PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 12(2): 1-7
https://doi.org/10.14719/pst.3871

HORIZON
e-Publishing Group

RESEARCH ARTICLE

Hepatoprotective and antioxidant activity of *Garcinia mangostana* L. pericarp extract in acetaminophen-induced hepatotoxicity in human hepatic HepG2 cell lines

Asha Sasi Kumar[1], Ambili Savithri[2], Remya Ambika Suseelan[3] & Reshmi Suseela[4]*

[1]Department of Chemistry, Sree Narayana College, Chathannur, Kollam 691 572, Kerala, India

[2]Department of Biochemistry, Sree Narayana College, Kollam, Kerala 691 001, India

[3]Department of Biochemistry, Sree Narayana College for Women, Kollam, Kerala 691 001, India

[4]Post Graduate Department of General Biotechnology, GEMS Arts and Science College, Malappuram 679 321,Kerala, India

*Email: reshmisuseela1233@gmail.com

OPEN ACCESS

ARTICLE HISTORY

Received: 10 May 2024
Accepted: 12 January 2025

Available online
Version 1.0 : 24 April 2025
Version 2.0 : 30 April 2025

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE

Asha SK, Ambili S, Remya AS, Reshmi S. Hepatoprotective and antioxidant activity of *Garcinia mangostana* L. pericarp extract in acetaminophen-induced hepatotoxicity in human hepatic HepG2 cell lines . Plant Science Today. 2025; 12(2): 1-7. https://doi.org/10.14719/pst.3871

Abstract

Garcinia mangostana L. belongs to the Guttiferae family prominently seen in South Asia. Its fruits were frequently acknowledged as the "queen of fruits". The Genus *Garcinia* comprises 35 genera and 240 species globally, among which 6 species were reported endemic to Western Ghats. The Pericarp and seed of *G. mangostana* are well known for their use in traditional systems of medicine against numerous ailments. In the current investigation, the hepatoprotective and antioxidant potential of methanolic extract of the pericarp of *Garcinia mangostana* L. were investigated against the acetaminophen-induced hepatotoxicity in HepG2 human liver cell lines. The qualitative analysis of methanol extract of *Garcinia mangostana* depicted the presence of immense phytoconstituents such as alkaloids, phenols, triterpenoids and flavonoids. It was observed that *Garcinia mangostana* acts as a potential hepatoprotective agent by reducing lipid peroxidation while significantly increasing the level of Glutathione (GSH) and superoxide dismutase (SOD) in dose-dependent manner. The hepatoprotective property of *Garcinia mangostana* was confirmed by the histopathological analysis and the results revealed that extract of *G. mangostana* recovered the liver cell lines towards almost normal level in a dose dependant manner from the histopathological alterations such as necrosis, vacuolation, etc., produced by acetaminophen.

Keywords

cytotoxicity; *Garcinia mangostana*; hepatoprotective activity; MTT assay; superoxide dismutase

Introduction

Garcinia mangostana L., widely recognised as Mangosteen in South East Asia, is prominently cultivated in India, Sri Lanka, Thailand, Malaysia, Philippines and Myanmar. *G. mangostana* L. is frequently acknowledged as "the queen of fruits" due to its pleasant aroma and sweet flavour. The Genus *Garcinia* comprises 35 genera and nearly 240 species globally and about 6 species were reported endemic to Western Ghats. *G. mangostana* reaches up to 6–25 m in height. The leaves are leathery, glabrous, opposite, short stalk, ovate-oblong or elliptic in shape. The fruits were dark reddish or purple in colour with fleshy, delicate, inner edible pulp of pleasant aroma (1). Fruits may contain 1–5 fully developed ovoid-oblong-shaped seeds or

sometimes seedless. Only 25% of the fruit is sweet and fleshy, whereas the remaining part is hard, tough and bitter pericarp, which is of greater pharmacological importance. According to WHO, the human consumption of *G. mangostana* was safe without any reported mutagenicity and teratogenicity for over a hundred years (2). The pericarp and seed of *G. mangostana* are well known for their use in traditional systems of medicines against various ailments like inflammation, gastrointestinal, urinary tract infections etc. (3). It is also used to treat wounds and skin infections (4), amoebic dysentery, leucorrhoea (5) and arthritis (6).

The Genus *Garcinia* is a rich source of alkaloids, phenols, flavonoids, benzophenones, proanthocyanins and xanthones. It has been reported that xanthone was one of the major phytoconstituents present in *G. mangostana*, which exhibits a plethora of biological activities such as anti-bacterial (7), anti-viral (8), anti-cancer, anti-inflammatory (9), anti-oxidant, analgesic and anti-allergic (10). Xanthone derivatives such as α mangostin, β mangostin, γ mangostin, mangostinone and dihydroxy-3-methoxy xanthone were isolated from the pericarp of *G. mangostana* have immense pharmacological properties including antioxidant and hepatoprotective. It has been reported that among the xanthones, alpha-mangostin suppresses the proliferation and enhances the apoptosis of HL-60 and HT116 colon cancer cell lines, moreover, it exhibits anti-proliferative activity against human breast adenocarcinoma cell lines, SKBR3 (11). Currently, tremendous research has been made in modern medicine to develop new drugs that stimulate or rejuvenate the liver cells and offer protection for the liver cells from damage (12). Due to its healthcare potential, recently, there has been an increase in the use of mangosteen products in the form of juices and diet supplements such as Xango, Verve and TriaXan. This has attracted the attention of researchers to analyse the phytochemical constituents of *G. mangostana* and its biological activities. Therefore, the present investigation was undertaken to analyse the antioxidant and hepatoprotective potential of the pericarp of *G. mangostana* in acetaminophen-induced hepatotoxicity in human HepG2 cell lines.

Materials and Methods

Plant materials and sample preparation

Plant samples were collected from *Garcinia mangostana* tree grown in Elavinthitta region of Pathanamthitta, Kerala, India (Fig. 1a). Ripened fresh fruits (Fig. 1b) with a completely purple-coloured pericarp of *G. mangostana* were collected and cleaned under running tap water to remove dust particles thoroughly. The pulp and pericarp were separated and the pericarp was shade-dried. The dried pericarp was ground into fine powder. The pericarp powder (4 g) was kept for 3 days with 200 mL of 95% methanol in a stopped flask with continuous shaking. The extracts were collected every 24 h and fresh methanol was added to the powder. The methanol extracts were pooled, filtered and dried under vacuum by using a rotary evaporator and the yield concentration was noted (13). The total extract of the plant sample was stored in the refrigerator (4 °C) for further experiments. The percentage yield of *G. mangostana* was 15.6% (w/w).

Fig. 1. *Garcinia mangostana* L. **a**. Habitat **b**. Fruit.

Phytochemical analysis

The phytochemical constituents of *G. mangostana* pericarp were qualitatively analysed using standard methods (14).

Total alkaloid, phenol and flavonoid analysis

Total alkaloid contents were estimated by the spectrophotometric method using Dragendroff's reagent (15), total phenol contents were estimated by Folin-Ciocalteau analysis (16) and flavonoid contents by aluminium chloride assay (17).

Determination of hepatoprotective activity

Cell line culture

HepG2 Liver Hepatic cell lines were purchased from the National Centre for Cell Sciences and grown in Dulbecco's Modified Eagles Media (DMEM) (Himedia, India). HepG2 cell line was cultured in Dulbecco's Modified Eagles media along with 10% FBS, sodium bicarbonate, L-glutamine and antibiotics such as Penicillin (100 µg/mL), Streptomycin (100 µg/mL) and Amphotericin B (2.5 µg/mL). Cultures were incubated at 37 °C in a 5% CO_2 incubator (NBS Eppendorf, Germany). The viability of cells was evaluated using an inverted phase contrast microscope and MTT assay (18).

After 2 days of incubation, a monolayer of cells was suspended in 10% growth medium (DMEM), 100 µL cell suspension was harvested and seeded at approximately 5×10^4 cells in 96 well microtiter plates (Nunclon, Denmark) and incubated in a humidified 5% CO_2 incubator at 37 °C for 24 h.

After 24 h, the cells were in an exponential phase, attaining sufficient growth; the cells were then treated with Acetaminophen (20 µM) to induce toxicity and incubated for 1 h. Some cells were cultured without Acetaminophen as a control. Methanol extracts of *G. mangostana* in various concentrations (25 µg, 12.5 µg, 6.25 µg, 3.1 µg and 1.5 µg) in 100 µL of 5% DMEM were prepared. The experiments were performed in triplicates. Prior to inoculation, the extract was filtered using a 0.22 µm millipore syringe filter and incubated at a temperature of 37 °C for 2 h.

Cytotoxicity analysis

Observation of the entire plate was done at a regular interval of 24 h using an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and the observations were recorded. Variations in the morphology of the cells in the form of folding, shrinking, granulation and vacuolisation were considered as an indication of cytotoxicity (19).

Detection of cytotoxicity by MTT method

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma, M-5655) (15 mg) was prepared by dissolving in 3 mL PBS, filtered and sterilised. After 24 h of incubation, the samples were removed and a fresh medium was added to avoid the direct interaction of the test extract with MTT. About 3.0 µL of MTT solution was added to all the culture wells. Prior to incubation at 37 ºC in a humidified 5% CO_2 incubator for 4 h, the plates were slightly shaken. After incubation, the supernatant was discarded and MTT solubilisation solution (DMSO) (100 µL) was added to the wells and mixed slightly by pipetting to solubilise the blue-coloured formazan crystals. The absorbance was measured by using a microplate reader at a wavelength of 540 nm (20).

Assessment of lipid peroxidation (LPO)

To analyse the degree of damage to hepatocytes, a lipid peroxidation (LPO) assay was performed (21). The treated cell samples were trypsinised with Trypsin-EDTA solution (Himedia, India) and centrifuged at 5000 rpm for 5 min. After centrifugation, the pellet was suspended in 200 µL of lysis buffer (0.1 M tris, 0.2 M EDTA, 2 M NaCl, 0.5% Triton). Samples were then incubated at 4 °C for 20 min and after incubation, cell lysate (50 µl) was added with 70% (500 µl) alcohol and 1.0% (w/v) TBA (1 mL). Then, all the tubes were transferred to a boiling water bath for 20 min. The vials were cooled under running water. After cooling the vials, an equal volume of trichloroacetic acid (10%) was added to the sample and centrifuged at 1000 rpm for 15 min. The supernatant was compiled and absorbance was read at 532 nm in a spectrophotometer (Beckman DU 650 Spectrophotometer). Control sample validation was performed in the same manner in which the distilled water was used instead of the TBA solution. The end product of lipid peroxidation is the formation of malonyldialdehyde (MDA) that reacts with thiobarbituric acid and forms a pink chromogen.

Reduced glutathione (GSH) assessment

GSH level was estimated according to a previously established protocol (22). Briefly, 1 mL of cell lysate was added to phosphate buffer (0.2M) (0.5 mL) (pH 8). The homogenate was added with an equal quantity of 20% trichloroacetic acid. The mixture was incubated for 5 min and then centrifuged at 200 rpm for 10 min. The supernatant (200 µL) was then added with 0.2 mL of DTNB (0.6 mM) (Ellman's reagent), mixed well and the absorbance was read at 420 nm. The GSH levels were compared with a standard reduced glutathione (23).

Superoxide dismutase assay (SOD)

The superoxide dismutase assay was performed according to the standard methods (24). For the analysis of SOD, cell lysate (50 mL) was mixed with the reaction mixture containing phosphate buffer (50 mM) (pH 7.8), methionine (45 µM), riboflavin (5.3 mM), potassium ferric cyanide (84 µM) and NBT (0.1M). The reaction mixture was stirred well and then incubated at 25 °C for 10 min and the absorbance was taken at 600 nm. The absorbance was compared with the standard curve obtained from the known SOD.

$$\text{Percentage of inhibition} = \frac{\text{Control - Test}}{\text{control}} \text{ X } 100$$

Statistical analysis

Statistical analysis was done using the software Graph pad Prism 5.01 software (GraphPad Software, Inc., San Diego, CA). The results were represented as mean ± SE (n = 3) and were analysed by one-way ANOVA (analysis of variance) followed by Dunnet's test. A value of $p < 0.05$ was considered to be statistically significant.

Results

Qualitative phytochemical analysis

Phytochemical analysis of the crude methanolic extract of *G. mangostana* pericarp revealed the presence of alkaloids, saponins, terpenoids, flavonoids and coumarin negative (Table 1).

Table 1. Preliminary phytochemical screening of methanolic extract of *G. mangostana* pericarp

Phytochemicals	Tests	Sample Pericarp
Alkaloids	Dragendorff´s	++
	Mayer´s	++
	Wagner´s	+
	Hager´s	++
Saponins	Foam	++
Phenol	Folin´s	++
Flavonoid	Shinoda´s	+++
Carbohydrate	Molisch´s	++
Terpenoid	Salkowski	+++
Steroid	Leibermann Buchard	++
Caumarine	Caumarine	-

+ mild, **++** moderate, **+++** abundance, **-** absence.

Total alkaloid, phenol and flavonoid content

Total alkaloid content, phenol and flavonoid contents were estimated as 36.26 mg/g dry weight, 38.17 mg gallic acid equivalents/gram dry weight of extract and 42.27 mg quercetin equivalents/gram dry weight of extract.

In vitro hepatoprotective activity of G. mangostana using HepG2 cell lines

The hepatoprotective activity of *G. mangostana* was evaluated by using different concentrations of methanol extract

of *G. mangostana* in acetaminophen-intoxicated human-derived liver cell-HepG2 cell lines. Acetaminophen is a prototype hepatotoxin that was generally used to analyse metabolite-dependent toxicity. In the present investigation, there was a markable reduction in viability of acetaminophen-treated cell lines compared with the untreated control (Fig. 2). Acetaminophen (20 µM) treated human HepG2 cell lines grown in DMEM showed a reduced percentage (50.52%) of viability compared to control (100%) (Table 2). The methanol extract (100 µL) of *G. mangostana* was tested at various concentrations ranging from 1.5 µg/mL to 25.0 µg/mL against acetaminophen-treated HepG2 liver cell lines and incubated for 72 h. After incubation, it was observed that the methanol extract (100 µL) of *G. mangostana* at various concentrations of 1.5 µg/mL, 3.1 µg/mL, 6.25 µg/mL, 12.5 µg/mL and 25.0 µg/mL showed an increased cell viability of 58.75%, 75.12%, 80.37%, 86.54% and 95.45%, respectively. The HepG2 cells treated with acetaminophen showed liver necrosis with inflammation, whereas the *G. mangostana*-treated cell lines showed less

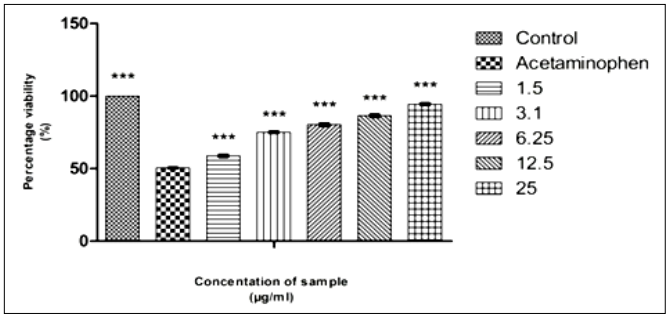

Fig. 2. Graphical representation depicting the hepatoprotective effect of the sample by MTT assay. All experiments were done in triplicates and results are represented as Mean ± SE. One-way ANOVA and Dunnet's test were performed to analyse data. ***p < 0.001 compared to acetaminophen exposed group.

Table 2. Effects of methanolic extract of *Garcinia mangostana* on acetaminophen intoxicated HepG2 cell lines

Sample (µg/ml)	Average absorbance @ 540nm	Percentage viability
Control	0.7905	100
Acetaminophen	0.3994	50.52
G. mangostana		
1.5	0.4644	58.75
3.1	0.5938	75.12
6.25	0.6354	80.37
12.5	0.6842	86.54
25	0.7467	94.45

cell necrosis and minimal inflammatory condition. The *G. mangostana* sample of concentration 25 µg/mL treated cell lines showed 94.45% cell viability and showed normal cellular morphology with less cellular necrosis with higher hepatoprotective activity (Fig. 3). Curative treatments with plant samples showed cytoprotection against acetaminophen-induced cell damage. Based on the findings of the current study, *G. mangostana* extract was considered safe in different concentration for HepG2 cell lines and also proved to be non-cytotoxic. The study signifies the hepatoprotective potential of *G. mangostana* and similar hepatoprotective properties were also reported in related species (25).

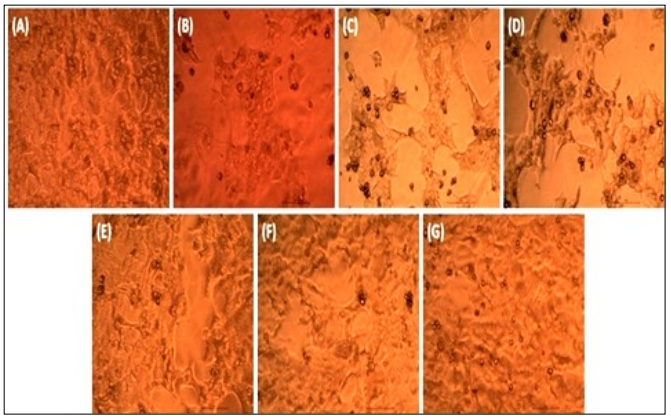

Fig. 3. Effect of *Garcinia mangostana* on cultured HepG2 cell lines treated with acetaminophen (**A**) Untreated control liver cells (normal architecture). **(B)** Acetaminophen treated liver cells: necrosis, loss of cellular boundaries. (**C**) Acetaminophen-treated cells co-administrated with varied concentrations of *Garcinia mangostana* sample 1.5 µg/mg. **(D)** sample 3.1 µg/mg. **(E)** sample 6.25 µg/mg. **(F)** sample 12.5 µg/mg. **(G**) Liver cells were treated with acetaminophen and *Garcinia mangostana* sample 25 µg/mg showing minimal inflammatory infiltration almost similar to normal cellular architecture showing hepatocyte regeneration.

Estimation of LPO, GSH and SOD

The current study depicted a significant increase in the level of MDA as a final product of lipid peroxidation in acetaminophen-intoxicated HepG2 cell lines. The level of lipid peroxides in acetaminophen-derived HepG2 cell lines is 0.64 compared with the control groups. The LPO content increased significantly in acetaminophen-intoxicated HepG2 cell lines, whereas the treatment of pericarp extract of *G. mangostana* at different concentrations ranging from 1.5, 6.25, 12.5 and 25.0 µg/mL showed a decreased level of MDA (0.91, 0.88, 0.73 and 0.64, respectively) (Fig. 4). Among the different concentrations of *G. mangostana*, extract 25.0 µg/ml showed the maximum protection. The enhanced range of lipid peroxides (MDA) showed the structural and functional alteration of the cellular membrane that leads to tissue damage (26). In the current study, the elevation of lipid peroxides was observed in acetaminophen-treated HepG2 liver cell lines, depicting the tissue damage and inefficient antioxidant defence mechanism against excessive free radical formation. It has been observed that treatment with *G. mangostana* significantly inversed these conditions and prevented tissue damage. The reduced LPO activity after the treatment with the *G. mangostana* extract

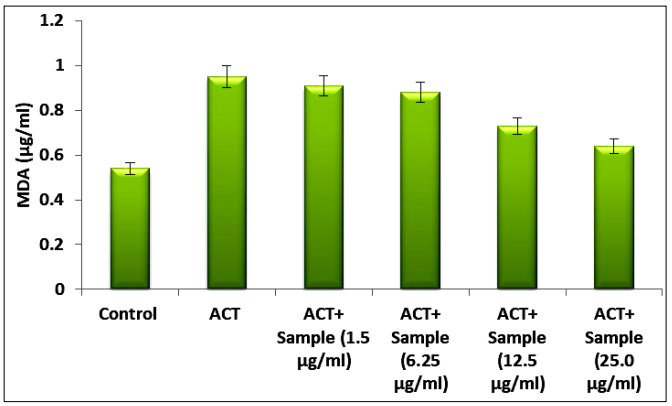

Fig. 4. Effect of methanol extract of *Garcinia mangostana* on the MDA level in HepG2 cell lines treated with acetaminophen. ACT represents the acetaminophen-treated samples. All experiments were done in triplicates and results are represented as Mean ± SE. One-way ANOVA and Dunnet's test were performed to analyse data, p < 0.001 compared to the acetaminophen-exposed group.

may be attributed to the antioxidant potential of *G. mangostana* by scavenging the free radicals produced in liver cell lines.

The enzymatic antioxidants, SOD and GSH activity were found to be significantly reduced ($P < 0.001$) in acetaminophen-treated HepG2 liver cell lines when compared with the control. The level of SOD (0.48) and GSH (1.99) was reduced in acetaminophen-treated cell lines, whereas the content of SOD was significantly increased in the liver cells treated with different concentrations (1.5, 6.25, 12.5 and 25.0 µg/mL) of *Garcinia mangostana*. The percentages of SOD (0.48) and GSH (1.99) in acetaminophen-treated samples were very much lesser than the normal untreated control, 1.24 and 4.25, respectively. The percentage protection in SOD was 0.51, 0.73, 0.92, 1.02 and GSH was 2.32, 2.87, 3.17, 3.98 at the plant sample concentration of 1.5, 6.25, 12.5 and 25.0 µg/mL, respectively (Fig. 5). Among the plant sample concentrations 25.0 µg/mL has shown maximum protection. In acetaminophen-initiated hepatotoxicity, the equilibrium between Reactive Oxygen Species formation and antioxidant defence mechanism may be hindered, resulting in oxidative stress through a series of activities that deregulate the normal cellular function, which finally leads to cellular necrosis. In the current study, it was clearly depicted that when the acetaminophen-added liver cell lines were treated with *G. mangostana*, the level of SOD and GSH were significantly increased, which indicates the antioxidant property of *G. mangostana.*

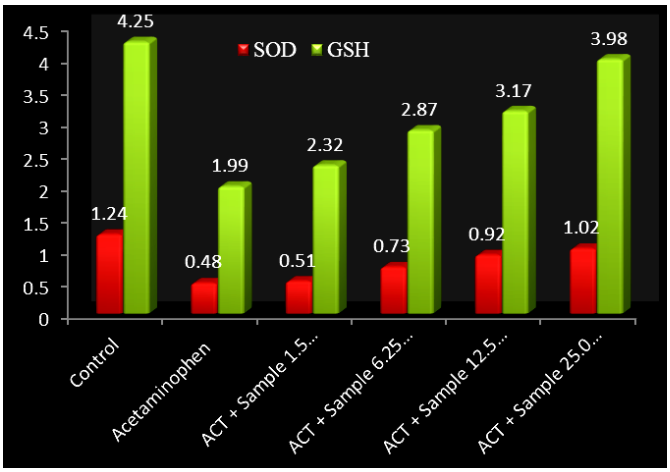

Fig. 5. Effect of methanol extract of the pericarp of *Garcinia mangostana* on antioxidant enzyme activity (SOD and GSH) in the acetaminophen-treated HepG2 liver cell lines. All experiments were done in triplicates and results are represented as Mean ± SE. One-way ANOVA and Dunnet's test were performed to analyse data, $p < 0.001$ compared to the acetaminophen-exposed group.

Discussion

Many plant species were claimed to have hepatoprotective properties and these properties greatly rely upon its phytoconstituents like phenols, flavonoids, alkaloids, terpenes, glycosides and xanthones. The preliminary phytochemical screening gained greater importance in the analysis of the antioxidant and hepatoprotective potential of plant species. In the present study, the overall phytoconstituents analysis of the methanol extract of *G. mangostana* pericarp revealed the presence of alkaloids, saponins, terpenoids, flavonoids, steroids, etc., and this can be attributed to the antioxidant and hepatoprotective potential of *G. mangostana*. Phenolic compounds are the major source of antioxidants or free radical scavengers that transform free radicals into stable ones (27). Earlier it had been reported about the involvement of free radicals in the destruction of liver cells and the free radical scavenging potential of phytoconstituents. HepG2 are immortalised human hepatoma cell lines, which have been commonly used as an *in vitro* model for hepatoprotective studies and drug metabolism. Moreover, it is nontumorigenic and exhibits a high rate of active proliferation (28). Incubating HepG2 cell lines with acetaminophen for 24 h caused a remarkable reduction in cell viability. The major mechanism behind acetaminophen-induced hepatotoxicity is the mitochondrial dysfunction caused due to the action of acetaminophen. As a result, it causes oxidative stress due to the overproduction of superoxide (O_2-). Overproduction of superoxide results in loss of cell function and ultimately leads to apoptosis or necrosis (29). The study focuses on the protective effect of *G. mangostana* against acetaminophen-induced cytotoxicity in HepG cell lines to analyse the hepatoprotective potential of the plant. The effect of methanol extract of G. mangostana at various concentrations ranging from 1.5 µg/mL to 25.0 µg/mL against acetaminophen-treated human HepG2 liver cell lines observed that methanol extract of *G. mangostana* exhibited a dose-dependent cytoprotection against acetaminophen treated cell lines.

Lipid peroxidation has been detected as evidence of liver injury due to acetaminophen administration and, the incapability of an antioxidant defense mechanism to reduce the formation of excessive free radical production. It was observed that the treatment of HepG2 cell lines with plant extract of *G. mangostana* remarkably reversed these variations. The increased level of malondialdehyde (MDA) in the liver highlighted the enhanced lipid peroxidation that resulted in tissue destruction and loss of the antioxidant defence mechanism. Treatment with *G. mangostana* pericarp significantly reversed all such transformations and this may be possible due to the antioxidant potential of *G. mangostana*. The body has a prominent mechanism to prevent and mitigate cellular damage induced by free radicals and this is achieved by an array of endogenous antioxidant enzymes such as SOD, GSH, CAT, GPX and GR. The decline in levels of enzymes such as SOD and GSH observed in acetaminophen-treated human HepG2 is a real manifestation of an enhanced formation of hepatic lipid peroxides content. It has been confirmed that the hepatoprotective potential of *G. mangostana* is mainly due to its antioxidant activity. It has been reported that glutathione is one of the most abundant tripeptides and non-enzymatic antioxidants in liver cells. The main function of glutathione is to scavenge free radicals like superoxide radicals and hydrogen peroxides and act as a substrate for glutathione peroxidase. In the present investigation, the decreased level of GSH is connected with the increased level of lipid peroxides in acetaminophen-treated cell lines and confirmed that the administration of *G. mangostana* significantly enhanced the level of glutathione in a dose-dependent manner (30). Reduced enzymatic activity of

SOD is the actual measure of hepatocellular damage in acetaminophen-treated cell lines but the treatment with different concentrations of *G. mangostana* showed a significant increase in the level of SOD which confirms the antioxidant potential of *G. mangostana*. SOD scavenges the superoxide anion and transforms it to hydrogen peroxide which were rapidly converted to water molecules by the action of CAT and GSH, thus reducing the toxic effects raised by free radicals (31). Moreover, the *G. mangostana* extract enhanced the activity of antioxidant enzymes (SOD and GSH) and reduced the quantity of lipid peroxide against the acetaminophen-induced hepatotoxicity in HepG2 cell lines. Thus, it was proved that the *G. mangostana* extract could scavenge the reactive free radicals that might cause damage to liver tissue and enhance the activities of hepatic antioxidant enzymes. Methanol extract of *G. mangostana* showed dose-dependent hepatoprotective activity and the sample (25.0 µg/mL) showed maximum hepatoprotection. The hepatoprotective and antioxidant activities of *G. mangostana* may be associated with flavonoid and phenolic compounds of the samples (32). The possible mechanism for the hepatoprotective activity of the pericarp extract of *G. mangostana* may be due to its ability to inhibit lipid peroxidation and enhance the activity of antioxidant enzymes (SOD and GSH) (33).

Conclusion

The results of the present study suggest that the methanolic extract of *G. mangostana* pericarp exhibits antioxidant potential against free radicals, prevents oxidative damage and affords significant protection against acetaminophen-treated human HepG2 cell lines. Immense phytochemicals in *G. mangostana* extract may be responsible for its antioxidant and hepatoprotective activities. It has been proved that the methanol extract of *G. mangostana* pericarp can be utilised as a source of natural antioxidant and hepatoprotective agents. Further studies on animal models are needed to evaluate their potential benefits.

Acknowledgements

We would like to thank the research team of Sree Narayana College, Kollam for the technical support

Authors' contributions

ASK and AS had supervised the current study, RAS and RS had performed and analysed the experiments. All the authors contributed equally in writing, reviewing and submitting the manuscript. All authors read and approved for the final submission of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

References

1. Jung HA, Su BN, Keller WJ, Mehta RG, Kinghorn AD. Antioxidant xanthones from the pericarp of *Garcinia mangostana* (Mangosteen). J Agri and Food Chem. 2006;54(6):2077–82. https://doi.org/10.1021/jf052649z
2. World Health Organization, Global Buruli Ulcer Initiative. Report of the 7th WHO advisory group meeting on Buruli ulcer: 8-11 March 2004, WHO headquarters, Geneva, Switzerland. World Health Organization; 2004
3. Tavera PTH, Thomas JB, editors. The medicinal plants of the Philippines. Philadelphia, USA: Blakiston's son and co.; 1901.
4. Pierce S, Vianelli A, Cerabolini B. From ancient genes to modern communities: the cellular stress response and the evolution of plant strategies. Funct Ecol. 2005;19(5):763–76. https://doi.org/10.1111/j.1365-2435.2005.01028.x
5. Moongkarndi P, Kosem N, Kaslungka S, Luanratana O, Pongpan N, Neungton N. Antiproliferation, antioxidation and induction of apoptosis by *Garcinia mangostana* (mangosteen) on SKBR3 human breast cancer cell line. J Ethnopharmacol. 2004;90 (1):161–66. https://doi.org/10.1016/j.jep.2003.09.048
6. Ming-Hui WANG, Zhang KJ, Qin-Lan GU, Xiao-Ling BI, Jin-Xin WANG. Pharmacology of mangostins and their derivatives: A comprehensive review. Chinese J Nat Med. 2017;15(2):81–93. https://doi.org/10.1016/s1875-5364(17)30024-9
7. Gopalakrishnan G, Banumathi B, Suresh G. Evaluation of the antifungal activity of natural xanthones from *Garcinia mangostana* and their synthetic derivatives. J Nat Prod. 1997;60 (5):519–24. https://doi.org/10.1021/np970165u
8. Chen SX, Wan M, Loh BN. Active constituents against HIV-1 protease from *Garcinia mangostana*. Planta Medica. 1996;62 (04):381–82. https://doi.org/10.1055/s-2006-957916
9. Chen LG, Yang LL, Wang CC. Anti-inflammatory activity of mangostins from *Garcinia mangostana.* Food and Chem Toxicol. 2008;46(2):688–93. https://doi.org/10.1016/j.fct.2007.09.096
10. Nakatani K, Atsumi M, Arakawa T, Oosawa K, Shimura S, Nakahata N, et al. Inhibitions of histamine release and prostaglandin E2 synthesis by mangosteen, a Thai medicinal plant. Biol and Pharma Bull. 2002;25(9):1137–41. https://doi.org/10.1248/bpb.25.1137
11. Setiawati A. Anticancer activity of mangosteen pericarp dry extract against MCF-7 breast cancer cell line through estrogen receptor-α. Ind J Pharma. 2014;25(3):119. https://doi.org/10.14499/indonesianjpharm25iss3pp119
12. Chattopadhyay R. Possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract: part II. J Ethnopharmacol. 2003;89(2–3):217–19. https://doi.org/10.1016/j.jep.2003.08.006
13. Bindu S, Rameshkumar KB, Kumar B, Singh A, Anilkumar C. Distribution of reserpine in *Rauvolfia* species from India-HPTLC and LC-MS studies. Industrial Crops and Prod. 2014;62:430–36. https://doi.org/10.1016/j.indcrop.2014.09.018
14. Kokate CK. Practical pharmacognosy, 4th edition, Vallabh Prakashan Publication, New Delhi; 1988.
15. Sreevidya N, Mehrotra S. Spectrophotometric method for estimation of alkaloids precipitable with Dragendorff's reagent in plant materials. J AOAC Internat. 2003;86(6):1124–27. https://doi.org/10.1093/jaoac/86.6.1124
16. Vernon LS, Orthofer R, Raventos LRM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 1999;299:152–78. https://doi.org/10.1016/S0076-6879(99)99017-1
17. Almulaiky YQ, Alshawafi WM, Al-Talhi HA, Zeyadi M, Anwar F, Al-abbasi FA, et al. Evaluation of the antioxidant potential and

antioxidant enzymes of some Yemeni grape cultivars. Free Radicals and Antioxidants. 2017;7(1):74–79. https://doi.org/10.5530/fra.2017.1.11

18. Reddy PR, Thiruvanavukkarasu P, Rajesh S, Karunakaran S, Hari R. Effect of ethanolic extract of *Carica papaya* leaves and their cytotoxicity and apoptotic potential in human ovarian cancer cell lines-PA-1. Pharmacogn Mag. 2020;16(05):524-30. https://doi.org/10.4103/pm.pm_117_20
19. Niu J, Li M, Wang Y. Cell proliferation and cytotoxicity assays, the fundamentals for drug discovery. Int J Drug Discov Pharmacol. 2024;3(3):100013. https://doi.org/10.53941/ijddp.2024.100013
20. Zheleva-Dimitrova D, Simeonova R, Kondeva-Burdina M, Savov Y, Balabanova V, Zengin G, et al. Antioxidant and hepatoprotective potential of *Echinops ritro* L. extracts on induced oxidative stress *in vitro*/*in vivo*. Int J Mol Sci. 2023;24(12):9999. https://doi.org/10.3390/ijms24129999
21. Li Y, Si D, Sabier M, Liu J, Si J, Zhang X. Guideline for screening antioxidant against lipid-peroxidation by spectrophotometer. eFood. 2023;4(2):e80. https://doi.org/10.1002/efd2.80
22. Ellman GL. Tissue sulfhydryl groups. Arch Biochem and Biophys. 1959;82(1):70–77. https://doi.org/10.1016/0003-9861(59)90090-6
23. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimic Biophys Acta (BBA) -Gen Subj. 1979;582(1):67–78. https://doi.org/10.1016/0304-4165(79)90289-7
24. Misra HP, Fridovich I. Superoxide dismutase: “positive” spectrophotometric assays. Anal Biochem. 1977;79(1–2):553–60. https://doi.org/10.1016/0003-2697(77)90429-8
25. HS, Singh SK. Phytochemical analysis, antioxidant and anti-inflammatory activities of *Phyllanthus simplex*. J Ethnopharmacol. 2011;137(3):1337–44. https://doi.org/10.1016/j.jep.2011.07.069
26. Sahoo S, Rath D, Kar DM, Pattanaik S. Hepatoprotective potency of *Litsea glutinosa* (L.) CB Rob. leaf methanol extract on H_2O_2-induced toxicity in HepG2 cells. J Ethnopharmacol. 2023;304:116076. https://doi.org/10.1016/j.jep.2022.116076
27. Donato MT, Tolosa L, Gómez-Lechón MJ. Culture and functional characterization of human hepatoma HepG2 cells. Protocols in *in vitro* Hepatocyte Res. 2015;77–93. https://doi.org/10.1007/978-1-4939-2074-7_5
28. Meister A, Anderson ME. Glutathione. Ann Rev Biochem. 1983;52(1):711–60. https://doi.org/10.1146/annurev.bi.52.070183.003431
29. Hsiao G, Shen MY, Lin KH, Lan MH, Wu LY, Chou DS, et al. Antioxidative and hepatoprotective effects of *Antrodia camphorata* extract. J Agri Food Chem. 2003;51(11):3302–08. https://doi.org/10.1021/jf021159t
30. Abood WN, Bradosty SW, Shaikh FK, Salehen NA, Farghadani R, Agha NF, et al. *Garcinia mangostana* peel extracts exhibit hepatoprotective activity against thioacetamide-induced liver cirrhosis in rats. J Funct Foods. 2020;74:104200. https://doi.org/10.1016/j.jff.2020.104200
31. Amresh G, Reddy GD, Rao CV, Singh PN. Evaluation of anti-inflammatory activity of *Cissampelos pareira* root in rats. J Ethnopharmacol. 2007;110(3):526–31. https://doi.org/10.1016/j.jep.2006.10.009
32. Wu YH, Zhang XM, Hu MH, Wu XM, Zhao Y. Effect of *Laggera alata* on hepatocytes damage induced by carbon tetrachloride *in vitro* and *in vivo*. J Ethnopharmacol. 2009;126:50–56. https://doi.org/10.1016/j.jep.2009.08.030
33. Mursal M, Saidi N, Yahya M, Murniana M, Ginting B. Antioxidant profile of ethyl acetate extract from *Garcinia mangostana* L. stem barks and its fractions: investigation using DPPH inhibition assay. Res J Pharm Technol. 2024;17(9):4461–64. https://doi.org/10.52711/0974-360X.2024.00689