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Protective Effect of Zizphus Vulgaris Extract, on Liver Toxicity in Laboratory Rats

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ABSTRACT

Some of natural and synthetic products have antioxidant properties which protect the liver against the destructive factors.This study aimed to investigatethe effect ofZizphus Vulgaris extracts on mouce liver. This experimental study was conducted at Yasouj University of Medical Sciences in 2010on 30healthy adult maleWistar rats.Animalswere randomly divided intofiveequal groups: the control group (receiving olive oil), control group(receiving olive oilandcarbon tetrachloride)and threeintervention groups(receiving different does of carbon tetrachlorideandolive oil. The intervention group was given daily doses of 200, 400 and 600mg per Kg of Zizphus Vulgaris extract by gavage respectively. After 45 days, the amount of liver enzymes, total protein, albuminandbilirubin inanimal's serawere measured. Data were analyzed by the SPSS software, using ANOVAandt-test. The concentration of total protein, albumin, AST, ALT, ALP in test groups I, II and III receiving Z.Vulgaris extract (200, 400 and 600 mg/kg weight) compared with control group were statistically not significant. Consumption of Z.Vulgaris reduced the bilirubin concentration in test groups I and II but this decrease was significant only in the test group I. Increasing of Z.Vulgaris dose in the test group III (600 mg Z.Vulgaris per kg body weight) showed increase in the level of serum bilirubin. Increase in the ratio of liver weight to body weight of rats in groups I and III in comparison with control groups was noticed although this difference was not statistically significant. Findings of this study revealed that dosage of 600 mg/kg extract of Z.Vulgaris caused significant improvements in CCl_4, induced liver necrosis ($P < 0.01$) and reduced portal cells inflammation ($P < 0.01$). Dose of 400 mg/kg of Z.Vulgaris induced some destruction and necrosis of liver cells in animals but significant reduction of portal cells inflammation was seen. Considering the obtained results, it seems that Ziziphus vulgaris fruit extract has shielding effects against toxins on liver cells.

Key words: Carbon-tetrachloride, Liver, Protection, Zizphus Vulgaris.

INTRODUCTION

Liver plays a significant role through many necessary physiological processes such as homeostasis glucose, necessary proteins preparation for plasma ,lipoprotein ,lipid preparation and secretion of bill acids ,and saving vitamins[1] .there is no certain reason for liver disease, but oxidation factors definitely are instrumented in providing liver pathology changes ,especially through poisoned and alcoholic livers. It occurs when those compounds bring about disorder in biological membranes of cell membranes structure and finally cause pathological changes. Mostly different restraint mechanisms of the body are not able to operate as mere protective role and need some assisting compounds especially nutritional anti-oxidants.usually some natural compounds

have the anti-oxidant qualities in which they have great importance in liver protection against destructive factors[2,3].

Zizphus jujube is a suitable example,including naturaland herbalcompounds which has a long story for medication usage. Jujube was applied in Eastern Asian countries for healing diseases like liver disorders, Anemia and asthma[4,5].observations have demonstrated that Jujube plant has active compounds and these compounds include protective effect on Histamine release ,activation of Cyclooxygenases*1 and11*,Cholinestrase[6].Jujube includes mucilage, malic acid ,citric acid ,vitamin C[7] and along with sugar elements up 2.17-6.5 per cent, protein elements , organic materials, compounds tripnoid, Flanoid and jojuphnoenosid[8].

Gong Cheng et al (2000)have attributed some medical feature of ZiZphus Jujube plant to its anti-oxidant qualities by extracting eight flanoeid types out of jujube[9].

Welli et at (2007),through an observation ,along with description of some vast medicinal features of Jujube in traditional and modern Chinese medicine ,have added that due to the presence of a type of proteoglycan in the fruit up this plant,Jujube stands out as a good source of medicinal qualities'[10].Based on the presence of various chemical compounds and anti-oxidants features of Jujube , this observation conducted on rats(rice wistar) regarding the effect of Jujube fruit essence on liver protection.

MATERIALS AND METHODS

An experimental observation was conducted in Yasuj medical sciences university in 201o.thirty mature rats heads ,male healthy ,were chosen from wistar species and were accidentally divided into five equal groups as follow:

Control groups that received olive oil twice a week- Sunday and Wednesday –for amount of one cc(cubic centimeter) of body weight for each kilo grams through peritoneum injection. Simultaneously this group accepted 0.5cc distilled water through gavage. Another group was witness group that also got 50-50 olive oil solution and carbon tetrachloride in amount of one milliliter for each kilo gram of body weight through peritoneum injection. witness group received o.5 cc distilled water through gavage as well . Finally,three intervention groups of 1, 2 and 3 that simultaneously received 50-50- olive oil and carbon tetrachloride in amount of one milliliter for each kilo gram of body weight.in addition , hydro alcoholic essence made from plants was gavaged in different doses of 200, 400, 600 milligram respectively to groups of 1 ,2 ,and 3.

To get essence from jujube fruit, it first was dried and ground to powder. Then 500 grams of powder was added to water,ethanol blended with the ratio of 1 to 1 to be soaked and strained for 24 hours .this action was operated into two processes.Jujube fruit was dried under vacuum condition and 50 centigrade of heat.The essence was again dried in incubator and was distilled with distilled water to the final volume of 500 milliliter .this product was kept in 10 milliliter vials in freezer -20 centigrade –till the time of usage .the rats were weekly weighted .the doses of essence and carbon tetrachloride kept changing based on new weight.After 45days.The rats were anaesthetized by diethyl ether and blood sampling was directly done from their hearts. The result was spinal cord paralysis from. Innards were macroscopic observed .each rats liver was weighted and sent to laboratory for pathology. A serum was produced out of the blood of each rat. These serums applied for measuring Aspartat amino transfer, Alanine amino transfers enzyme ,alkaline phosphates enzyme ,total protein ,albumin and bilirubin. liver samples were fixed after weighting in natural formalin . buffered solution -10 per cent .sample were put in paraffin, and cut in wide wage, painted by hematoxilineosin. Inflammation degree of portal and necrosis in liver cells –in half form –were observed by two pathologists.

The consequence in fact was divided into four categorises of no histologist change (degree Zero) ,slight histologist changes (degree one),average histologist changes(degree two),intense histologist (degree three),through SPSS soft ware along with statistical tests analyses variance(one way) and T-test.

RESULTS

Finding consequences indicated that the average and deviation standard for protein and albumin density in rats stand respectively out 7/11+_o/37 and 3/82+_o/13milligram per cent for control group,3/76+_0//49 and 3/76+_0/021, or witness group ,7.06+_0/09and7.06+_0/09 for first intervention(experimental) group ,6/9+_0/25 and 3/75+_0/91 for second intervention(experimental) group, 6/65+_0/91and 3/7+_0/28 for third invention g(experimental) group. According to consequence, significant difference was seen between control groups ,witness and intervention.

Theaverage densityof total and direct bilirubin also were respectively determination as 0/16+_0/13 and 0/28+_0/07 for control group, 0/22+_0/17and 0/48+_0/21for witness group, 0/07+_0/09and 0/27+_0/05 for first intervention (experimental) group, 0/10+_0/11 and 0/30+_0/08 for second group intervention(experimental), and 0/25+_0/12 and 0/52+_0/17 for third intervention group.this shows that witnessgroup in comparison with control group has significant difference in total bilirubin (p<0/05).

There is significant deference between first intervention (experimental) group and witness group in total and direct bilirubin (p<0/05) as well ,but there seems no important difference between second and third intervention(experimental) groups in comparison with witness group(p<0/05).in table 1, the average of standard deviation for liver enzymes, density group is shown, so that there is not found significant difference in density of alkaline phosphates and Asparate transfer and Alanine transfer enzyme in groups of control ,witness ,and intervention (experimental) (p<0/05).

The research findings show that the average for liver weight to body weight in control group,3/93+_0/25, witness group 4/25+-0/36 ,intervention ((experimental) group one 4/67+0/94, intervention ((experimental) group two 4/10+-0/29 and intervention ((experimental) group three was 4/71+-10 mg.

That no significant difference was observed among the control group, witness group, and intervention (experimental) groups(p<0/05). histopathologicalobservation results demonstrated that width wise cut of liver in normal animals shows,normal cells along with healthy cytoplasm and central nucleus and vein. In animals,those with usage of tetrachloride carbon,livercells show high rate of rain in necrosis and portal.

In poisonous cells by tetrachloridecarbon and cured by Jujube very slight alteration was found along with almost normal structure in third intervention (experimental) group. Treatment by Jujube -600milligram as body weight in kilo gram – was remarkable in liver necrosis due to tetra chloride carbon .It also causes reduction in inflammation of portal cells .but in second intervention(experimental) group it was seen more rain in cells and reduction in inflammation of portal cells .to same extent , alteration in liver cells was normal. in the first intervention (experimental) group necrosis cells and liver portal inflammation was of higher and less rats of treatment in comparison with other two groups .It remarkable differencewas also seen between this group and other groups regarding liver cells necrosis (p<0/05),but there was no difference in inflammation of portal cells.the main difference was the general rate of liver structure ruin with second and third

Table 1: the average of standard deviation for liver enzymes

group	Alkaline phosphatas enzyme	Asparat transfras enzyme	Alanine transfras enzyme
Control	837+_155	139+_16	160+_20
witness	852+_145	143+_29	163+_10
First intervention	875+_137	137+_50	194+_53
Second intervention	792+_103	138+_30	160+_12
Third intervention	486+_156	160+_63	183+_26
Significant	<0/05	<0/05	<0/05

intervention(experimental) groups (p<0/ 05).treatment -200and 400 milligram as body weight in kilogram –causes reduction in liver necrosis and inflammation of portal area.

DISCUSSION AND CONCLUSION

Liver is the main organ of metabolism ,splash and excretion of materials and is continuously exposed to different types ofexternal and internal compounds .the outbreak of liver diseases is universally expanding and not only man-made chemical medication are not completely useful and function in curing of diseases but also undesirable and risky have vital effects ;therefore there is a need to a suitable substitution entering to medical science for curing liver diseases(2) .the aim of this experiment was the effect of Jujube essence for liver protection on rats.

The results demonstrated that alteration of enzyme rate in witness group had a slight increase in comparison control group. In intervention(experimental) group-received 200 milligram of Jujube essence –liver enzyme rate had shown an increase comparing with witness group but in intervention(experimental) group received 400 and 600 milligram of Jujube essence the rate of liver enzymes were decreased in comparison with witness group. This reduction was even more in intervention (experimental) group received 400 milligram of essence. Disorder in unity of plasma membrane -liver cells-causes enzymesentrance-which are naturally in cytosol -into blood circulation,that is the indictor and standard for observing liver condition. One of the major standards of liver damages due to toxin is increasing of liver enzymes activity in serum. reactivity of liver enzymes to natural state and also reduction of bilirubin density and increasing of total protein and albumin serum are of main standards in liver cure and recovery of this prominent organ(1).

Another important consequence indicates that total and direct bilirubin density in witness group, received just tetra chloride carbon increased comparing with control group.

It happens due to liver cells ruin and lack of protection substance .In addition, application of Jujube essence within first and second intervention (experimental) groups provided less bilirubin density. This density also within third intervention (experimental) group increased comparing with witness group. It can indicat the opposit influence of Jujube in more dose on bilirubin degree[5,6,7].

In study conducted by Nabavi Zadeh et al(2004) the influence of drug plants hydro alcoholic extract was observed on hyperbilirubinemia kid.the result showed not sufficient decrease in bilirubin density by the plant[11].

In this study no difference between the average of albumin and total protein density within observed groups was demonstrated, since protein and albumin serum are impressed through persistent liver disease[11]. the study of Abrahimi at el (2010) showed that The results indicate that *Zizyphus jujuba* was effective for the treatment of neonatal jaundice in the first 12 hours of treatment compared to controls which could be due to higher effect of Z. jujuba extract to reduce bilirubin concentration with different mechanisms[12] and rats kidney damages were sharp and remarkable; hence these consequences appear greatly acceptable.

Rats weights increased within witness and intervention (experimental) groups. These alterations were more within the first and second groups.

Theaverage percent of liver comparing with rats, body weight was observed throughinvestigating groups. Since injection of tatra chroride carbon causes damage to liver and provides disorder metabolism in rats bodies,it also causes less rat body weight and liver enlargement,that is one of the characteristics of hepatotoxic compounds. Liver damages and particularly fibrotic damages prepare enlargement and firmness[13].

In this research,too, the ratio has increased in witness group in comparison with the control group. The revision percent of the livers weight

attribution to the rats body weight has been demonstrated in the intervention ((experimental) group one and three to the witness group.

Histopathology results showed there was not remarkable difference in groups under observation. Consequence of observation done by Pervaded et al (2002)showed that alcoholic resin of pistachio essence has protection effects on liver against tetra chloride carbon that it might be related to flanoid per cent in resin[14]. In another study,it has been indicated that alcoholic essence ofplant is able to decrease necrosis, fat changes and hepatocyte swelling.This plant might be able to some effects of gathering free radicals or able to control these productions[9].

The results of observations carried out by Ayato Ilahi et al (2007)indicated that alcoholic essence of Cilimarine plant in 50 milligram to kilogram dose helps to a great extent in prevention from liver necrosis expansion and increasing of enzyme activity due to tatra chroride carbon injection. It also helps and prepares the recovery for degenration process and textural damages[15].

Generally,the results of this observation demons treated that Jujube is highly,able to have protection effects against carcinogenic and toxic factors on liver cells.

It seems that more studies and research are required to show these compounds effects on mechanism of protection influencing on poisonous liver cells.

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