ORIGINAL ARTICLE

Hepato-Protective Effects of Silymarin and Coffee in Rats

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ABSTRACT

Objective: To compare the effects of silymarin and coffee on liver enzymes in acetaminophen-induced hepatotoxicity in rats.

Study Design: Experimental-randomized control study.

Place and Duration of Study: The research was conducted from October 2018 to October 2019 in Pharmacology Department at IIMCT in mutual collaboration with National Institute of Health (NIH) Islamabad.

Material and Methods: At day 0 after initial blood sampling, Acetaminophen (300 mg/kg) by intraperitoneal route was given to 30 rats to induce hepatotoxicity. These rats were further divided into three experimental groups on day 8. Group 2 was a disease control group, Silymarin (100mg/kg) was given to group 3 rats and group 4 rats were treated with Coffee (200 mg/kg) through intragastric gavage for fourteen days. Terminal blood sampling was done at day 21 through cardiac puncture for biochemical estimation on same day. Mean± SEM was calculated and analyzed through SPSS 20. P value less than 0.05 was considered statistically significant.

Results: Our results showed major elevation ($p<0.05$) in alanine aminotransferase and aspartate aminotransferase levels in group 2 when compared to normal control -group. The rats treated with silymarin & coffee considerably ($p <0.05$) lowered biomarker enzymes in comparison to disease control group 2 respectively.

Conclusion: Coffee lowers ALT and AST levels as compared to Silymarin in Acetaminophen induced hepatotoxicity in rats.

Key Words: *Acetaminophen, Alanine Transaminase, Aspartate Aminotransferases, Coffee, Silymarin.*

Introduction

Liver injury has a diffused pathology and if not controlled effectively then may lead to fibrosis, cirrhosis and hepatocellular carcinoma.[1,2] There is high prevalence of all sorts of hepatitis in Pakistan. Also, Pakistan has been named as "Cirrhotic state" because both HBV and HCV are responsible for more than 75% of cirrhosis which further leads to hepatocellular carcinoma.[3,4] These all challenging diseases with high prevalence around the globe have less effective long term treatment which make them financial burden and cause of death.[2] Steroids, vaccines, interferons, antiviral drugs and many other conventional drugs, which are usually used for treating liver ailments, when administered chronically or sub-chronically have been found to have adverse effects.[5,6] Therefore in recent years exploration of antioxidants of plant source and their hepatoprotective potential is being under solemn consideration. So that people may consume them because the use of natural medication and nutritional habits is appreciated by general public usually.[7] Flavonoids are considered good antioxidant compounds generally due to their phenolic structures and inhibition of free radical mediated processes.[8] One of the flavonolignane extracted from milk thistle, "Silymarin" has been utilized for the treatment of various liver disorders that portray functional impairment or degenerative necrosis. It is familiar for its antioxidant, anti-inflammatory and anti-fibrotic properties and exhibits protective effects in different liver issues. It acts as a free radical scavenger which consequently prevents lipid peroxidation and its related cell injury by stabilizing the membrane.[9] Among the most commonly consumed beverages worldwide prepared from plants, coffee holds a significant position specifically among working people. It is the 2nd most traded commodity in the world having lots of diversities in

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types and ways of preparation from seeds. Over the last period of years immediate development has been seen in the Pakistan's beverage industry. There is growing market and prompt opening of different coffee shops for the people to approach it easily.[10] Coffee is not only an aromatic flavored drink but is also a rich source of dietary antioxidants.[7] It is a multiplex mixture of varying compounds including caffeine (1, 3, 7trimethylxanthine) and up to 1000 described phytochemicals which help in combating reactive oxygen species.[11] Epidemiological data over the last years shows the inverse relationship of coffee and the risk of several liver diseases.[7] Large amount of chlorogenic acid present in Coffee reduce the risk of glucose intolerance and Non-alcoholic fatty liver disease.[12] Redox equilibrium is restored as well as expression of pro-inflammatory cytokines is reduced. Specifically nicotinic acid present in Coffee is a potent anti-fibrotic agent while caffeine blocks TGF-b as well as suppression of DNA synthesis and enhances apoptosis of hepatic stellate cell (HSC). All these processes account for hepatoprotection. Thus it is proposed that two or more cups of coffee in a day protects the liver and ameliorates almost all liver ailments.[7,13,14,15]

So the present study was performed to compare the efficacy between Silymarin and Coffee in terms of reversal of hepatic damage.

Material and Methods

This randomized-control trial done by balloting method was conducted at the Pharmacology department with Multidisciplinary -Research -Lab at IIMCT in mutual collaboration of Animal -House in NIH, Islamabad from ctober 2018-Oct 2019. Accredited Ethical Review Committee of the institute approved the research proposal before starting the study. Research Grade Acetaminophen and Silymarin were obtained from Sigma Aldrich. Coffee beans were procured from Al-Fatah super store in Centaurus mall, Islamabad and sent to herbarium section of National Agriculture Research Centre (NARC) for identification and validation through proper taxonomic rules. Coffee beans were then grinded, powdered and kept airtight in cool and dry place. The present study included forty adult healthy male albino rats weighing 300-350 grams with normal baseline liver function tests. Exclusion criteria was female rats and abnormal liver function tests**.** Rats were kept under controlled environment with temperature of 20-25 degree Celsius and constant twelve hour dark and light cycle. No mortality or morbidity was observed during the whole period of experiment. Division was done into four groups having ten rats in each cage. Blood samples were drawn from two rats of every group at day 0 by intracardiac blood sampling method. Group 1 was a control group which received normal diet and tap water. Rats in other three groups were given Acetaminophen 300 mg/kg injection through intraperitoneal route (just once at day 0) for the induction of hepatotoxicity. To assess the advancement of study, second blood sampling of two rats from three groups was done on 8th day. After the confirmation, no treatment was given to group 2 (disease control) rats. Rats in group 3 received Silymarin 100 mg/kg[16] through intragastric gavage once daily and group 4 was treated with Coffee once daily in the morning. Preparation of coffee involved mixing of coffee powder in boiling water and then filtering it on paper. 200mg/kg[17] dose was given to the rats through gavage method. On day 21 after giving anesthesia with chloroform, blood samples from all the rats which were not in the fasting state, were drawn through cardiac puncture by 3 cc syringe. After clot formation, blood samples were centrifuged at 3500 RPM for 5 min[18] by Bench top centrifuge. In tubes serum was separated for final biochemical estimation which was done on same day by using ALT kit (Merck) and AST kit (Merck) on Chemistry analyzer. Mean± SEM of all four groups was calculated and for comparison post-hoc Tukey test was done. All this was analyzed statistically by using SPSS 20. P value less than 0.05 was chosen significant for the results obtained.

Results

Transaminase levels were increased significantly ($p<0.05$) in rats of group 2 (disease control) due to treatment with Acetaminophen as compared to group A (normal control). Results obtained in group 4 (Coffee treated) showed significant reduction in serum biomarkers as compared to group 3 rats who were treated with Silymarin. Summary of results is as followed:

Discussion

Raised levels of transaminases i.e. ALT and AST makes them important for diagnosis, confirmation as

Table I: Mean ± SEM of ALT and AST Values among All Groups

Groups n = 10	"ALT"	"AST"
Group 1	36.40 ± 3.655	40.20 ± 3.397
Group 2	157.60 ± 7.827	129.00 ± 8.637
Group 3	91.00 ± 1.517	75.80 ± 2.709
Group 4	69.60 ± 3.600	65.40 ± 2.337
p value	<0.05*	

*= Sig value

ALT = Alanine aminotransferase,

AST= Aspartate aminotransferase

Table II: Post -Hoc -Comparison of "ALT" and "AST" B/W Groups

Comparison Of Groups	ALT "Mean difference"	AST "Mean difference"
Group 1 vs. group 2	-121.200*	-88.800*
Group 1 vs. group 3	-54.600*	35.600*
Group 1 vs. group 4	-33.200*	-25.200*
Group 2 vs. group 3	66.600*	53.200*
Group 2 vs. group 4	88.000*	63.600*
Group 3 vs. group 4	21.400*	10.400*

*= Significant

ALT= Alanine –Amino -transferase,

AST= Aspartate –amino –transferase

well as for determining the extent of liver damage clinically and experimentally. The present study showed reduction in transaminase levels of group 3 and group 4 rats which were given silymarin and coffee. But coffee ameliorated the hepatic damage more significantly as compared to silymarin.

In this study, single intraperitoneal injection of Acetaminophen 300mg/kg is used to induce acute liver injury in rats of experimental groups (group 2 to group 4) which resulted in significant increase in serum ALT and AST levels as compared to normal control group 1. And then group 2 is taken as disease control group. This study is supported by the study done by Jersiah and colleagues who used 300 mg/kg dose of Acetaminophen intraperitoneal injection in rats to cause the acute hepatotoxicity and increased "ALT" and "AST" levels in rats.[19]

Silymarin[9] and Coffee[20], both are derived from plants and each one has hepatoprotective activity owing to their antioxidant potential. Special attention has been given to Coffee in the present study as it is also commonly consumed beverage by the people.[10] Comparison has been done with Silymarin, a standard drug that is not something new for liver patients but its bad taste and low bioavailability are the limitations of its usage. According to results, reversal of hepatic damage in the rats was seen with the usage of Silymarin 100mg/kg in group 3. The reversal is due to its membrane stabilizing activity which prevents leakage of intracellular enzymes. This is supported by the study of Godswill J.Udom who investigated the hepatoprotective properties of ethanol seed extract of *Citrus paradisi Macfad* (Grape Fruit) against paracetamol-induced hepatotoxicity in wistar rats.[21] Our results of ALT and AST of group 3 are also in concordance with the study of Bektur and colleagues who studied the Protective effects of Silymarin against acetaminophen-induced hepatotoxicity and nephrotoxicity in mice.[8]

Significant difference in the means of biochemical parameters of group 4 (Coffee 200mg/kg) as compared to the means of group 2 showed that Coffee improved the signs of liver damage and showed reversal of inflammatory signs. Hepatoprotective activity is due to the phenolic content in the coffee that prevents lipid peroxidation against free radicals. Moreover Caffeine and NA block TGF-b and enhance apoptosis of hepatic stellate cells.[22] These results are in accordance with Ibrahim Halil Bahcecioglu who studied serum marker levels and histopathology in *Pistacia terebinthus* Coffee protects against thioacetamide-induced liver injury in rats which showed the preventive effect against experimental hepatotoxicity.[23] Jonathan Arauz studied Coffee consumption prevents fibrosis in a rat model that mimics secondary biliary cirrhosis in humans and concluded that increase in liver function tests was completely mitigated by Coffee in accordance with results of this research.[24] Federico Salomone studied that Coffee enhances the expression of chaperones and antioxidant proteins in rats with non-alcoholic fatty liver disease and concluded that there was a reduction in serum markers in the rats fed on high fat diet plus coffee to healthy control levels.[25]

There was also a significant difference of results between group 3 and group 4 which showed that both agents used for the reversal of APAP induced hepatotoxicity showed improvement in the biochemical parameters but Coffee was better than Silymarin in improving the LFTs.

Conclusion

Silymarin and Coffee individually ameliorate the hepatotoxic effects but Coffee has more beneficial hepatoprotective effects than Silymarin in Acetaminophen induced hepatotoxicity in rats. For future, histopathology aspect can be explored .Further, individual constituents of Coffee can be explored for hepatoprotection.

REFERENCES

1. Ali Saber Sarg N. The Role of Cymbopogon Citratus Extract in Protecting the Liver Against Injurious Effect of Dimethylnitrosamine in Rats. Int J Clin Dev Anat. 2015;1(4):89.
2. Furtado KS, Prado MG, Aguiar e Silva MA, Dias MC, Rivelli DP, Rodrigues MAM, et al. Coffee and Caffeine Protect against Liver Injury Induced by Thioacetamide in Male Wistar Rats. Basic Clin Pharmacol Toxicol. 2012;111(5):339–47.
3. Butt AS. Epidemiology of Viral Hepatitis and Liver Diseases in Pakistan. Euroasian J Hepato-Gastroenterology. 2015;5:43–8.
4. Parkash O, Hamid S. Next big threat for Pakistan Hepatocellular Carcinoma (HCC). 2016;66:735–9.
5. Koh PH, Mokhtar RAM, Iqbal M. Antioxidant potential of Cymbopogon citratus extract: Alleviation of carbon tetrachloride-induced hepatic oxidative stress and toxicity. Hum Exp Toxicol. 2012;31(1):81–91.
6. Freitag AF, Cardia GFE, da Rocha BA, Aguiar RP, Silva-Comar FM de S, Spironello RA, et al. Hepatoprotective Effect of Silymarin (Silybum marianum) on Hepatotoxicity Induced by Acetaminophen in Spontaneously Hypertensive Rats. Evidence-Based Complement Altern Med. 2015;2015:1–8.
7. Xiong F, Guan YS. Cautiously using natural medicine to treat liver problems. World J Gastroenterol. 2017;23(19):3388–95.
8. Bektur NE, Sahin E, Baycu C, Unver G. Protective effects of silymarin against acetaminophen-induced hepatotoxicity and nephrotoxicity in mice. Toxicol Ind Health. 2013;32(4):589–600.
9. Jalali SM, Najafzadeh H, Bahmei S. Protective role of silymarin and D-penicillamine against lead-induced liver toxicity and oxidative stress. Toxicol Ind Health. 2017;33(6):512–8.
10. Manan A, Shahid B, Sultan A, Ahmed UI. Consumer Demand for Beverages in Pakistan. Cercet Agron Mold. 2017;50(2):109–20.
11. Rybakowska IM, Milczarek R, Slominska EM, Smolenski RT. Effect of decaffeinated coffee on function and nucleotide metabolism in kidney. Mol Cell Biochem. 2018;439:11–8.
12. Katayama M, Donai K, Sakakibara H, Ohtomo Y, Miyagawa M, Kuroda K, et al. Coffee consumption delays the hepatitis and suppresses the inflammation related gene expression in the Long-Evans Cinnamon rat. Clin Nutr [Internet]. 2014;33(2):302–10.
13. Saleem M, Latif A. Multiple Beneficial effects Associated with Coffee Consumption on Human Liver. J Hepatol Gastrointest Disord. 2017;03(02):1–7.
14. Arauz J, Ramos-Tovar E, Muriel P. Coffee and the Liver. Liver Pathophysiology: Therapies and Antioxidants. Elsevier Inc.; 2017. 675–685.
15. Dranoff JA. Coffee consumption and prevention of cirrhosis: In support of the caffeine hypothesis. Gene Expr. 2018;18(1):1–3.
16. Das S. Hepatoprotective activity of methanol extract of Fenugreek seeds on rats. Int J Pharm Sci Res. 2014;5(4):1506–13.
17. Arauz J, Zarco N, Hernández-Aquino E, Galicia-Moreno M, Favari L, Segovia J, et al. Coffee consumption prevents fibrosis in a rat model that mimics secondary biliary cirrhosis in humans. Nutr Res [Internet]. 2017;40:65–74.
18. Ebrahimi E, Shirali S, Talaei R. The Protective Effect of Marigold Hydroalcoholic Extract in STZ-Induced Diabetic Rats: Evaluation of Cardiac and Pancreatic Biomarkers in the Serum. J Bot. 2016;2016:1–6.
19. Jarsiah P, Nosrati A, Alizadeh A, Hashemi-Soteh MB. Hepatotoxicity and ALT/AST enzymes activities change in therapeutic and toxic doses consumption of acetaminophen in rats. Int Biol Biomed J. 2017;3(3):118–24.
20. Aleksandrova K, Bamia C, Drogan D, Lagiou P, Trichopoulou A, Jenab M, et al. The association of coffee intake with liver cancer risk is mediated by biomarkers of inflammation and hepatocellular injury: Data from the European Prospective Investigation into Cancer and Nutrition. Am J Clin Nutr. 2015;102(6):1498–508.
21. Udom GJ, Yemitan OK, Umoh EE, Mbagwu HOC, Ukpe E, Thomas PS. Journal of Herbal Drug. 2018;8(4):219–25.
22. Katayama M, Donai K, Sakakibara H, Ohtomo Y, Miyagawa M, Kuroda K, et al. Coffee consumption delays the hepatitis and suppresses the inflammation related gene expression in the Long-Evans Cinnamon rat. Clin Nutr. 2014;33(2):302–10.
23. Bahcecioglu IH ali., Ispiroglu M, Tuzcu M, Orhan C, Ulas M, Demirel U, et al. Pistacia Terebinthus Coffee Protects against Thioacetamide-Induced Liver Injury in Rats. Acta Medica (Hradec Kralove). 2015;58(2):56–61.
24. Arauz J, Zarco N, Hernández-Aquino E, Galicia-Moreno M, Favari L, Segovia J, et al. Coffee consumption prevents fibrosis in a rat model that mimics secondary biliary cirrhosis in humans. Nutr Res. 2017;40:65–74.
25. Salomone F, Li Volti G, Vitaglione P, Morisco F, Fogliano V, Zappalà A, et al. Coffee enhances the expression of chaperones and antioxidant proteins in rats with nonalcoholic fatty liver disease. Transl Res. 2014;163(6):593–602.