Freund´s adjuvants. *Journal of Allergy & Therapy*, **5**: 183. [*doi:10.4172/2155-6121.1000183*].

Nili-Ahmadabadi, A., Ghobadi, S., Dastan, D. and Soleimani, M. 2018. Hepatoprotective potential and antioxidant activity of Allium tripedale in acetaminophen-induced oxidative damage. *Research in Pharmaceutical Sciences*, **14**(6): 488. [*doi:10.4103/1735-5362.272535*].

Ghosh, M.N. 1984. *Fundamentals of Experimental Pharmacology* (2nd edn.). Scientific Book Agency; Kolkata, India.

Ha, K.T., Yoon, S.J., Choi, D.Y., Kim, D.W., Kim, J.K. and Kim, C.H. 2005. Protective effect of *Lycium chinense* fruit on carbon tetrachloride-induced hepatotoxicity. *Journal of Ethno-pharmacology*, **96**(3): 529-535.

Hinson, J.A., Roberts, D.W. and James, L.P. 2010. Mechanisms of acetaminophen-induced liver necrosis. *Handbook of Experimental Pharmacology*, **196**: 369-405. [*doi:10.1007/978-3-642-00663-0_12*].

Iorga, A., Dara, L. and Kaplowitz, N. 2017. Drug-induced liver injury: Cascade of events leading to cell death, apoptosis or necrosis. *International Journal of Molecular Sciences*, **18**(5): 1018. [*doi:10.3390/ijms18051018*].

Islam, M.T., Quispe, C., Islam, Md.A., Ali, E.S., Saha, S., Asha, U.H., Mondal, M., Razis, A.F.A., Sunusi, U., Kamal, R.M., Kumar, M. and Sharifi-Rad, J. 2021. Effects of nerol on paracetamol-induced liver damage in Wistar albino rats. *Biomedicine & Pharmacotherapy*, **140**: 111732. [*doi:10.1016/j.biopha.2021.111732*].

Janghel, V., Patel, P. and Chandel, S.S. 2019. Plants used for the treatment of icterus (jaundice) in Central India: A review. *Annals of Hepatology*, **18**(5): 658-672.

Jing, Y., Wu, K., Liu, J., Ai, Q., Ge, P., Dai, J., Jiang, R., Zhou, D., Che, Q., Wan, J. and Zhang, L. 2015. Aminotriazole alleviates acetaminophen poisoning via downregulating P450 2E1 and

suppressing inflammation. *PLOS ONE*, **10**(4): 0122781. [*doi:10.1371/journal.pone.0122781*].
Katarey, D. and Verma, S. 2016. Drug-induced liver injury. *Clinical Medicine*, **16**(6): S104-S109.
Keshari, P., Pradeep, P. and Bhat, S. 2019. Evaluation of hepatoprotective potential of *Rhododendron arboreum* Sm. stem bark as *Abhava pratinidhi* Dravya (Substitute) of *Rohitaka* (Tecomella undulata (Sm.) Seem.) against paracetamol induced hepatotoxicity in experimental rats. *Pharmacognosy Journal*, **11**(5): 1148-1154.
Khatib, N.A., Digaswala, P., Gupta, C.D. and Varghese, A. 2010. Hepato-protective potential of *Garcinia indica* fruit rind extract against acetaminophen induced hepatic damage in Wistar rats. *Pharmacology [online]*, **1:** 561-566.
Kullak-Ublick, G.A., Andrade, R.J., Merz, M., End, P., Benesic, A., Gerbes, A.L. and Aithal, G.P. 2017. Drug-induced liver injury: Recent advances in diagnosis and risk assessment. *Gut,* **66**: 1154-1164. [*doi:10.1136/gutjnl-2016-313369*].
Lim, S.H., Lee, H.S., Lee, C.H. and Choi, C.-I. 2021. Pharmacological activity of *Garcinia indica* (Kokum): An updated review. *Pharmaceuticals*, **14**(12): 1338. [*doi:10.3390/ph14121338*].
Livak, K.J. and Schmittgen, T.D.2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods*. **25**: 402-408.
Mahendran, P. and Devi, C.S. 2001. Effect of *Garcinia cambogia* extract on lipids and lipoprotein composition in dexamethasone administered rats. *Indian Journal of Physiology & Pharmacology*, **45**(3): 345-350.
Manyike, P., Kharasch, E., Kalhorn, T. and Slattery, J. 2000. Contribution of CYP2E1 and CYP3A to acetaminophen reactive metabolite formation. *Clinical Pharmacology & Therapeutics*, **67**(3): 275-282.
Mathew, G.E., Mathew, B., Shaneeb, M.M. and Nyanthara, B. 2011. Diuretic activity of leaves of *Garcinia cambogia* in rats. *Indian Journal of Pharmaceutical Sciences*, **73**: 228-230.
Mazzio, E.A. and Soliman, K.F.A. 2008. *In vitro* screening for the tumoricidal properties of international medicinal herbs. *Phytotherapy Research*, **23**(3): 385-398.
McGill, M.R., Sharpe, M.R., Williams, C.D., Taha, M., Curry, S.C. and Jaeschke, H. 2012. The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. *Journal of Clinical Investigation*, **122**(4): 1574-1583.
Mundugaru, R., Varadharajan, M.C. and Basavaiah R. 2014. Hepatoprotective activity of fruit extract of *Garcinia pedunculata*. *Bangladesh Journal of Pharmacology*, **9**: 483-487.
Nili-Ahmadabadi, A., Alibolandi, P., Ranjbar, A., Mousavi, L., Nili-Ahmadabadi, H., Larki-Harchegani, A., Ahmadimoghaddam, D. and Omidifar, N. 2018. Thymoquinone attenuates hepatotoxicity and oxidative damage caused by diazinon: An *in vivo* study. *Research in Pharmaceutical Sciences*, **13**(6): 500. [*doi:10.4103/1735-5362.245962*].
Noureddin, N. and Kaplowitz, N. 2018. Overview of mechanisms of drug-induced liver injury (DILI) and key challenges in DILI research. pp. 3-18. **In**: *Drug-induced Liver Toxicity. Methods in Pharmacology and Toxicology* (eds. Y. Will and M. Chen). Humana Press, New Jersey, USA.
Okokon, J., Simeon, J. and Umoh, E. 2016. Nephroprotective activity of *Homalium Letestui* stem extract against paracetamol induced kidney injury. *Journal of Experimental and Integrative Medicine*, **6**(1): 38. [*doi:10.5455/jeim.250216.or.147*].
Prasanth, N.V., Rasheed, S.P, Thomas, T., Joseph, S. and Varghese, C. 2013. Evaluation of *in vitro* and *in vivo* anti-inflammatory activity of *Garcinia cambogia.* L. *International Journal Pharmacy and Pharmaceutical Sciences*, **5**: 263-264.
Raghavendra, M., Yatish, K.V. and Lalithamba, H.S. 2017. Plant-mediated green synthesis of ZnO nanoparticles using *Garcinia gummi-gutta* seed extract: Photoluminescence, screening of their catalytic activity in antioxidant, formylation and biodiesel production. *The European Physical Journal Plus*, **132**(8): 358. [*doi:10.1140/epjp/i2017-11627-1*].
Raskovic, A., Milanovic, I., Pavlovic, N., Cebovic, T., Vukmirovic, S. and Mikov, M. 2014. Antioxidant activity of rosemary (*Rosmarinus officinalis* L.) essential oil and its

hepatoprotective potential. *BMC Complementary and Alternative Medicine*, **14**: 225. [*doi: 10.1186/1472-6882-14-225*].

Reddy, M.K., Reddy, A.G., Kumar, B.K., Madhuri, D., Boobalan, G. and Reddy, M.A. 2017. Protective effect of rutin in comparison to silymarin against induced hepatotoxicity in rats. *Veterinary World.* **10**(1): 74-80.

Rehna, A., Neethu, K.P. and Deepa, A.K. 2021. Phytochemical analysis and antimicrobial activity of ethanolic extract of dried fruit rind of *Garcinia gummi-gutta*. *Journal Ayurveda and Herbal Medicine,* **7**(2): 81-85.

Sallie, R., Tredger, J. and William, R.1991. Drugs and the liver. *Biopharmaceutics* & *Drug Disposition*, **12**: 251-259.

Samarajeewa, U. and Shanmugapirabu, N. 1983. A cheap method for preservation of fish. pp. 80-81. **In:** *Proceedings of the 6th International Congress of Food Science and Technology, Vol. 1,* Boole Press, Dublin, Ireland.

Sarvankumar, G., Lalitha, V., Sengottuvelu, S., Sharif, S.H. and Sivakumar, T. 2011. Hepatoprotective activity of *Vitex negundo* Linn bark against chemical induced toxicity in experimental rats. *International Journal of Advanced Pharmaceutical Sciences,* **2**: 534-540.

Semwal, R.B., Semwal, D.K., Vermaak, I. and Viljoen, A. 2015. A comprehensive scientific overview of *Garcinia cambogia*. *Fitoterapia*, **102**: 134-148.

Singh, H., Prakash, A., Kalia, A.N. and Majeed, A.B.A. 2016. Synergistic hepatoprotective potential of ethanolic extract of *Solanum xanthocarpum* and *Juniperus communis* against paracetamol and azithromycin induced liver injury in rats. *Journal of Traditional and Complementary Medicine*, **6**(4): 370-376.

Smith, Y.R.A. and Adanlawo, I.G. 2015. Protective effect of saponin extract from the root of *Garcinia kola* (Bitter kola) against paracetamol-induced hepatotoxicity in Albino rats. *International Journal of Biological, Food, Veterinary and Agricultural Engineering*, **9**(2): 130-134.

Stanger, B.Z. 2015. Cellular homeostasis and repair in the mammalian liver. *Annual Review of Physiology*, **77**(1): 179-200.

Subramanya, S.B., Venkataraman, B., Meeran, M.F.N., Goyal, S.N., Patil, C.R. and Ojha, S. 2018. Therapeutic potential of plants and plant derived phytochemicals against acetaminophen-induced liver injury. *International Journal of Molecular Sciences*, **19**(12): 3776. [*doi:10.3390/ijms19123776*].

Sumayya, P.A., Murugan, M., Gopukumar, S.T. and Praseetha, P.K. 2016. Estimation of phytochemical, antimicrobial and molecular comparison of fruit extracts of *Garcinia cambogia* and *Tamarindus indica*. *Research Journal of Pharmacological, Biological and Chemical Sciences,* **7**(1): 1256-1263.

Sarkar, J., Kumar, N. and Gupta, P. 2020. Evaluation of hepatoprotective action of *Solanum melongena* L. peel extract against paracetamol induced liver damage in albino rats. *Medical Science*, **24**(104): 2013-2024.

Tungmunnithum, D., Thongboonyou, A., Pholboon, A. and Yangsabai, A. 2018. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines*, **5**(3):93. [*doi:10.3390/medicines5030093*].

Wang, X., Wu, Q., Liu, A., Anadón, A., Rodríguez, J.L., Martínez-Larrañaga, M.R., Yuan, Z. and Martínez, M.A. 2017. Paracetamol: Overdose-induced oxidative stress toxicity, metabolism, and protective effects of various compounds *in vivo* and *in vitro*. *Drug Metabolism Reviews*, **49**(4): 395-437. [*doi:10.1080/03602532.2017.1354014*].

Yamaura, K., Nakayama, N., Shimada, M. and Ueno, K. 2011. Protective effects of goldenseal (*Hydrastis canadensis* L.) on acetaminophen-induced hepatotoxicity through inhibition of CYP2E1 in rats. *Pharmacognosy Research*, **3**(4): 250. [*doi:10.4103/09748490.89745*].

Yuan, L. and Kaplowitz, N. 2013. Mechanisms of drug-induced liver injury. *Clinics in Liver Disease*, **17**(4): 507-518.