Hepatoprotective Effect of *Lagenaria Siceraria* (Linn) in Carbamazepine Induced Hepatotoxicity in Rabbits

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ABSTRACT

OBJECTIVE: To determine the hepatoprotective effect of *Lagenaria Siceraria* in carbamazepine induced hepatotoxicity in rabbits
STUDY DESIGN: A Randomized controlled trial (experimental animal study).
PLACE AND DURATION: Department of Pharmacology, Federal Urdu University, from 4th April 2016 to 10th July 2016.
METHODOLOGY: Animals (Rabbits) were divided into four groups G1, G2, G3 and G4; each group consisted of five rabbits. Normal saline was administered to G1. However, CBZ (6 mg/kg/day), *L. Siceraria* (400 mg/kg/day) and a combination of 1:1 CBZ and *L. Siceraria* were administered to G2, G3 and G4 respectively for a period of 90 days. Blood samples were analyzed for liver enzymes and total proteins. Moreover, histopathological study was also performed on liver specimen collected from animals of all groups to see morphological and pathological changes in the tissue.
RESULTS: *L. Siceraria* fruit extract significantly reduced liver enzymes, SGPT (66%), ALP (47%), γ – GT (14%) and total proteins (5.5%), whereas the levels of SGPT (7.2%), ALP (80%), γ – GT (7.6%) and total proteins (22.1%) in CBZ treated group were elevated. While a combination of *L. Siceraria* with CBZ showed a considerable increase in SGPT (31%), but ALP (15%) and total proteins (8.4%) were lessened, with no any change observed in γ – GT. Results of liver tissue histology did not show necrosis and cholestasis in G3 and G4 but these were found in G2.
CONCLUSION: Hepatoprotective potential of *L. Siceraria* suggested its use to prevent elevation of hepatic enzymes due to long term administration of carbamazepine.
KEYWORDS: *Lagenaria Siceraria*, Carbamazepine, Hepatotoxicity, Hepatoprotection, Liver function test, Total protein.

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INTRODUCTION

Lagenaria Siceraria Linn. (Mol.) belongs to family cucurbitacea. This plant contains a wide range of bioactive compounds such as sterols, terpenoids, flavonoids, and saponins it also contained dietary fibers. The fruit of *L. Siceraria* is reported as hepatoprotective and antioxidant[1] whereas; it also possesses cardioprotective, anti-inflammatory, anti-hyperlipidimic and antihyperglycemic effects[2-5]. Literature also reported that it is used as purgative, sedative and in gastrointestinal disorders. Therapeutically *L. Siceraria* is good source for treatment of diabetes, constipation, and urinary problems[6].

Carbamazepine (CBZ) is an effective antiepileptic drug and a potent inducer of microsomal enzymes in liver. A transient and asymptomatic elevation of liver enzymes occurs in 25-61% of patients receiving CBZ. Hepatotoxic reactions of CBZ usually occur within 3-4 weeks after the initiation of therapy and are independent of serum CBZ levels[7]. It is metabolized by hepatic microsomal enzymes belonging to P_{450} 3A family. The most important metabolic product of CBZ is 10,11-CBZ epoxide, which has been shown to be pharmacologically active. CBZ-associated hepatotoxicity manifests as granulomatous hepatitis, abnormal liver function tests, hepatocellular necrosis, lymphadenopathy and biliary tract infection[8].

Liver injuries and hepatotoxicties are the side effect of many drugs used in different ailments therefore, it is necessary to explore the remedy which prevent liver damage. Owing to its hepatoprotective prospective, the *L. Siceraria* fresh juice was compared with carbamazepine induced hepatotoxic effects in

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rabbits. For this purpose various parameters; macroscopic liver examination, microscopic liver examination, hepatic enzyme analysis and total blood proteins were observed.

These findings would evolve a novel paradigm to determine the hepatoprotective effect of *Lagenaria Siceraria* in carbamazepine induced hepatotoxicity in rabbits.

METHODOLOGY

This Randomized controlled trial (experimental animal study) was carried out at department of Pharmacology,Federal Urdu University, Karachi from 4th April 2016 to 10th July 2016. For this purpose healthy animals (Rabbits) weighing 1.0 – 1.3 kg were used and divided into four groups (n=5) as; Group 1 = Negative control (Saline treated, 3.5ml), Group 2 = Positive control (CBZ (6mg/kg) treated), Group 3 = *L. Siceraria* treated group (3.5ml fresh juice equivalent to 400 mg of dried aqueous extract), Group 4 = CBZ + *L. Siceraria* treated group (6mg/kg, 3.5ml). All the treatments were given orally once daily for the period of 90 days.

For liver function analysis animals were anesthetized by using chloroform. Approximately 6 ml of blood was drawn via cardiac puncture. Later on, the serum was separated and analyzed for biochemical estimation of serum glutamic pyruvte transaminase (SGPT), Gama Glutamyl transferase (ϒ-GT) and alkaline phosphatase (ALP) was performed by using Ecoline 500, GPT (ALT) Tris of Merck, Ecoline 25 ϒ-GT of Merck and Ecoline 25 AP of Merck respectively. Total plasma protein and bilirubin levels were also measured through routine laboratory procedure[9].

Immediately the liver was removed from sacrificed rabbits for its size measurement. Besides, tissues specimens were maintained in formalin for 12 hours. Paraffin-embedded sections were stained with hematoxylin and eosin (H&E). Bright-field microscopy was performed using a Zeiss Axioskop microscope, and digital images were obtained on a Spot camera (Advanced Spot Software; Diagnostic Instruments Inc., Sterling Heights, Michigan, USA). Morphometric analysis of H&E-stained tissues was performed using Zeiss Image software at 400X[9].

Data Analysis: One way analysis of variance (ANOVA) was used to compare treated groups. Value of P<0.05 and P<0.001 were considered as significant and highly significant respectively. Results were expressed as Mean ± SEM.

RESULTS

Liver enzymes were determined in all animal groups. Values of SGPT and ALP were significantly reduced in G3 and G4 when compared with G2. In all groups, values of total bilirubin, direct bilirubin and γ – GT did not change significantly (Table-I).

CBZ – Carbamazepine, SGPT – Serum glutamic-pyruvic transaminase, ALT – Alanine transaminase, ALP – Alkaline Phosphatase, γ – GT – Gamma Glutamyl transferase. Values are expressed in Mean ± SEM; n=5

Results of G2 showed low albumin levels which was also reflected by reduction in total proteins. Increased albumin and total proteins were observed in G3 and G4 in comparison with positive control (Table-II).

CBZ – Carbamazepine, A/G – Albumin/globulin. Values are expressed in Mean ± SEM; n=5

Gross anatomy and histology of liver specimens were measured in centimeter. Average dimension of liver specimen was 7x4x2.5 cm, 8x6x3 cm, 7x5x4 cm, 7x7x2.5 cm for control, CBZ, *L. Siceraria* and CBZ+ *L. Siceraria* respectively.

DISCUSSION

Increased level of liver enzymes is a useful tool to diagnose hepatotoxicity induced by drugs. Liver enzymes like SGPT (serum glutamate-pyruvate transaminase) and ALP (Alkaline Phosphatase) are markers of hepatocellular injury[10,11]. High values of γ – GT and ALP may be attributed to the rare condition of ductopenia which is characterized by loss of small bile ducts and risk of jaundice as well as impaired liver function[12].

Table–I: Effect of *Lagenaria Siceraria* with Carbamazepine on liver functions (N=20)

Treatment	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)	SGPT (U/L)	ALP (U/L)	γ – GT (U/L)
G1 (n=5)	0.79 ± 0.01	0.05 ± 0.01	55 ± 0.32	36 ± 1.92	13 ± 1.00
G2 (n=5)	0.82 ± 0.01*	0.05 ± 0.01	51 ± 3.61*	65 ± 1.61**	14 ± 1.30*
G3 (n=5)	0.81 ± 0.01	0.04 ± 0.01	17 ± 1.18**	34 ± 1.30**	12 ± 1.00*
G4 (n=5)	0.84 ± 0.03*	0.05 ± 0.01	35 ± 1.59*	55 ± 1.16*	14 ± 1.10

* Significant (P<0.05). ** Highly significant (P<0.001)

Table–II: Effect of *Lagenaria Siceraria* with Carbamazepine on plasma proteins (N=20)

Treatment	A/G ratio	Albumin (g/dl)	Globulin (g/dl)	Total Protein (mg/dl)
G1 (n=5)	17.51 ± 1.54	6.83 ± 0.32	0.39 ± 0.02	7.22 ± 0.32
G2 (n=5)	16.11 ± 1.46	6.12 ± 0.71	0.38 ± 0.03	6.50 ± 0.37**
G3 (n=5)	12.64 ± 1.24	6.32 ± 0.71	0.5 ± 0.05	6.82 ± 0.63*
G4 (n=5)	12.03 ± 0.6*	6.26 ± 0.24	0.52 ± 0.02*	6.78 ± 0.55**

* Significant (P<0.05). ** Highly significant (P<0.001)

G2 treated with carbamezapine showed significant increase in SGPT (7.2%), ALP (80%) and γ – GT (7.6%) when compared with

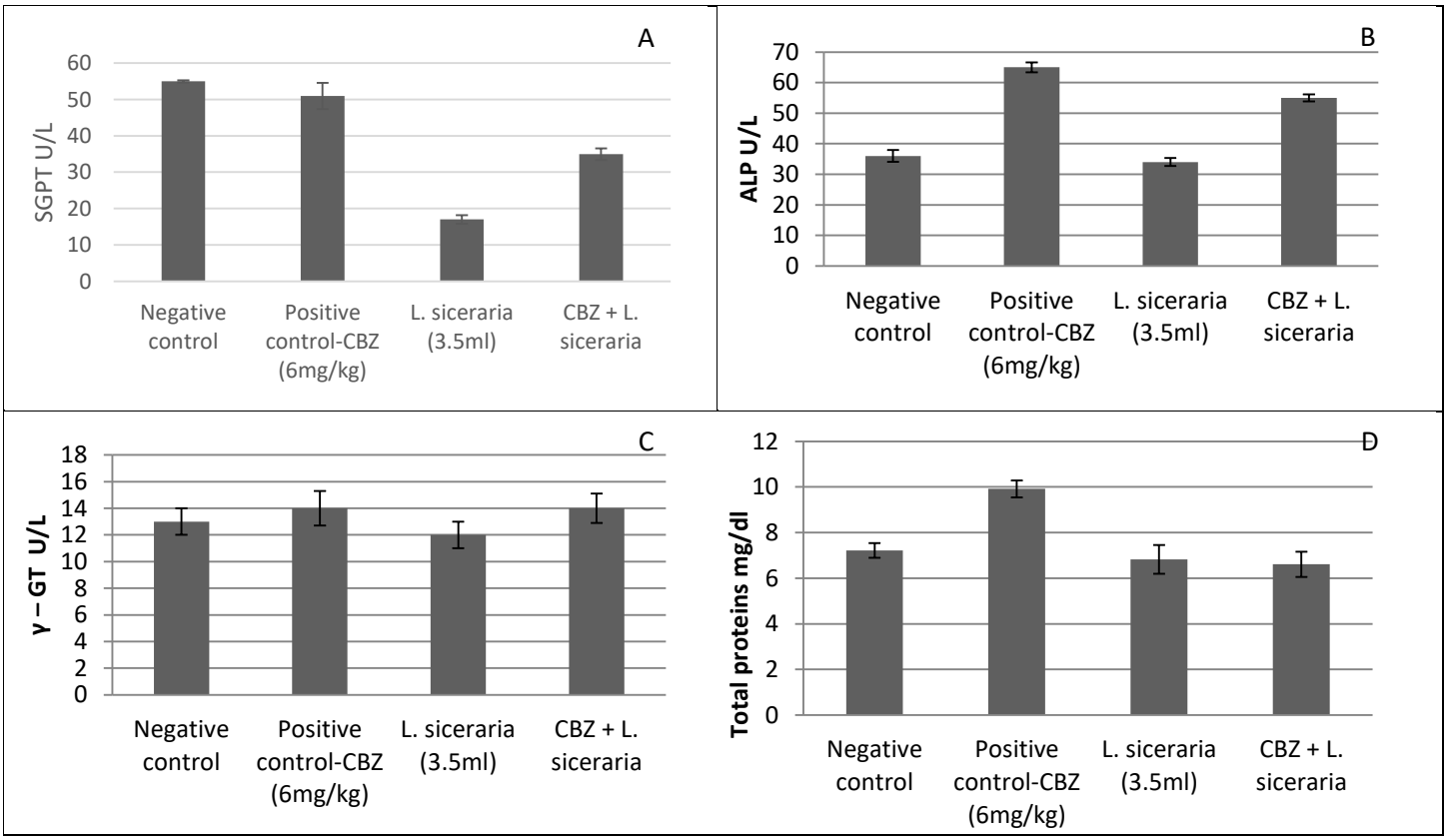
negative control. These high values indicated obstruction in bile flow. Significant reduction in all liver enzymes was observed in G3 treated with Lageneria siceraria (Fig-1). i.e SGPT (66%), ALP (47%) and γ – GT (14%). Reduction in SGPT and ALP with high significance, suggested hepatic protective effect of *L. Siceraria*. In G4- combination of CBZ and *L. Siceraria*, significant decreased in SGPT (31%) and ALP (15%) was found.

CBZ is a $CYTP_{450}$ enzyme inducer therefore, it has tendency to increase liver enzymes[13]. Hepatotoxic effects of carbamezapine also depend upon the individual susceptibility, which are unable to detoxify the secondary metabolite of carbamezapine such as 10, 11 – carbamazepine epoxide[14]. *L. Siceraria* due to its antioxidant property combats with free radical hence protecting lipid peroxidation which is one of the reasons for hepatotoxicity[5]. *L. Siceraria* have also been reported to possess vitamin C, phenolic group and flavonoid group as its constituents; these act as antioxidant[7]. Hepatoprotective activity of *L. Siceraria* is supported by its antioxidant property[15], which may play a role in detoxification of carbamezapine's metabolites.

Another biomarker for hepatotoxicity is the total proteins and albumin which are predominantly produced by the liver. Hypoalbuminaemia is frequently seen in advanced chronic liver diseases. Hence decline in total protein content can be deemed as a useful index of the severity of cellular dysfunction in chronic liver diseases[16]. CBZ treatment showed significant decrease in total proteins (9.9%) and albumin (10.3%) which may be an indication of impaired protein synthesis. *L. Siceraria* treatment showed slight increase in total proteins (4.9%) and albumin (3.2%) levels. Combination of CBZ+*L. Siceraria* treated group was able to increase the levels of total protein (4.3%) and albumin (2.3%). It was found that *L. Siceraria* produced hepatoprotective activity by elevating total proteins and albumin levels.

Groups treated with *L. Siceraria* and combination of CBZ+*L. Siceraria* did not show any significant deviations in hepatic parameters when compared with control group. On the other hand larger dimension of liver specimen with pale colour was observed in CBZ treated group. Increase weight of rabbits was also observed in CBZ treated group (data already presented in previous paper) [5].

Histological findings of G2-CBZ treated group showed that few hepatocytes exhibit ballonic degeneration associated with 5% to 15% steatosis, 10-12 portal tracts were present which exhibit moderate amount of periportal inflammation associated with interface hepatitis. Foci of spotty necrosis and choalestasis were also observed. Hepatic necrosis, granulomatous hepatitis and immunoallergic hepatitis in patients using carbamezapine have been reported[17]. G3- *L. Siceraria* treated group, did not show necrosis and cholestiasis. Moreover mild macrovascular steatosis less than 10% was observed. G4-CBZ + *L. Siceraria* treated group- did not show any sign of necrosis, minimum fibrosis was observed with mild periportal inflammation whereas; steatosis was less than 5%. These findings may provide evidence for the potential hepato-protective effect of *L. Siceraria*.



Fig–1: Effect of Lagneria siceraria, carbamezapine and its combination on liver enzymes. A – Serum glutamic-pyruvic transaminase, B–Alanine transaminase, C–Gamma Glutamyl transferase, D – Total proteins. (N=20)

CONCLUSION

On the basis of above results it is concluded that *L. Siceraria* have hepatoprotective effect as well as reduces the hepatotoxicity induced by carbamezapine. Therefore, *L. Siceraria* may be used in prevention of elevated hepatic enzymes during long term administration of carbamazepine as a supportive adjuvant.

CONTRIBUTION OF AUTHORS

Owais F: Introduction, Data analysis, Literature search, Reference Citation.
Mehjabeen: Experimental Design, Data analysis, Result and data interpretation, Final formatting of manuscript**.**

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REFERENCES

1. Saha P, Mazumder U, Haldar P, Gupta M, Sen SK, Islam A. Antioxidant and hepatoprotective activity of *Lagenaria Siceraria* aerial parts. Pharmacog J. 2011;3(23):67-74.
2. Minocha S. An overview on *Lagenaria Siceraria* (Bottle gourd). J Biomed Pharm Res. 2015;4(3):4-10.
3. Sharma NK, Yadav P, Singh HK, Shrivastava AK. In vitro antioxidant activity of *Lagenaria Siceraria* leaves. Malays J Pharm Sci. 2013; 11(1):1-11.
4. Saha P, Mazumder UK, Haldar PK, Sen SK, Naskar S. Antihyperglycemic activity of *Lagenaria Siceraria* aerial parts on streptozotocin induced diabetes in rats. Diabetol Croat. 2011;40(2):49-60.
5. Mehjabeen, Owais F, Jahan N, Khan M. Protective effect of *Lagenaria Siceraria* (mol.) standl. against carbamezapine induced hyperlipidemia: comparative study between male and female animal model. Int J Biol Pharm Allied Sci. 2016; 5(10):2455-63.
6. Amit K, Sangh P, Neeraj K S , Jha K K. Phytochemical, Ethnobotanical and Pharmacological Profile of *Lagenaria Siceraria*: - A Review. J of Pharmacog and Phytochem. 2012; 1 (3): 24-31.
7. Strolin Benedetti M, Ruty B, Baltes E. Induction of endogenous pathways by antiepileptics and clinical implications. Fundam Clin Pharmacol. 2005; 19(5):511-29.
8. Maheswari E, Saraswathy GRL, Santhranii T. Hepatoprotective and antioxidant activity of N-acetyl cysteine in carbamazepine-administered rats. Indian J Pharmacol. 2014; 46(2):211-15.
9. Chiu H-C, Kovacs A, Ford DA, Hsu F-F, Garcia R, Herrero P, et al. A novel mouse model of lipotoxic cardiomyopathy. J Clin Invest. 2001; 107(7): 813-22.
10. Nyblom H, Berggren U, Balldin J, Olsson R. High AST/ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking. Alcohol Alcohol. 2004;39(4):336-39.
11. Nyblom H, Björnsson E, Simrén M, Aldenborg F, Almer S, Olsson R. The AST/ALT ratio as an indicator of cirrhosis in patients with PBC. Liver Int. 2006; 26(7):840-45.
12. Ramos AMO, Gayotto LCC, Clemente CM, Mello ES, Luz KG, Freitas ML. Reversible vanishing bile duct syndrome induced by carbamazepine. Eur J Gastroenterol Hepatol. 2002;14(9):1019-22.
13. Ahmed SN, Siddiqi ZA. Antiepileptic drugs and liver disease. Seizure. 2006;15(3):156-64.
14. Pirmohamed M, Leeder JS. Anticonvulsant agents. In: Kaplowitz N, Laurie DD, editors. Drug-induced liver disease. 3 ed. London: Academic press; 2013. p. 425-46.
15. Mayakrishnan V, Veluswamy S, Sundaram KS, Kannappan P, Abdullah N. Free radical scavenging potential of *Lagenaria Siceraria* (Molina) Standl fruits extract. Asian Pac J Trop Med. 2013; 6(1): 20-26.
16. Venukumar M, Latha M. Hepatoprotective effect of the methanolic extract of Curculigo orchioides in CCL4 -treated male rats. Indian J Pharmacol. 2002; 34(4):269-75.
17. Ganesh S, Shukla L, Kandasamy A, Benegal V. A case of carbamazepine-induced severe cholestatic hepatitis: Case report and review of literature. Indian J Psychol Med. 2017; 39(5):688-90.