Hepato-protective Nutraceutical Formulation

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ABSTRACT

Purpose of study: To formulate a hepato-protective nutraceutical formulation for the inflammatory conditions related to the liver, mainly hepatitis, and to study the recovery profile of the liver after its administration.

Introduction: There are many conditions involved in liver inflammation, such as hepatitis mainly caused by viruses (HVA, HVB, HVC, HVE, etc.) but there may be several other causes like autoimmune hepatitis and hepatitis that occurs due to secondary causes of some medications, toxins, alcohols, drugs, etc. The condition, if not treated properly can progress to fibrosis (scarring), cirrhosis, or liver cancer. Hepatitis B and C cases are increasing day by day in Pakistan. According to WHO, 23,720 people died of hepatitis-related causes in Pakistan in 2016. Our main objective is to enhance liver recovery after hepatitis. The current Nutraceutical formulation (NF) can be used as a measure of adjunct therapy along with allopathic treatment as well as preventive care for the progression of the disease.

Methodology: Pre-Clinical studies are being carried out on Albino rats divided into four groups; control, the standard that is silymarin, the experimental group in which CCl4 was used to induced liver inflammation and nutraceutical formulation containing *Vitis vinifera, Tamarindus indica, Elettaria cardamomum* was administered daily and the fourth group in which only CCl4 was administered. Histopathology and Liver function tests were performed to study and verify the effects of nutraceutical formulation on the liver. Standard procedures and guidelines are being followed to perform this experiment specifically pathological induction of liver inflammation.

Result: LFTs of all the groups were compared and the changes in the pathological profile were noted. The experimental group in which the nutraceutical formulation was administered showed signs of liver recovery within 2 weeks.

INTRODUCTION:

There are many conditions involving inflammation of the liver such as hepatitis mainly caused by viruses (HVA, HVB, HVC, HVE, etc.) but there may be several other causes like autoimmune hepatitis and hepatitis that occur due to secondary causes of some medications, toxins, alcohols, drugs, etc. The condition, if not treated properly can progress to fibrosis (scarring), cirrhosis, or liver cancer. Approximately, 5 to10 million people are affected with hepatitis B and C respectively in Pakistan[1-3]. According to WHO, 23,720 people died of hepatitis-related causes in Pakistan in 2016. Our main objective is to enhance liver recovery after hepatitis (World Health Organization). Our Nutraceutical formulation can be used as a measure of adjunct therapy along with allopathic treatment as well as preventive care for the progression of the disease.

Liver diseases are among the top ten killer diseases mostly in Asia, causing millions of deaths every year. The liver helps to break down certain medicines in your blood. Increased serum levels of medicine will lead to liver damage. This can lead to drug-induced hepatitis. The severity of drug-induced liver injury varies from minor nonspecific changes in the hepatic structure to fulminant hepatic failure, cirrhosis, and liver cancer. Herbal drugs are inexpensive, possess minimal adverse effects and people have faith in them, therefore hepato-protective drugs of botanical origin are getting importance and being promoted in the global market. Different types of chemical ingredients are present in hepato-protective plants such as phenols, coumarins, lignans, essential oils, monoterpenes, carotenoids, glycosides, flavonoids, etc[4]. which may contribute to their hepato-protective effects. *Vitis vinifera, Tamarindus indica, Elettaria cardamomum* have been proven to show anti-oxidant activity[5] and multiples research studies provide evidence of their anti-inflammatory, hepato-protective, and regeneration enhancing abilities on liver degenerative diseases as they contain phenol and its derivatives as well as vitamins to provide healthy nourishment to help the liver in quick recovery[6].

METHODOLOGY:

The randomized controlled preclinical study was carried out on groups of NMR albino male rats for a fortnight. The batch was divided into four groups namely; Normal, control, Standard, and experimental. Similar food, environmental and physical conditions were provided to all groups. Hepatitis was induced chemically by carbon tetrachloride (CCl4) in control, standard, and experimental groups[7-9]. The control group was left untreated, the standard was treated with the silymarin (Silliver by Abbott) and experimental was treated with the nutraceutical formulation (NF) containing *Vitis vinifera, Tamarindus indica, and Elettaria cardamomum*; prepared as syrup by extraction from the fruits and pods of the named plants in distilled water, whereas rock candy was used for sweetening purpose. Carbon tetrachloride was administered twice to the batch, throughout the experiment. Animals of normal groups were dissected on the 7th day of practice because carbon tetrachloride would have led to death ultimately if not treated. Standard and treated groups were dissected in the following manner, one animal from each group was dissected on the 7th day with the control group to compare the difference between the two,

another dissection was performed on 10th day, each animal from both groups, on 14th day normal and rest of the animals from standard and experimental groups were dissected. Blood of dissected animal was drawn from the apex of heart. Liver function tests (LFTs) for the samples were performed and the following observations were obtained.

RESULTS:

Average level of ALT (alanine transaminase) is 44.3 U/L in the normal group whereas 104.3 U/L in control, 37.6 U/L in standard, and 49.6 U/L in the experimental (tested) group. The level of AST (aspartate aminotransferase) is 166 U/L whereas 347.3 U/L in control, 101 U/L in standard, 180 U/L in the experimental (tested) group. Gamma GT levels indicated in normal, standard, experimental (tested) groups are almost 4 U/L whereas in control the level is 8.6 U/L.

The difference in the levels of AST, ALT, Gamma GT between normal and control group clearly shows the hepato-toxic and degenerated inflamed liver, respectively the difference between control and standard as well as experimental groups proves the recovery and regeneration of hepatocytes, with much more extended in the experimental (tested) group in comparison to standard.

Statistical analysis performed using SPSS version 16.0 and by applying ANOVA proved the significant difference between Normal control (healthy group), Negative control (disease induced group), standard and treated group. For statistical results refer to Table 1.

The mean difference at significant level of 0.05 is indicated by *. The results are compared with negative control group.

Table 1: Effect of Nutraceutical Formulation on Serum Marker Enzymes of Control, Standard and Treated Animals

Group	**ALT (U/L)**	**Alkaline Phosphatase (U/L)**	**GGT (U/L)**	**AST (U/L)**	**Total Bilirubin (mg/dL)**	**Direct Bilirubin (mg/dL)**	**Indirect Bilirubin (mg/dL)**
Normal Control (Saline)	44.33±2.5	171±1.0	4±0.0	166±2.0	0.18±0.02	0.12±0.02	0.07±0.015
Negative Control (CCl_4)	104.33±3.5*	160.66±2.08*	8.66±0.57*	347.33±2.5*	0.16±0.02	0.20±0.02*	0.06±0.041*
Standard (Silymarin)	37.66±2.08*	74.66±2.5*	4±0.0*	101±2.0*	0.14±0.05	0.11±0.005*	0.09±0.015*
Treated (NF)	49.66±2.5*	119.66±1.5*	4± 0.0*	180±4.5*	0.15±0.04	0.10±0.005*	0.05±0.036*

Values are presented in Mean±SD. *= Significant at ≤ 0.05 compared with CCl_4 Group. Abbrevitions: CCl_4=Carbontetrachloride; NF=Nutraceutical Formulation; ALT=Alanine aminotransferase; AST=Aspartate transaminase; GGT=Gamma Glutamyl Transferase

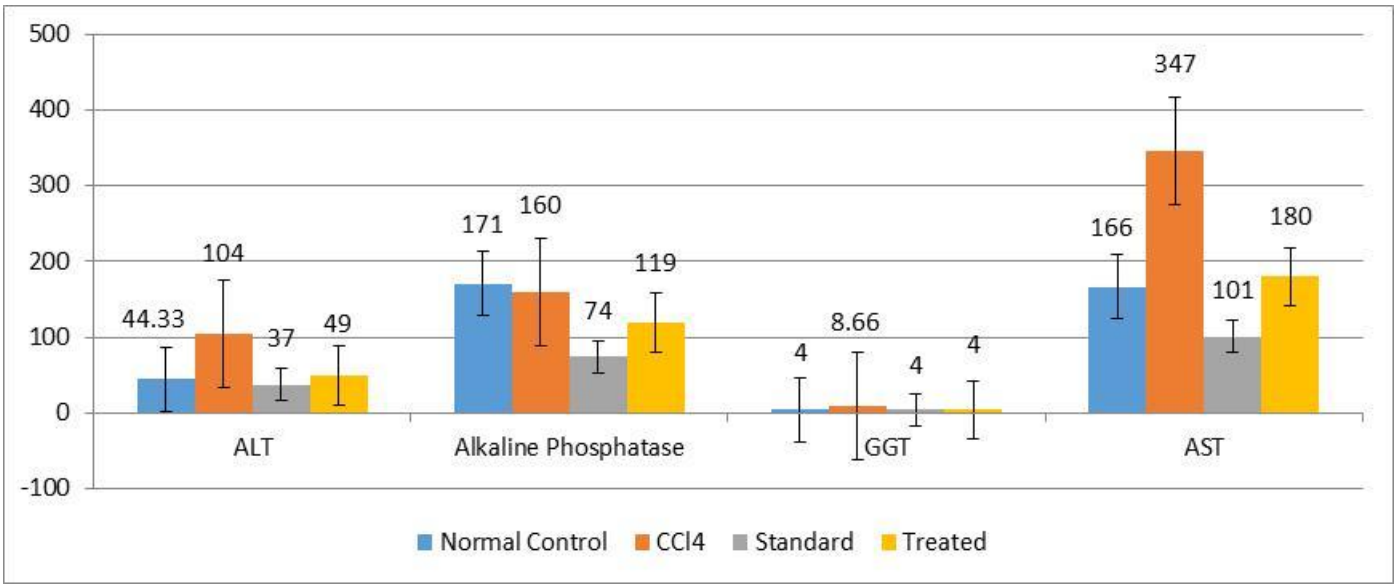

Figure 1: Showing Effect of Nutraceutical Formulation on ALT, Alkaline Phosphatase, GGT and AST of Normal Control, CCl_4, Standard and Treated Animal Groups

DISCUSSION:

Liver function test interpretation as a primary screening plays an important role in the diagnosis of liver diseases. The elevation or reduction in levels of different LFT parameters helps in the evaluation of major liver diseases and non-hepatic diseases. There are three to four major parameters in LFT that act as initial markers in the diagnosis of hepatitis. Elevation in the levels of ALT, AST, alkaline phosphatase, and in-direct bilirubin reports about hepato-cellular damage[10]. Hepatitis specifically in its chronic stages produces more hepato-cellular enzymes that are why elevated levels of AST and ALT predominate mostly in chronic viral hepatitis cases. Whereas in the case of acute hepatitis levels of alkaline phosphate enzyme is much higher than that of ALT and AST. In-direct bilirubin levels are raised more than normal in cases of viral hepatitis whether acute or chronic[11].

In this study, we chemically induced liver damage which is further confirmed via LFT that indicates raised levels of alkaline phosphatase in the control group animals. Hepatitis induce using CCl4 was acute whereas, hepato-cellular damage caused due to antibiotics specially cephalosporin second generation was chronic.

This study shows that the use of a nutraceutical formulation that contains *Vitis vinifera, Tamarindus indica, Elettaria cardamomum* has significant hepato-protective and hepato-

regeneration enhancing effects on hepato-toxic and degenerated (inflamed) liver which happens in most of the liver diseases such as hepatitis[12]. Vitis vinifera (fruit) contains polyphenols that show hepato-therapeutic activity. It acts as a synergistic anti-oxidant as well as anti-hepatotoxic proved via animal trials, in vitro, and in vivo studies. Multiple research studies and trials show that the synergistic functions of VVF polyphenols could be a promising new anti-hepatotoxic agent for targeting both necroptotic and profibrotic mediators. It reduces oxidative stress and inflammation enhancing liver recovery. The major volatile constituents of tamarind pulp include furan derivatives (44.4%) and carboxylic acids (38.2%), the components of which are furfural (38.2%), palmitic acid (14.8%), oleic acid (8.1%), and phenyl acetaldehyde (7. 5%)[13]. It is one of the most that holds important multipurpose nutritional value. It is renowned for its antioxidant and hepato-protective activity. Many research studies conclude that *Vitis vinifera, Tamarindus indica, Elettaria cardamomum* play a major role in liver recovery with no toxicities reported as they contain phenolic compounds and their derivatives along with nutritional constituents [14].

Statistical analysis shows a clear difference between negative control (disease induced group) and normal control (healthy group), treated and standard group, representing the effectiveness of herbal formulation (in treated group) in case of serious liver injury comparable to that of the standard group. This difference indicates that this herbal formulation could be a better choice and could give much better results than that of standard if included as an adjunct therapy in case of liver injuries and malnutrition providing rejuvenating and multi nutritional values with regenerative effects.

CONCLUSION:

Worsening conditions of liver inflammation are all due to negligence leading to fibrosis (scarring), cirrhosis, or liver cancer. So the major focus of this study is to prevent the progression and enhance the recovery of the disease. As proved in this study, Liver recovery was enhanced when the nutraceutical formulation was used. It can also be used as a nutritional value and for detoxification. Proper lifestyle measures should be taken to maintain a healthy liver and to aid the recovery in case of liver diseases.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES:

1. Memon, A.R., Shafique, K., Memon, A., Draz, A.U., Rauf, M.U.A. and Afsar, S., 2012. Hepatitis B and C prevalence among the high risk groups of Pakistani population. A cross sectional study. Archives of Public Health, 70(1):1-6.

2. Butt, A.S., 2015. Epidemiology of viral hepatitis and liver diseases in Pakistan. Euroasian journal of hepato-gastroenterology, 5(1):43.

3. Aziz, S., Khanani, R., Noorulain, W. and Rajper, J., 2010. Frequency of hepatitis B and C in rural and periurban Sindh. JPMA-Journal of the Pakistan Medical Association, 60(10):853.

4. Zokirova, U.T., Khidyrova, N.K., Tursunova, N.V., Syrov, V.N. and Shakhidoyatov, K.M., 2015. Polyprenols from Vitis vinifera leaves and their hepatoprotective activity. Chemistry of Natural Compounds, 51(3):423-426.

5. Aboubakr, M. and Abdelazem, A.M., 2016. Hepatoprotective effect of aqueous extract cardamom against gentamicin induced hepatic damage in rats. Int J Basic Appl Sci, 5(1):1.

6. Badolati, N., Masselli, R., Sommella, E., Sagliocchi, S., Di Minno, A., Salviati, E., Campiglia, P., Dentice, M., Tenore, G.C., Stornaiuolo, M. and Novellino, E., 2020. The Hepatoprotective Effect of Taurisolo, a Nutraceutical Enriched in Resveratrol and Polyphenols, Involves Activation of Mitochondrial Metabolism in Mice Liver. Antioxidants, 9(5):410.

7. Khattab, A.A., Taweek, A.M., Abo-EL-Sooud, K., Ahmed, K.A., El-Gendy, A.N. and Ahmed, A.R., 2020. Elettaria cardamomum essential oil rescues paraceta-mol-induced hepatorenal damage via modulating oxidative stress in rats. Adv. Anim. Vet. Sci, 8(s2):24-33.

8. Orhan, D.D., Orhan, N., Ergun, E. and Ergun, F., 2007. Hepatoprotective effect of Vitis vinifera L. leaves on carbon tetrachloride-induced acute liver damage in rats. Journal of ethnopharmacology, 112(1:145-151.

9. Clichici, S., Olteanu, D., Filip, A., Nagy, A.L., Oros, A. and Mircea, P.A., 2016. Beneficial effects of silymarin after the discontinuation of CCl4-induced liver fibrosis. Journal of medicinal food, 19(8):789-797.

10. Shakya, A.K. and Shukla, S., 2011. Evaluation of hepatoprotective efficacy of Majoon-e-Dabeed-ul-ward against acetaminophen-induced liver damage: A Unani herbal formulation. Drug development Research, 72(4):346-352.

11. Liu, J.Y., Chen, C.C., Wang, W.H., Hsu, J.D., Yang, M.Y. and Wang, C.J., 2006. The protective effects of Hibiscus sabdariffa extract on CCl4-induced liver fibrosis in rats. Food and Chemical Toxicology, 44(3):336-343.

12. Elguindy, N.M., Yacout, G.A., El Azab, E.F. and Maghraby, H.K., 2016. Chemoprotective effect of Elettaria cardamomum against chemically induced hepatocellular carcinoma in rats by inhibiting NF-κB, oxidative stress, and activity of ornithine decarboxylase. South African journal of botany, 105:251-258.

13. Shammi, N.J., Choudhry, Z.K., Khan, M.I. and Hossain, M.M., 2013. A comparative study on the hepatoprotective effect of Tamarindus indica and vitamin E in long Evans rats. Bangladesh Journal of Medical Biochemistry, 6(2):63-67.

14. Meena, S.Z., Rahman, M.A., Bagga, P. and Mujahid, M., 2019. Hepatoprotective activity of Tamarindus indica Linn stem bark ethanolic extract against hepatic damage induced by co-

administration of antitubercular drugs isoniazid and rifampicin in Sprague Dawley rats. Journal of basic and clinical physiology and pharmacology, 30(1):131-137.