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ORIGINAL ARTICLE

Effect of aqueous extract of garlic and licorice on Carbon Tetra Chloride induced Liver fibrosis by evaluating serum Aspartate Amino Transferase (AST) and Serum Alanine Amino Transferase (ALT).

ASIA FIRDAUS[1], SHAZIA ASIM[2], AMMARAH HASNAIN[3],SHAHID HAMID[4], SANA TUFAIL[5], HAMMAD HUSSAIN[6]
[1]*Assistant Professor, Department of Pharmacology, Lahore Medical & Dental College, Lahore*
[2]*Associate Professor, Department of Pharmacology, Lahore Medical & Dental College, Lahore*
[3]*Assistant Professor, Institute of Molecular Biology and Biotechnology, University of Lahore*
[4]*ex. Assistant Professor, KEMU, Lahore*
[5]*Associate professor pharmacology, SheikhZayed Medical College, Rahim Yar Khan*
[6]*Deputy registrar University of Health Sciences, Lahore*
Correspondence to Dr. Shazia Asim Email: shazia.asim@lmdc.edu.pk Cell: 03349911022

ABSTRACT

Background: Hepatic fibrosis results from various chronic insults such as chronic hepatitis B and C, parasitic disease, autoimmune hepatitis, nonalcoholic steatohepatitis (NASH) and hereditary metal overload e.g. iron and cupper and it is linked with remarkable morbidity and mortality.
Aim: To evaluate and compare the antifibrotic effect of Aqueous Garlic extract and Licorice Aqueous extract on carbon tetrachloride induced hepatic fibrosis in rats.
Method: 4 groups of rats were taken. In group A and group B, rats were given injection of normal saline intraperitonally. In group C and D, rats were given injection of Aqueous garlic extract (AGE) and licorice aqueous extract (LAE) 01 ml / Kg body weight of rat /day intraperitonally for next four weeks respectively. At the end, all rats were sacrificed. Blood and liver were taken for biochemical examination.
Results: This study results showed that the use of aqueous garlic extract and aqueous licorice extract reduces CCl_4 induced liver fibrosis in rats.
Keywords: Aqueous garlic extract (AGE), licorice aqueous extract (LAE), hepatic fibrosis, anti-fibrotic effect

INTRODUCTION

Liver in our body is the site to regulate storage of glycogen, decay of RBCs, hormone production, plasma protein synthesis and detoxification (Mustafa et al., 2015). As liver plays an important role in detoxifying chemicals so it is exposed to their deleterious effects enhancing its sensitivity to different diseases. Therefore, more than 10% people in the world are suffering from liver diseases (Zhang et al., 2013). Liver diseases cause's 1.2 million people deaths in 2013. The fifth most common cancer with more than 1 million annual mortality worldwide is Hepatocellular carcinoma (HCC) (Jemal and Murray, 2005). In the majority of patients progression to cirrhosis occurs after an interval of 15-20 years. In many patients, the cirrhosis results in major complications like ascites, hepatic encephalopathy, renal failure and bleeding from esophageal varices. The patients may remain free of major complications for many years (compensated cirrhosis). But if it progresses to decompensated cirrhosis then patient survival is short and liver transplantation is only effective treatment (Davis et al., 2003)

CCl_4 is one of the potent hepatotoxins causing degenerative changes in the liver. It is extensively used in research for evaluation of protective agents for liver. Exposure to high concentrations of CCl_4 including its vapors can damage brain, liver and kidney (Seifert et al., 1994, Masuda, 2006, Rood et al., 2001) and can result in cancer especially in people working in chemical laboratories (Ahmad and Ahmad, 2014).

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Complementary and alternative medicine (CAM) is used in medicine to treat diseases but CAM is not the part of conventional medicinal system. It is on record that herbal remedies play a major role in health care (Organization, 1993). Almost 80% of the people in the world for their primary health care rely on CAM, especially herbal medication (Mirghafourvand et al., 2016). was called "The Threriac for the peasants" by Galen in 129-199 A.D. to cure numerous diseases (Pasteur, 1858). In 1858, Louis Pasteur stated that garlic could have antimicrobial effect. In the beginning and mid-20th century, garlic was used to treat cholera and typhoid in Africa (Edwards et al., 2005). It was known as Russian penicillin during World War II because it was very effective when adequate antibiotics were not available (Durak et al., 2004). It is also claimed that garlic has anti-hyperlipidemic effects so reduces coronary heart disease. It also has antihypertensive and antifungal effects (Anwar and Meki, 2003). The complications of diabetes mellitus can be delayed effectively with garlic and melatonin because these act by scavenging free radicals and by stimulating antioxidant system (Anwar and Meki, 2003).

Licorice is one of the most frequently used herbal drug for treatment of liver diseases in traditional medicine of China. Chinese herbal medicine Sho-saiko-to is a mixture of seven herbal preparations, which is widely administered in Japan to patients with chronic hepatitis and cirrhosis (Li et al., 2019). Various bioactive components have been isolated and identified from the licorice like licochalcone A, glycyrrhizin, glycyrrhetinic acid. Newer evidence suggested that multiple mechanisms including anti-oxidative, anti-steatosis, anti-inflammation, antifibrotic, and anticancer effects of these natural herbal compounds are involved to help in liver diseases (Jung et al., 2015).

Jung and his colleagues in their study showed licorice have protective effects in alcohol-induced liver injury. It may be due to its anti-inflammatory and antioxidant activity (Bataller and Brenner, 2005).

A number of compounds have been identified in licorice. It contains many substances like flavonoids, polysaccharides, pectin's, simple sugars, amino acids, mineral salts etc. The sweet taste of root of licorice is due to glycyrrhizin which contains a mixture of potassium-calcium-magnesium salts of glycyrrhizic acid (Yamamura et al., 1992). The flavonoid content includes liquiritin, isoliquiritin etc (Vaya et al., 1997). The antioxidant activity of licorice is due to is isoflavones, glabridin and hispaglabridins A and B (Tamir et al., 2001). It also has estrogen-like activity which is due to isoflavones glabridin and glabrene (Crance et al., 1990). The anti-inflammatory activity of licorice like steroid hormones is due to inhibition of phospholipase A2 (Ohhuchi, 1982). In vitro, glycyrrhizic acid is responsible for inhibition of cyclooxygenase, results in decrease prostaglandin production and platelet aggregation. Carbon tetrachloride (CCl_4), is frequently used agent to induce hepatic fibrosis in different liver-related researches (Wu et al., 2019). Previously it was shown in different studies that CCl_4 treated rats show remarkable increas in serum lipids, liver enzymes and oxidative stress markers like Reactive oxygen species(ROS), Methylene Dioxy Amphetamine (MDA), Glutathione Peroxidase (GSH): Glutathione disulfide (GSSG) (Aleynik et al., 1997).

MATERIAL AND METHODS

This study was conducted at the Experimental Research Laboratory of the University of Health Sciences Lahore from September 2019 to December 2019. Forty male albino rats, weighing 200-250 grams were kept in animal house of University of Health Sciences, Lahore at 23-250C room temperature, and 60% of humidity and 12 hour's cycles of light and dark for 10 weeks. They were provided with standard rat diet and water, weighed before start of experiment. Randomly rats were divided into four groups.

All rats in group A (control group) were given the subcutaneous(S/C) injection of liquid paraffin (0.3 ml/100grams of body weight of rats) every 3rd day for first six weeks. All rats in group B, C and D were given the subcutaneous injection of 40% Carbon tetrachloride (CCl_4) (0.3 ml/100grams of body weight of rats) every 3rd day for first six weeks.

After six weeks, Rats in groups A and B were given intraperitoneal injection of normal saline and rats in groups C and D were given intraperitoneal injection of Aqueous garlic extract (AGE) and licorice aqueous extract (ALE) one ml / Kg body weight of rat /day for next four weeks respectively. After 4 weeks of administration of AGE and ALE. All rats were sacrificed. Blood samples and liver tissue were taken for biochemical examination.

Table: 1 Interventions and Dosage Schedule

GROUP	Treatment S/C Injection for 6 weeks	Treatment after 6 weeks I/p injection for next 4 weeks	Sacrified at 10 weeks
Group A	Liquid paraffin	Normal saline	Sacrificed
Group B	CCl_4	Normal saline	sacrificed
Group C	CCl_4	AGE	sacrificed
Group D	CCl_4	ALE	sacrificed

Liver damage assessment: Biochemical analysis was done by measuring
1- Serum aspartate amino transferase (AST)
2- Serum Alanine amino transferase (ALT)

Statistical analysis: By using SPSS (Statistical Package for Social Sciences) 19, the data was entered and analyzed. For quantitative variables. For qualitative variables, frequencies, percentages and graphs are given. To observe mean differences between groups, one way ANOVA was applied. To see which group mean differs; Post hoc Tukey's test was applied. Less than 0.05 p-valves were considered statistically significant

RESULTS

Post hoc Tukey test for multiple comparisons was used. This test showed that the animals mean body weight in group B was significantly less in comparison to group A, C and D. there was no statistically significant difference between group C and D as shown in table 4.

Fig 1: Animals mean body weight in grams at the beginning of experiment in various groups.

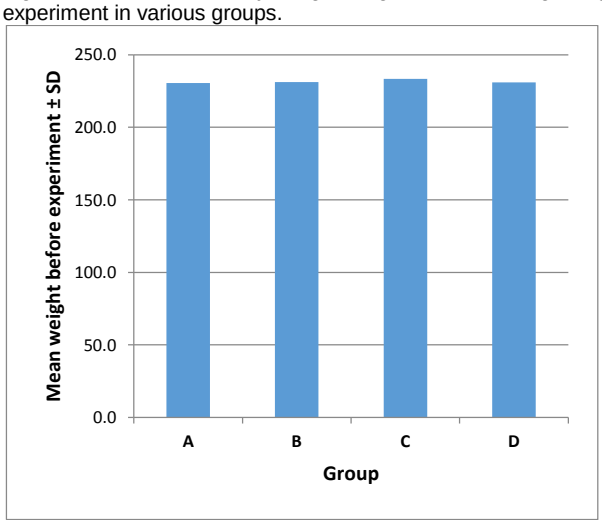
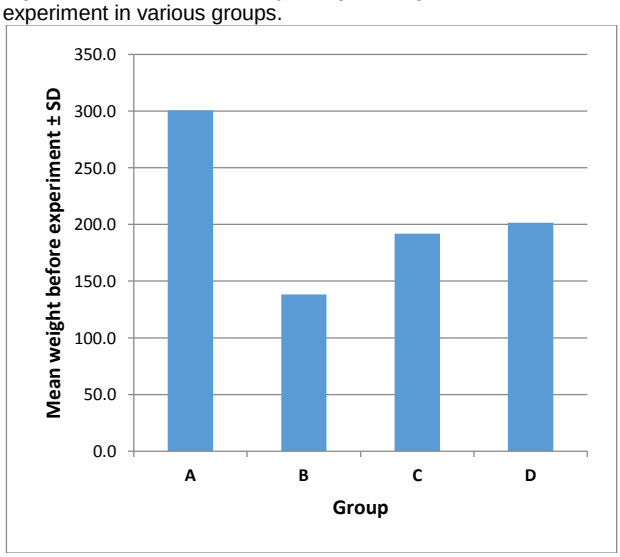


Fig 2: Animals mean body weight in grams at the end of experiment in various groups.



Liver function tests:

Serum Alanine amino transferase ALT (U/L) (Table 3): At the end of experiment ALT level of group A was 39.9±3.4, group B, C and D were 88.9±8.9, 43.0±7.6 and 37.6±5.4 respectively. The Shapiro Wilk test was used to assess the normality of the data. As according to Shapiro Wilk test, data distribution was normal. The mean difference in ALT level among groups was determined by one way ANOVA test. The difference among groups was significant with p-value < 0.001.

hoc Tukey test for multiple comparisons was used. This test showed that the animals mean ALT level in group B was significantly higher in comparison to group A, C and D. There was no statistically significant difference between group C and D as shown in table 4.

Fig 3: Comparison of mean ALT levels among groups

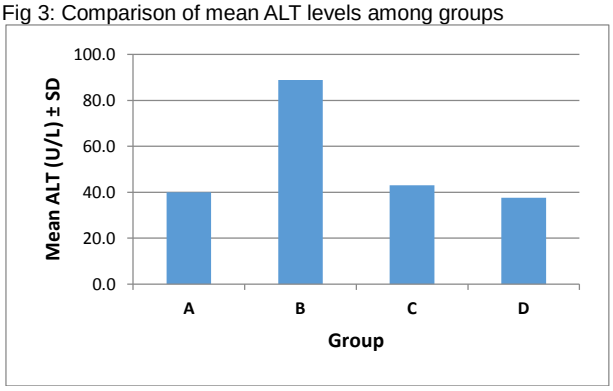
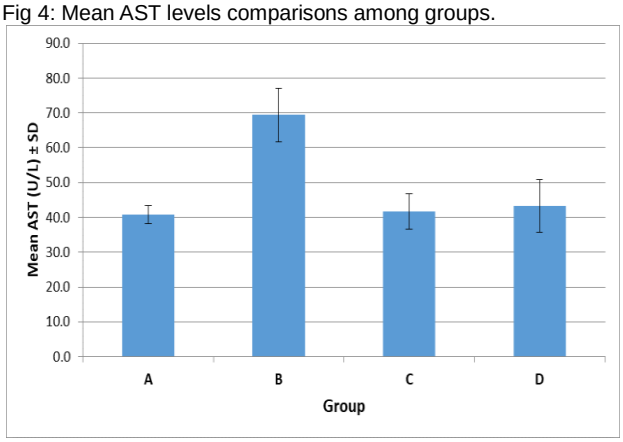


Fig 4: Mean AST levels comparisons among groups.



Serum aspartate amino transferase (AST) U/L (Table 5): At the end of experiment AST level of group A was 40.9 ± 2.6, group B, C and D were 69.4 ± 7.7, 41.6 ± 5.1 and 43.3 ± 7.6 respectively. The Shapiro Wilk test was used to assess the normality of the data. As according to Shapiro Wilk test, data distribution was normal. The mean difference in AST level among groups was determined by one way ANOVA test. The difference among groups was significant with p-value < 0.001.

Post hoc Tukey test for multiple comparisons was used. This test showed that the animals mean AST level in group B was significantly higher in comparison to group A, C and D. There was no statistically significant difference between group C and D as shown in table 6.

Table 2: Animals mean body weight at the beginning and end of experiment among groups

Mean Weight of animals (gm)	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	Group D (Mean ± SD)	p-value
At the start of experiment	230.6 ± 6.2	231.1 ± 4.8	233.3 ± 4.6	231.0 ± 6.1	0.520
At the end of experiment	300.7 ± 9.1	138.1 ± 9.6	191.9 ± 16.4	201.5 ± 13.2	< 0.001

Table 3: Serum alanine amino transferase of animals among groups

	Group A (Mean ± SD)	Group B (Mean ± SD)	Group C (Mean ± SD)	Group D (Mean ± SD)	p-value
ALT (U/L)	39.9 ± 3.4	88.9 ± 8.9	43.0 ± 7.6	37.6 ± 5.4	< 0.001

Table 4: Pair wise comparison of alanine amino transferase (ALT) levels among various groups

Groups	Groups	Mean difference	Std errors	P value
Group A	Group B	-48.93000*	2.43164	< 0.001
	Group C	-3.04333	2.43164	0.597
	Group D	2.32333	2.43164	0.775
Group B	Group C	45.88667*	2.43164	< 0.001
	Group D	51.25333*	2.43164	< 0.001
Group C	Group D	5.36667	2.43164	0.134

Table 5: Serum aspartate amino transferase of animals among groups

	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	Group D Mean ± SD	p-value
AST (U/L)	40.9 ± 2.6	69.4 ± 7.7	41.6 ± 5.1	43.3 ± 7.6	< 0.001

Table 6: Pair wise aspartate Amino Transferase (AST) levels comparisons among various groups

Groups	Groups	Mean difference	Std. Error	P value
Group A	Group B	-28.54733*	2.23058	< 0.001
	Group C	-0.76267	2.23058	0.986
	Group D	-2.38867	2.23058	0.709
Group B	Group C	27.78467*	2.23058	< 0.001
	Group D	26.15867*	2.23058	< 0.001
Group C	Group D	-1.62600	2.23058	0.885

DISCUSSION

In this study, the hepatoprotective effects of aqueous garlic extract (AGE) and aqueous licorice extract (ALE) were observed and compared. It was demonstrated that AGE and ALE may help to slow the progression of CCl_4 induced hepatic fibrosis. It was observed that the serum ALT and AST in group B (model group) were significantly raised as compared to group A (control group). Their level in groups C (AGE treated) and group D (ALE treated) were found to be reduced as compared to group B.

Nursal Gedik et al, in their study demonstrated that use of aqueous garlic extract (AGE) reduces liver fibrosis and oxidative injury produced by bile duct obstruction in rats. The BDL (bile duct ligation)-induced deterioration of the hepatic functions can be improved by AGE. The raised serum ALT, AST and LDH activity and TNF-α levels induced by BDL can be decreased by AGE. The BDL-induced increase in myeloperoxidase activity, lipid peroxidation, collagen content and decrease in glutathione levels were reverted to normal by AGE. In another study, it was shown that the liver injury induced by ischaemia-reperfusion (I/R) in rats is also alleviated by AGE. The multiple mechanisms are involved which lead to I/R induced liver injury like inflammation, hypoxia, and free radical production (Cotran, 1989).

The one of the most common causes of hepatotoxicity may be a change in liver and serum lipids. The most of the lipids and lipoproteins are synthesized by liver. The normal hepatic function is responsible for homeostasis of lipids and lipoprotein metabolism. Chronic liver disease and hepatocarcinoma are often associated with abnormal serum lipids. Any disturbance in lipid metabolism results in their storage as triglycerides which lead to steatosis and lipid peroxidation (Ginsberg, 2006). Further accumulation of lipids in liver results in inflammation, apoptosis and fibrosis so the hepatic steatosis progresses to steatohepatitis and cirrhosis (Skibola and Smith, 2000).

The sensitive markers of liver damage are serum AST, ALT, ALP and bilirubin which released into blood after cellular injury.109 High levels of these serum markers are consider as index of liver injury. The raised ALT is the most sensitive indicator (Tsai et al., 2008).

In this study, the raised serum levels of transaminases may be due to the leakage of enzymes from cytoplasm into the blood after cell injury in group B. This showed that AGE and ALE have ability to arrest hepatic injury by decreasing the leakage of enzyme into blood by preserving the cell membrane integrity thereby restoring enzymes as in group C and group D. The damaged hepatocyte membrane leads to liberation of cytosolic enzymes might have resulted from free radicals induced lipid peroxidation of membrane.

CONCLUSION

The results of this study indicate that the aqueous extract of garlic and aqueous licorice prevents liver fibrosis induced by CCl_4 in rats. These showed almost same results biochemically. Generally, the hepatoprotective action of AGE and ALE is likely due to a counteraction of free radicals by its antioxidant flavonoids. Further studies are required to see the hepatoprotective effect of both AGE and ALE.

Conflict of interest: Nil

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