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Original article

Effects of Monosodium glutamate and Citrus limon peel extract on the hepatic enzymes correlating with histopathological changes of liver

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ABSTRACT

Introduction: *Monosodium glutamate (MSG) is a taste enhancer used in wide range of food varieties, but on the other side it causes certain health issues. Citrus limon (C. limon) peels have wide spectrum of phytochemicals with therapeutic effects. Liver plays a vital role in the metabolism of certain chemical substances. The current study was aimed to find out the effect of MSG and Citrus limon peel extract on the enzymes serum glutamic pyruvic transaminase (SGPT) and serum glutamic-oxaloacetic transaminase (SGOT) produced from the liver and their correlation with histopathological changes of liver in adult albino rats.*

Materials & methods*: Citrus limon fruits were collected, authenticated and methanolic extract was prepared from the peels. 36 Male adult albino rats were divided into 9 groups, each group contain 4 rats and treated in 2 phases. Different concentrations of MSG and Citrus limon peel extract were administered. Blood samples were collected on day 0, 21 and 31 to measure the liver enzymes and the liver specimens were preserved in formalin for tissue processing.*

Results: *The liver enzymes were elevated in the MSG low and high dose groups and reduced to normal in Citrus limon peel extract groups. The MSG treated groups showed inflammatory changes and disintegration of hepatocytes and the Citrus limon peel extract administration reduced the inflammation effectively compared to natural recovery group.*

Conclusions*: The Citrus limon peel extract effectively lowered the SGOT and SGPT levels along with histological repair confirms that it has hepatoprotective property.*

Keywords*: Liver, Monosodium glutamate, Citrus limon peel extract, serum glutamic pyruvic transaminase, serum glutamic-oxaloacetic transaminase.*

INTRODUCTION

In most of the food products the additives are added for preservation purpose and also to enhance the taste. A widely used food additive Monosodium glutamate (MSG) which is commonly known as Ajinomoto is the sodium salt of glutamic acid that makes the food more palatable.[1,2] MSG is commonly used flavouring chemical agent in Chinese, Japanese and Taiwanese foods.[3] Food and drug administration (FDA) considered that the human consumption of MSG is safe in any quantity.[4] As per recent literature 3 to 4 grams of MSG per day is considered to be safe and any increase in its quantity even a gram may elevate the risk of onset of the metabolic disorders.[5] Chronic use of MSG may lead to minor illness such as weakness, dizziness, headache and flushes and also may cause major illnesses such as neurological problems include neuropathies, epilepsy, anxiety, schizophrenia, Huntington's disease, Parkinsonism, Alzheimer's disease and amyotrophic lateral sclerosis. MSG overuse may also cause cardiac arrhythmia, urticaria, retinal degeneration and endocrine disorders.[6,7]

Liver is an important organ that helps in metabolism and storage of many substances. The metabolic functions that the hepatocytes perform include detoxification, deamination, transamination, removal of ammonia in the form of urea, biosynthesis as well as release of non-essential amino acids, and plasma proteins apart from immunogamma globulins. The hepatocytes are also involved in gluconeogenesis, glycogen storage, cholesterol, phospholipid, and lipoprotein synthesis, oxidation of fatty acids, iron storage in the form of ferritin, and storage of vitamins A, D, and B12.[8-12] Liver is the most commonly affected organ as it metabolizes and stores most of the nutrients and chemicals which are consumed. The MSG gets absorbed into the small intestine by active transport system specific for amino acids. The glutamic acid undergoes transamination as it reaches the liver through blood stream and ultimately causes raised alanine levels in portal blood. When large quantity of glutamate is consumed that may lead to elevated portal glutamate and results in hepatotoxicity.[13] The liver enzymes specially serum glutamic pyruvic transaminase (SGPT) also known as aspartate aminotransferase (AST), and serum glutamic-oxaloacetic transaminase (SGOT) also known as Alanine aminotransferase (ALT) plays a key role in liver health and any elevation in these enzymes signals the potential dysfunction or damage to the liver.[14]

There are several synthetic drugs that possess hepatoprotective activity which usually have side effects. There is a need in developing new drugs from the natural and herbal resources. *Citrus limon* peel is a good source of polyphenols and flavonoids that possess anti-inflammatory, antioxidant, anticancer and antimicrobial properties.[15,16] The hepatoprotective activity of *Citrus limon* peel extract was not studied much. Thus, the present study was designed to find out the hepatoprotective activity of the *Citrus limon* peel extract on the MSG treated rats.

MATERIALS AND METHODS

The presents study was conducted at Systemic Life Sciences and Research Pvt Ltd, Hyderabad, Telangana. The study was approved by the ethical committee. CPCSEA approval number**:** 01/IAEC/SLSRPL/2023.

MSG was procured from authorized vendor (AVRA). *Citrus limon* fruits were purchased from the authorized vendor and authenticated at Biodiversity Research & Education Centre, bearing the code number: 4555. The *Citrus limon* peels were shade dried, powdered and the methanolic extract was prepared by Soxhlet method.

Animals: A total of 36 adult male albino rats were selected randomly and the average body weight was 250 to 300gms. All the rats were kept in cages under slandered hygienic conditions and with suitable atmosphere. The animals were provided with standard laboratory feed and water at regular intervals. The study was adhered closely to the criteria set forth by the National Institutes of Health (NIH) regarding the treatment and usage of rats. All the rats were numbered and were fasted for 1 hour before administering MSG or *Citrus limon* peel extract. The route of administration of drug was oral.

Experimental study design:

In the present study 36 male adult albino rats were selected. The study was conducted in 2 phases. The rats were divided into 9 groups and each containing 4 rats. Phase 1 rats were sacrificed on day 22 and phase 2 rats on day 32.

Phase 1: Group 1 to 5 were included in this phase and the period was of 1 to 21 days.

Group 1: Control group where the rats were treated with normal saline.

Group 2: The rats were treated with MSG low dose (100mg/kg body weight).

Group 3: The rats were treated with MSG high dose (200mg/kg body weight).

Group 4: The rats were treated with MSG low dose (100mg/kg body weight) & *Citrus limon* peel extract 100mg/ kg body weight;

Group 5: The rats were treated with MSG high dose (200mg/kg body weight) & *Citrus limon* peel extract 100mg/ kg body weight;

Phase 2: Group 6 to 9 were included in this phase and the period was of 1 to 31 days.

Group 6: The rats were treated with MSG low dose (100mg/kg body weight) for 21 days and only *Citrus limon* peel extract 100mg/ kg body weight was given from 22nd to 31st day

Group 7: The rats were treated with MSG high dose (200mg/kg body weight) for 21 days and only *Citrus limon* peel extract 100mg/ kg body weight was given from 22nd to 31st day

Group 8: The rats were treated with MSG low dose and *Citrus limon* peel extract (100mg/kg body weight) for 21 days and left for recovery from 22nd to 31st day.

Group 9: The rats were treated with MSG high dose and *Citrus limon* peel extract (200mg/kg body weight) for 21st day and left for recovery from 22nd to 31st day.

Liver enzyme analysis:

The blood samples were collected just before starting the study (Day 0) and at the end of the experiment for the Phase I at 21st day and for Phase II at 31st day. The rats were sequentially anaesthetized with isoflurane for about 50–60s, around 2ml of blood was collected into a well labelled 4ml plain sample vial using capillary tube from retro-orbital plexus for the estimation of Serum glutamic oxaloacetic transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT). The blood was centrifuged after 30 minutes to obtain the serum. Serum sample was analyzed in a fully automatic analyzer (Erba-EM200), using ERBA kits.

Histopathological study of liver:

The phase 1 animals that is group 1 to group 5 were sacrificed on 22nd day and the liver specimens were collected, washed and labelled. The liver specimens were stored in 10% formalin and the tissue processing was carried out in the automatic tissue processor and the paraffin tissue blocks were prepared. The paraffin embedded blocks were cut into 5µ thick sections and the hematoxylin and eosin staining carried out and slides were prepared for all animals. The slides were observed under low (10X) and high (40X) magnifications. From each slide 8 high magnification field were observed under light microscope to note the histological changes and photos were captured.

Statistical analysis:

The data was analyzed using SPSS software version 22**.** One way ANOVA followed by Tukey's multiple comparison tests were performed for bodyweight and liver parameters SGPT and SGOT.

RESULTS

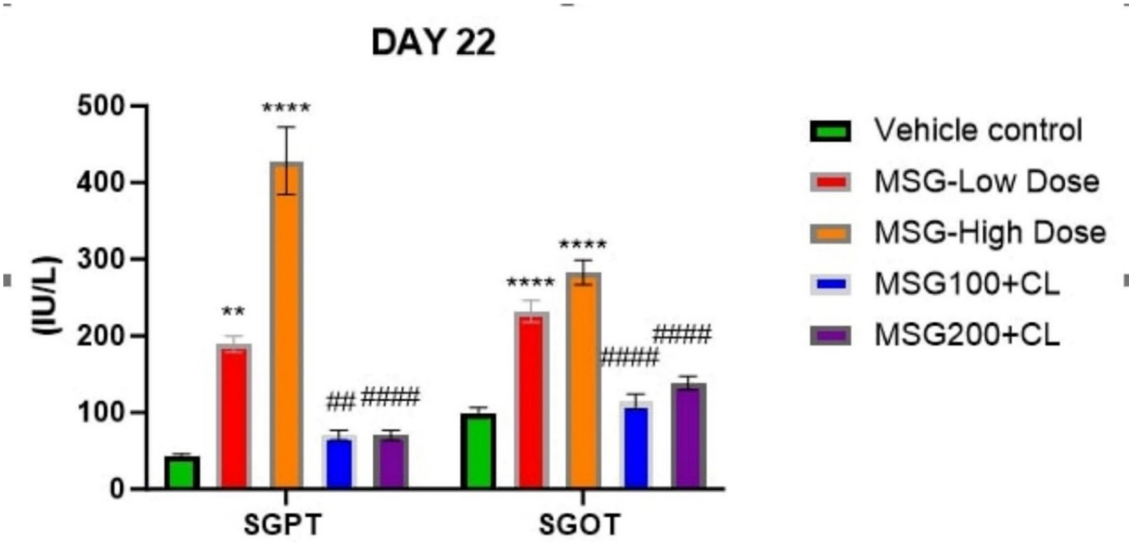
There was no significant difference in the body weights of all the groups in the 0th week that is before starting the treatment and also till the end of 1st week. In the 2nd week the MSG low as well as high dose treated groups showed significant decrease in the body weight whereas *Citrus limon* peel extract treated group did not show any significant difference in the body weight compared to vehicle control group. This same trend continued till 4th week.

Liver enzyme analysis: The liver enzymes SGPT and SGOT were analyzed and compared in 2 phases and in all study groups.

Phase I study:

There was no significant difference in SGPT, SGOT before starting the treatment in all the groups the values were within normal range, which satisfied the inclusion criteria of all rats in their respected groups. While On 22nd day in both low and high dose MSG intervention groups i.e., G2 & G3 there is a significant elevated levels of both SGPT and SGOT (**$p<0.01$ and ****$p<0.0001$) when compared to vehicle treated groups (Table I), (Fig. 1). Whereas *Citrus limon* peel extract treated animals in both MSG low dose and MSG high dose intervention groups i.e. G4 & G5 showed significant decrease in serum SGPT and SGOT levels (##$p<0.01$ and ####$p<0.0001$) when compared to MSG alone treated groups (Table 1), (Fig. 1). Further *Citrus limon* peel extract treated animals showed SGPT and SGOT values almost near normal range and has a non-significant difference when compared to the vehicle treated control animals.

Fig. 1: **Effect of Liver parameters, SGPT (IU/L) and SGOT (IU/L) in the Phase I rats given MSG orally and treated with CL medicine.**



Results were expressed as mean ± SEM (n = 8). *P < 0.05; **P < 0.01; ***P < 0.001; ****P <0.0001 when compared to Vehicle control group (G1), While #P < 0.05; ##P < 0.01; ###P < 0.001; ####P <0.0001 when G4 compared with G2 and G5 compared with G3.

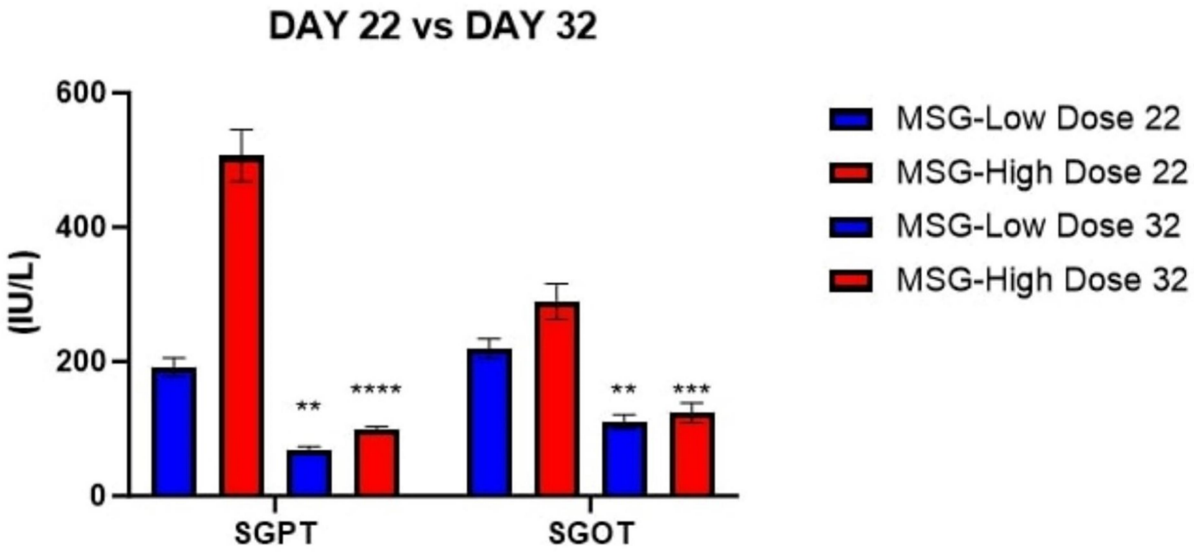
Table 1: Effect of Liver parameters in the Phase I rats given MSG orally and treated with CLPE.

Groups	Treatments	SGPT (IU/L)		SGOT (IU/L)	
		Before treatment	Day 22	Before treatment	Day 22
G1	Vehicle Control	40.00±2.08	42.25±3.68	101.0±9.23	99.75±6.96
G2	MSG-Low Dose	40.88±2.88	189.4±10.44**	99.50±5.96	232.3±14.01****
G3	MSG- High Dose	38.13±3.25	428.3±44.09****	105.1±9.12	282.8±15.99****
G4	MSG + CLPE	41.38±3.03	70.38±6.62$^{\#\#}$	106.9±8.17	114.4±9.69$^{\#\#\#\#}$
G5	MSG + CLPE	39.00±3.43	108.8±6.11$^{\#\#\#\#}$	108.8±6.61	138.5±8.77$^{\#\#\#\#}$

Phase II study:

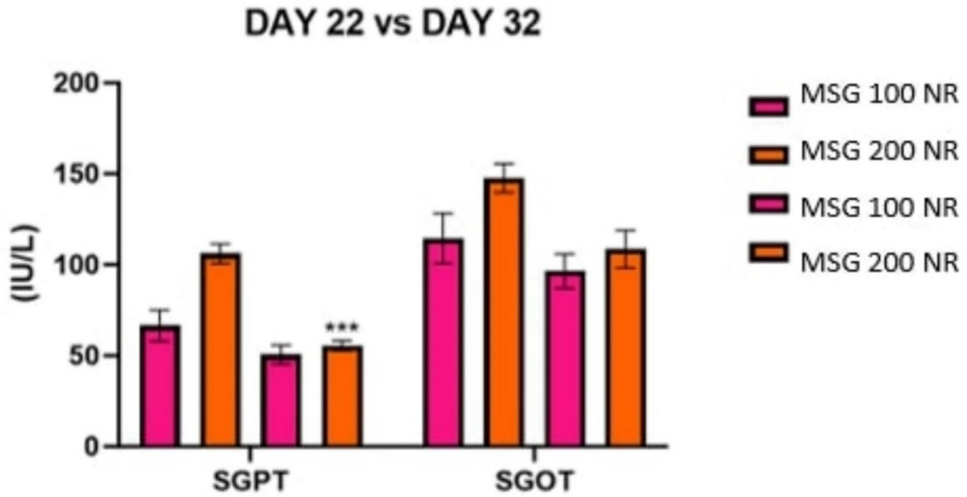
The G6 and G7 group animals has withdrawn from low and high dose MSG intervention on day 22 and started giving *Citrus limon* peel extract treatment till day 31, further looked for reversal of liver parameters, while G8 and G9 group animals were withdrawn from both MSG and *Citrus limon* peel extract on day 22 and looked for relapse of liver parameters. On day 32 there was a significant decrease in both SGPT and SGOT levels (**p<0.01, ***p<0.001 ****p<0.0001) in G6 and G7 groups compared to the elevated values of day 22 (Table II), (Fig. 2). Whereas, withdrawal of MSG and *Citrus limon* peel extract in G8 and G9 on day 22 did not relapse the elevation of liver parameters performed on day 32 (Table II), (Fig. 3).

Fig. 2: Effect of Liver parameters, SGPT (IU/L) and SGOT (IU/L) in the Phase II study.



Results were expressed as mean ± SEM (n = 4). *P < 0.05; **P < 0.01; ***P < 0.001; ****P <0.0001, significant when compared to values of day 22 of their respected groups.

Fig. 3: Effect of Liver parameters, SGPT (IU/L) and SGOT (IU/L) in the Phase II study natural recovery groups Group 8 and Group 9.



Results were expressed as mean ± SEM (n = 4). *P < 0.05; **P < 0.01; ***P < 0.001; ****P <0.0001, significant when compared to values of day 22 of their respected groups.

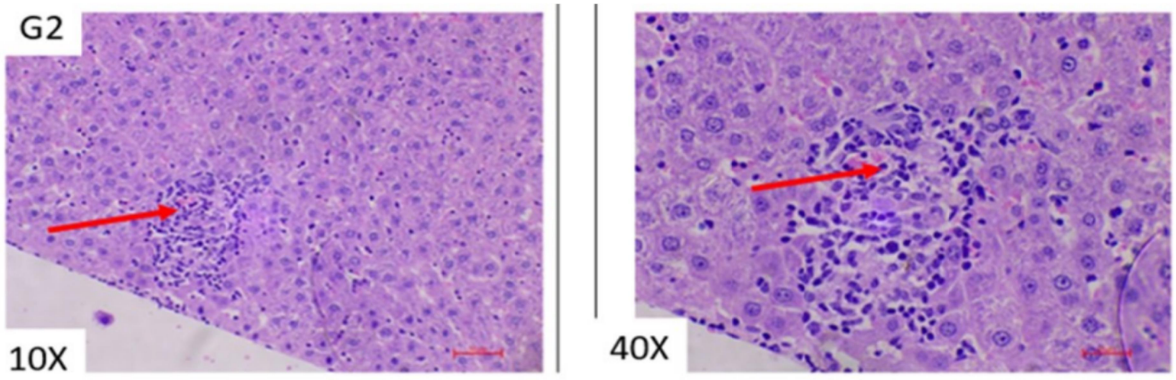
Table II: Effect of Liver parameters in the Phase II study

Groups	Treatment	SGPT (IU/L)		SGOT (IU/L)	
		Day 22	Day 32	Day 22	Day 32
G6	MSG Low Dose + CLPE	191.8±13.68	69.25±3.816**	219.5±14.95	110.8±10.39**
G7	MSG High Dose +CLPE	506.3±38.72	99.00±4.983****	289.3±26.49	123.5±14.64***
G8	MSG Low dose + left for recovery	66.50±8.627	50.50±5.204	114.5±13.67	96.50±9.215
G9	MSG High dose + Left for recovery	106.0±5.477	55.50±2.754***	147.8±7.836	108.5±10.22

Histopathological changes of liver:

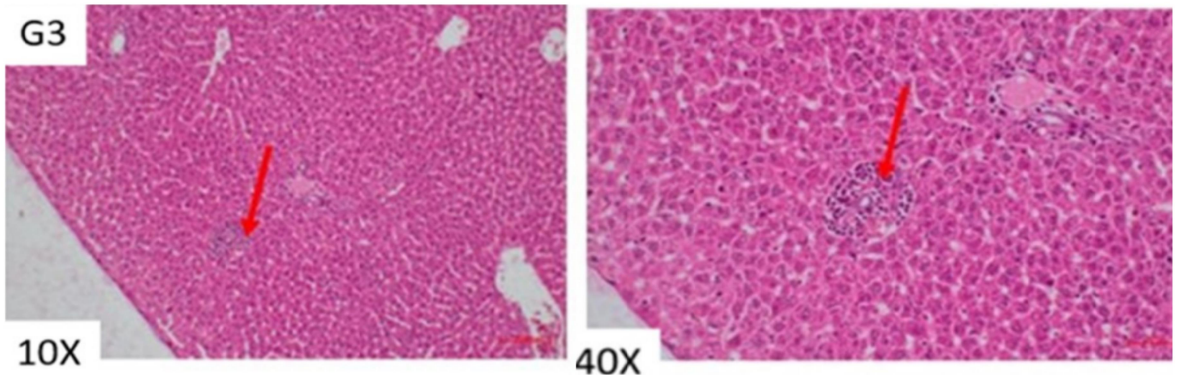
Phase 1: The control group G1 showed normal histology. G2 group showed Multifocal necrosis of hepatocytes with infiltration of inflammatory cells in the hepatocytes in the centri lobular region (Figure 4).

Figure 4: Showing multifocal necrosis of hepatocytes with infiltration of inflammatory cells in the hepatocytes in group G2.



G3 group presented with Foci of mild infiltration of inflammatory cells in the hepatocytes in the centri lobular/ peri biliary region of liver (Figure 5). In G2 and G3 groups dilatation of sinusoids with hemorrhagic changes in peri-sinusoidal spaces and the hepatocyte disintegration were also observed.

Figure 5: Foci of mild infiltration of inflammatory cells in the hepatocytes in the centri lobular/ peri biliary region of liver



The low dose MSG with *Citrus limon* peel extract combination groups G4 showed normal histology (Figure 6) and high dose MSG with *Citrus limon* peel extract combination group G5 showed mild inflammation

in liver along with mild vacuolar degeneration of hepatocytes with mild sinusoidal haemorrhages in between the hepatocytes on 22nd day (Figure 7).

Figure 6: Showing normal histology of the liver in G4 group treated with low MSG and *Citrus limon* peel extract

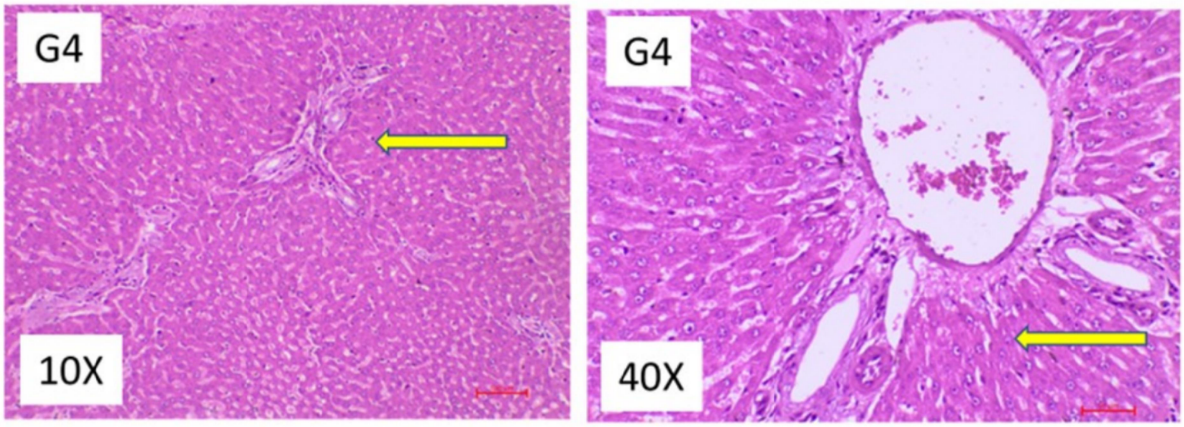
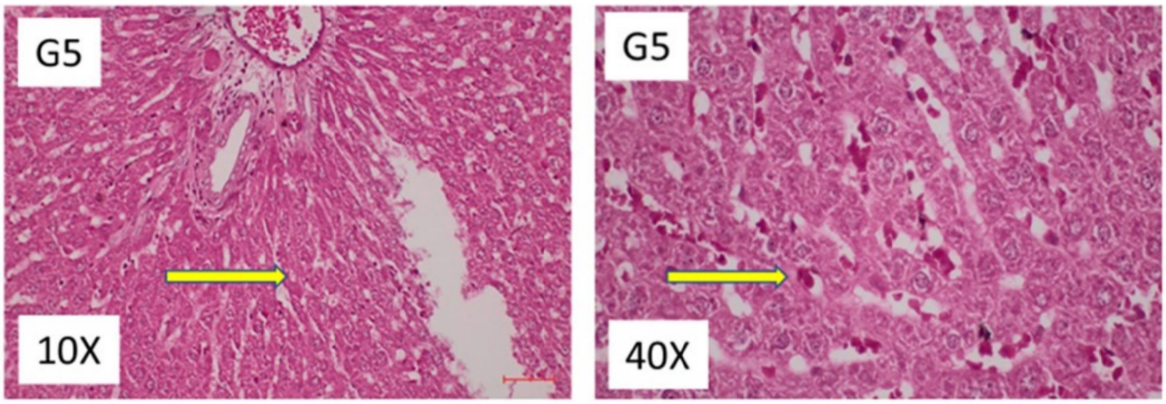


Figure 7: Showing mild vacuolar degeneration of hepatocytes with mild sinusoidal haemorrhages was noticed in between the hepatocytes in G5 group treated with high dose MSG and *Citrus limon* peel extract



Phase 2:

The low dose MSG for 22 days followed by only *Citrus limon* peel extract for next 10days (G6) group showed recovery changes and normal histology (Figure 8) whereas the high dose MSG for 22 days followed by only *Citrus limon* peel extract for next 10days (G7) showed mild foci necrosis of hepatocytes with infiltration of inflammatory cells in the hepatocytes in the centri lobular region and mild sinusoidal hemorrhages was also observed (Figure 9).

Figure 8: Showing normal morphology of hepatocytes in the portal, peri portal and centri lobular region of liver in G6 group

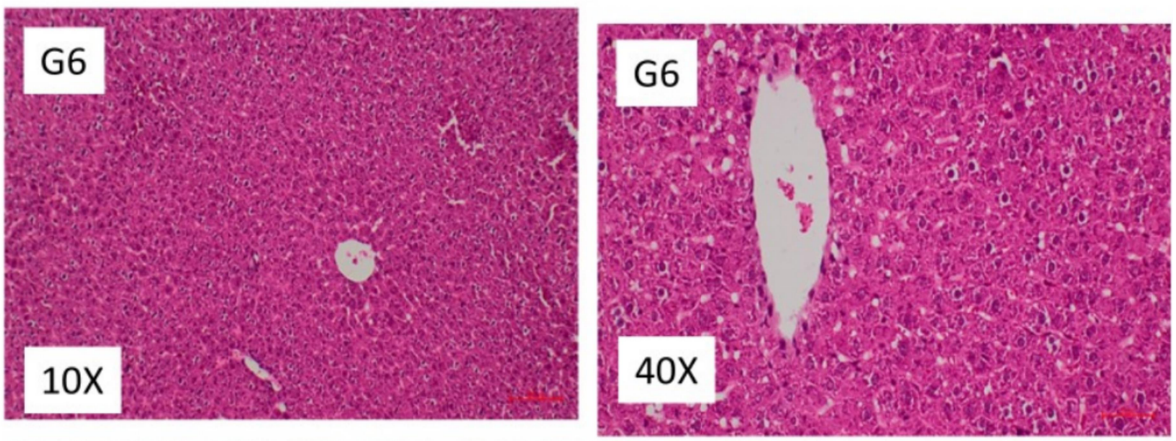
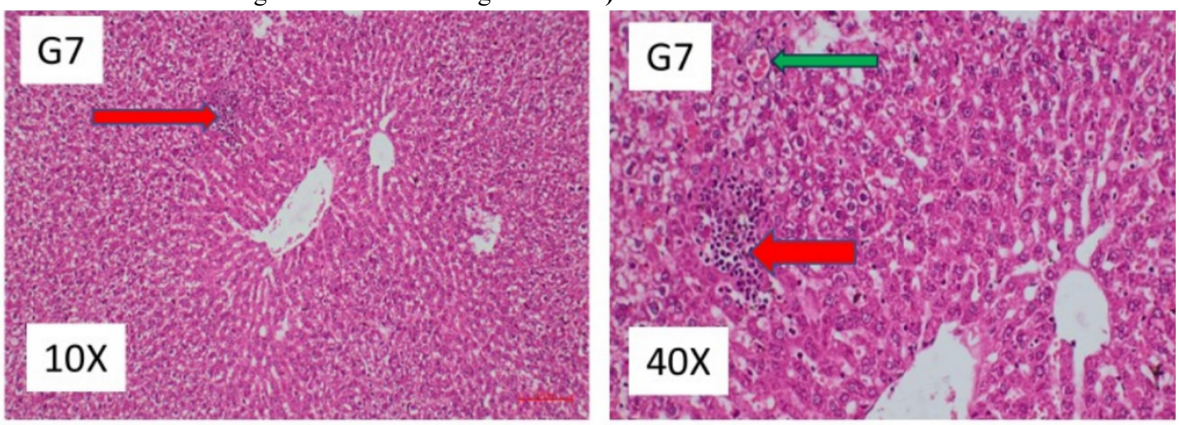
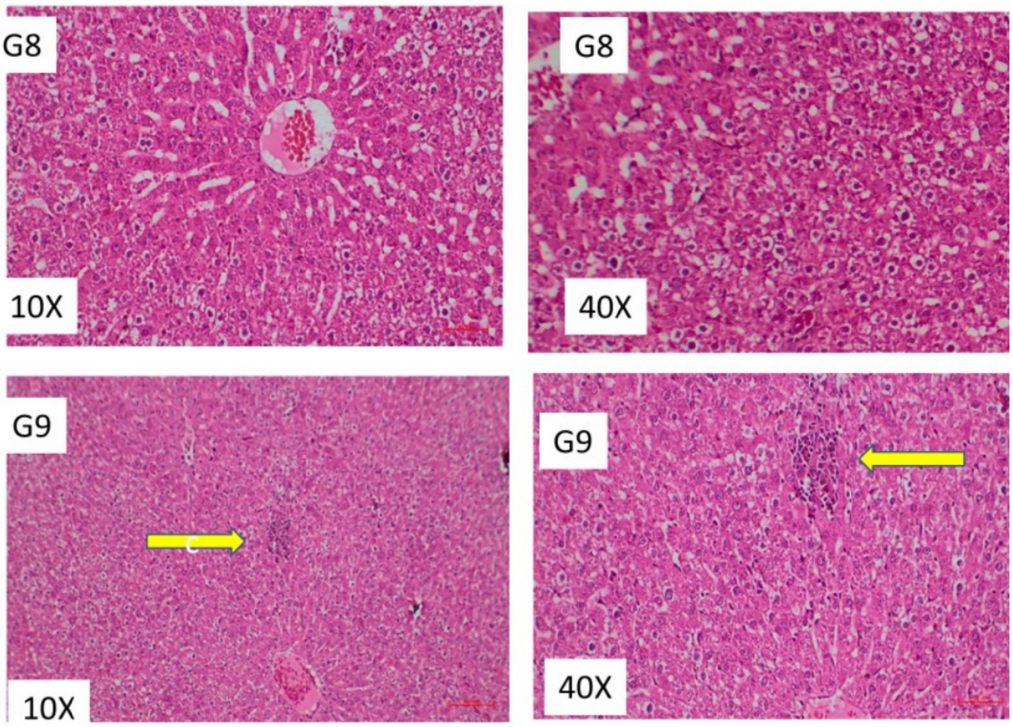


Figure 9: Showing mild focal necrosis of hepatocytes with infiltration of inflammatory cells in the hepatocytes in the centri lobular region and mild sinusoidal haemorrhage in G7 group. (Red arrow and mild sinusoidal hemorrhages were observed – green arrow**)**



In group 8 (G8) showed normal histology. In group 9 (G9) where high dose MSG and *Citrus limon* peel extract was given for 22 days and left for natural recovery for next 10 days the presented with multifocal mild necrosis of hepatocytes with infiltration of inflammatory cells in the hepatocytes in the centri lobular region (Figure 10).

Figure 10: Showing normal histology in G8 group and Multifocal mild necrosis of hepatocytes with infiltration of inflammatory cells in the hepatocytes in the centri lobular region in G9 group. (Yellow arrow showing inflammatory cells)



DISCUSSION

The liver performs a variety of functions and is the main organ involved in detoxification. It helps the body to clear the pathogens, harmful substances, and metabolic waste products while also ensuring that other organs function properly (17-19). Literature suggests that the chronic and high doses of MSG can induce oxidative stress that leads to liver damage and raised SGOT and SGPT levels (20, 21). The *Citrus limon* peel is a natural product that has potential anti-inflammatory, antioxidant and anticancer phytocomponents that can show hepatoprotective effect on the MSG induced liver. Thus, the present study carried out to find the hepatoprotective activity of *Citrus limon* peel extract.

The results of the present study shows that the SGOT and SGPT levels were normal in control group and significantly higher in MSG low as well as high dose treated groups and the enzyme levels were back to normal range after administering the *Citrus limon* peel extract. But the enzyme levels are still high in natural recovery groups where the *Citrus limon* is not administered after MSG, that confirms the *Citrus limon* peel extract has a hepatoprotective activity. Manal Said Tawfik et al. (2012) reported that, the MSG at doses of 0.6 and 1.6 mg/g of body weight showed adverse effects on liver and kidney function that could be due to oxidative stress and also reported that elevated levels of ALT, decreased levels of albumin and serum bilirubin in the MSG treated rats compared to the control rats and concluded that there was a significant hepatotoxicity caused by the MSG (22). Similar findings were observed in the present study where the ALT/SGPT levels were raised in MSG groups.

Eweka AO et al., (2011) reported that the AST/SGOT and ALT/SGPT were significantly high in MSG groups when compared to control group and also observed that the enzyme levels were increased in a dose dependent manner. In their study the histological changes observed were dilatation of the central vein with lysed red blood cells, distortion of cyto-architecture of the hepatocytes, along with degenerative as well as atrophic changes on the liver of the MSG feed received animals (23). These results were comparable and similar with the findings of the present study where the multifocal necrosis of the hepatocytes and the inflammatory infiltration of the cells were observed in the MSG group.

Shefalee K. Bhavsar et al., (2006) reported that the *Citrus limon* fruit extract showed hepatoprotective activity in dose dependent manner in carbon tetrachloride induced liver damage in Wistar rats and HepG2 cell line. This study is in contrary to present study where the MSG used to induce liver damage and *Citrus limon* peel extract was used not the fruit pulp (24). Literature on hepatoprotective activity of *Citrus limon* peel extract on MSG induced liver damage was not available. The *Citrus limon* peel has more phytocomponents than the fruit pulp. The phytocomponent abundantly found in *Citrus limon* peel is 2-Methoxy-4-vinylphenol has anti-inflammatory and anticancer activity (25,26). The other phytocomponents present in *Citrus limon* peel such as Heptadecane, Palmitic acid and Stigmasterol were known to have anti-inflammatory, antioxidant and anticancer activity (27-29). This could be the reason for the hepatoprotective activity of the *Citrus limon* peel on the liver.

CONCLUSION

Citrus limon peel can be thought of as a hepatoprotective herbal agent based on 2 factors. MSG caused elevation levels of the serum SGOT and SGPT in animals and the *Citrus limon* peel extract effectively lowered the SGOT and SGPT levels. On the other hand, histological changes that were shown in the MSG groups were inflammatory alterations and hepatocyte disintegration, and the *Citrus limon* peel extract groups effectively prevented the inflammation and cell necrosis. Hence, *Citrus limon* peel has hepatoprotective activity.

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