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Effects of concentrated prune juice extract against carbon tetrachloride induced liver injury in albino rat model

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ABSTRACT

To investigate hepatoprotective effect of concentrated prune juice extract (CPJ) against carbon tetrachloride (CCl_4) induced liver injury in albino rat model. Animal House, Isra University Hyderabad from May to December 2012. Subjects and Methods: Forty five albino rats were divided into three groups; Group1. Controls received 0.9% isotonic saline, Group 2. Received CCl_4 orally (1.9mg/kg) mixed in olive oil, and Group 3. received the CCl_4+CPJ. Blood samples were collected for liver biochemical assays. The animals were sacrificed, liver tissue, after fixation in 4% formaldehyde, was embedded in paraffin. Tissue sections of 5µ thickness were subjected to haematoxylin and eosin staining and were assessed by light microscopy. The data was analyzed on *Statistix 8.1* using one-way analysis of variance and post hoc test. A p-value of ≤ 0.05 was taken statistically significant. The liver biochemical and histological findings reveal statistically significant differences among the controls, CCl_4 and CCl_4+CPJ groups (p=0.0001). Liver enzymes and histology was deranged significantly in CCl_4 group compared to controls and CCl_4+CPJ group (p=0.0001).The CCl_4+CPJ group shows less elevation of liver enzymes and derangement in liver histology when compared to CCl_4 group (p=0.001). The histological findings of congestion, inflammatory cell infiltrate, vacuolar degeneration and necrosis are found prominent in CCl_4 group. The present study concludes that *Concentrated Prune Juice extract* showed hepatoprotective effect against carbon tetrachloride induced liver injury.

Introduction

Prune juice is a familiar sight on grocery shelves but until recently it ha not been used widely in concentrated form as a food ingredient. Successful applications of the concentrate have included a range of bakery products[1] as well as dairy and snack foods and some beverages.[2] In many cases, the properties derived from prunes are used to replace other additives, It has been theorized that prune juice might inhibit mold development in bakery products due to its high malic acid content as well as traces of benzoic and salicylic acids.[1,2] In addition, the concentrate has been used as a natural sweetener, colorant, and humectant. Since prune juice has traditionally been considered by consumers so be a healthful and natural food, the ongoing trend toward a more healthful diet and lifestyle should continue to stimulate increased use of prune products in the coming decade.

Although the nutritional properties of prunes are widely recognized, little work has actually been performed to elucidate their functional properties in bakery products. In this article, we discuss the results of laboratory tests to examine and quantify prune juice concentrate baking qualities, mold-inhibiting properties, and effects on final product quality of yeast-leavened baked goods, as well as suggestions as to how these properties might be used in development of new products. Prune juice concentrate is a standardized product made by concentrating a water extract of dried prunes. It has a natural dark brown color.

Unlike many other fruit juice concentrates, it does not require refrigeration and is free from sulfites and other artificial preservatives. The sucrose, fructose, and dextrose are readily available as fermentable sugars in yeast-leavened doughs Unique in fruit juices is the high level of sorbitol found in prune juice concentrate. While sorbitol is reported to be 60% as sweet as sucrose, ii is not fer in yeast dough's. It has been used to improve keeping properties of military canned bread.[3] Prune juice concentrate can provide a natural means by which sorbitol can be introduced into products to improve sweetness, humectancy, and lower water activity. Medically, sorbitol has been used as a cathartic and as a sugar substitute, for diabetics.[4] It is also a common ingredient in sugar-free, non carcinogenic gums and candies. The high potassium and low sodium content in prunes favors its use in products designed for individuals who arc taking diuretics to manage their elevated blood pressure.

The present study was designed to observe effects of concentrated prune juice extract on liver and possible protective role of concentrated prune juice extract in albino rat model at animal house of Isra University. MATERIALS AND

Methods

The present experimental study included forty five albino rats at animal house of Isra University from March to July 2013. Albino rats of 250-300 grams were included while female rats, and rats weighing <250 grams or >300 grams were excluded

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from the study. The Animals were housed in animal house at an optimal room temperature with 55-60% humidity and exposed to 12 hour light-dark cycles. The chaw like fresh alfalfa and clean water are provided freely.

Group 1. (n=15) Rats received 0.9% isotonic saline orally on alternate day for three successive weeks and served as control group,

Group 2. (n=15) Rats were given CCl_4 orally mixed in olive oil for three successive weeks and

Group 3. (n=15) Rats received 12% concentrated prune juice extract daily and CCl_4 for three successive weeks

Carbon tetrachloride was purchased from scientific drug store. The concentrated Prune Juice extract was purchased from chemist and druggist of Isra University hospital. Olive oil was used as vehicle to administer CCl_4. Twenty four hours after the end of experimental period, blood samples were collected from peripheral veins. Sera were separated by centrifugation at 300xs for ten minutes. Serum samples were used to determine liver enzymes. The animals were sacrificed by over-dose of Ketamine and Xylazil as described by Nayak et al. (2006)[5] and liver was removed promptly for histological study.

Liver enzyme assays were determined for alanine transaminase (ALT), aspartate transaminase (AST), alkaline phophatase (ALP) and lactate dehyderogenase (LDH) using commercially available diagnostic kits.

Each sample of liver obtained was washed in normal saline and tissues were fixed in previously marked containers, containing 10% formaldehyde as preservative. The tissues were embedded in paraffin, cut into 5 um thick sections and stained with Hematoxylin-Eosin (H & E) and Masson`s trichrome staining for histological examination. The histological criteria included vacuolar degeneration, inflammatory cell infiltrate, congestion and necrosis. The histological parameters were graded as follows; 0 = no abnormal findings, + = mild injury, ++ = moderate injury and +++ = severe injury.[6]

The data was analyzed on *Statistix`` 8.1.* (USA). The continuous variables were presented as mean±SD and range. The categorical variables were analyzed by Chi-square test. While the continuous variables among and between groups were calculated by one-way analysis of variance and post hoc testing. A p-value of ≤ 0.5 was taken statistically significant.

Result

In present study, we observed major differences in liver enzyme assays among groups. The ALT, AST, ALP and LDH in serum of Rats treated with carbon tetrachloride were found elevated compared with control group after three weeks, with a highly significant of p-value for all variables (p=0.001) The CCl_4+CPJ showed a significant reduction in the liver enzymes compared with the CCl_4 group (p=0.001) and control group (p=0.001). The CPJ when mixed with CCl_4 showed significant reduction in the liver enzyme elevation in blood sera. The finding shows significant hepatoprotection by the CPJ in CCl_4 induced injury. The liver enzyme assays among different groups are shown in table.1.

Different parameters of histological score of liver injury are shown in Table. II. The Liver sections from control group showed intact central portal venules and compact hepatocytes arrangement. Normal looking hepatocytes with prominent nucleus, nucleolus and well preserved cytoplasm were seen in control group. (Figure. 1).

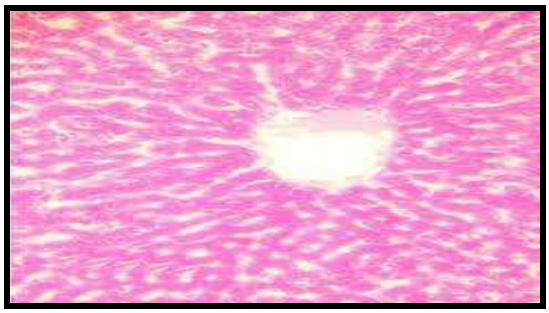

Figure 1. Liver slide of control group shows normal looking hepatocytes arranged in cords. Central vein is shown separated by sinuso

The CCl_4 group showed derangement of hepatocytes cords, hydropic changes with congestion of central venules and sinusoids, and abundant inflammatory cell infiltration (Figure.2).

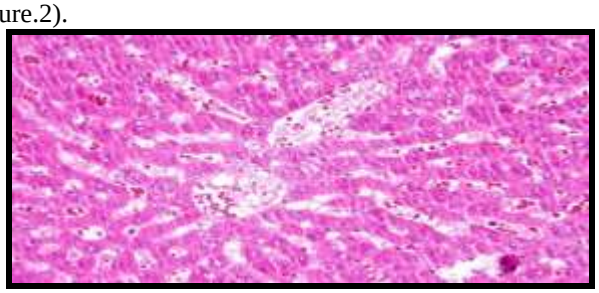

Figure 2. CCl_4 group showing hydropic degeneration, inflammatory cell infiltrate and necrosis

The centrilobular hepatocytes showed hydropic changes and necrosis, while midzonal and peripheral hepatocytes showed vacuolar degeneration and fatty changes in CCl_4 group. (Figure.3.).

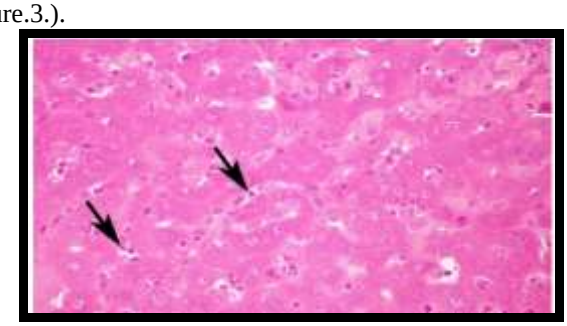

Figure 3. Concentrated Prune juice extract and carbon tetrachloride group showing normal hepatocyte arranged in cords with congested sinusoids, and few lymphocytic infiltrations.

In CCl_4+CPJ animals, liver tissue sections revealed least derangement of hepatocytes cords, hepatocytes damage and necrosis was limited compared with CCl_4 group. (Figure.4)

Discussion

Carbon tetrachloride is a commonly used hepatotoxin in experimental study of liver diseases in animal models.[7] The liver toxicity is produced by release of free radicals and lipid peroxidation[8] which causes hepatocyte necrosis, inflammation and fibrogensis.[9] The serum levels of ALT, AST, ALP and LDH reflect the physiological state of liver. The ALT, AST, ALP and LDH are released parallel to the distortion of liver, and cellular injury of the organ caused by toxic metabolites and diseases.[10] The present study indicates that the carbon tetrachloride caused an increase in serum levels of liver enzymes in rabbits as compared to control group; that is carbon tetrachloride induced a detectable damage to liver sufficient to release liver enzymes, as

previously reported by Hukkeri et al.[11] The Hukkeri proved elevation in the plasma level of cytoplasmic and mitochondrial enzymes due to liver injury induced by CCl_4 in animal models.[11] Increased blood levels of liver enzymes indicate rupture of the cell membrane and damage of hepatocytes sufficient to release cytoplasmic enzymes into blood circulation.[12] In the present study, damage of liver caused by CCl_4 was evident by the rise in serum marker enzymes beside the histological changes in liver tissue. Administration of CCl_4 significantly increased the serum levels of liver enzymes; AST, ALT, ALP and LDH, which are indices of liver cell damage and leakage of enzymes from cells.[13,14]It is reported that rise in ALT is almost always due to hepatocellular damage; accompanied by rise in AST and ALP.[15] The carbon tetrachloride is found to produce free radicals, which affect cellular permeability of hepatocytes leading to elevated levels of liver enzymes.[16] The histological examination of current work correlates with disturbance in biochemical markers of hepatocellualr damage. Histological examination of carbon tetrachloride group revealed disruption of normal structural organization of hepatic architecture, hepatic lobules and loss of the characteristic cord-like arrangement of the normal liver cells. The hepatic cells revealed characteristic appearance of cellular injury and showed marked cytoplasmic vacuolization. The nuclei of these cells were pyknotic. Lymphocytic infiltration and fatty change was also evident. Our findings are supported by previous studies which showed that carbon tetrachloride induces centrilobular hepatocellular vacuolar degeneration and necrosis.[17-19] The carbon tetrachloride induced hepatotoxicity has been attributed to the formation of free radicals during its detoxification in hepatocytes smooth endoplasmic reticulum by the cytochrome P450.[20] Balahoroglu et al.[21] reported that carbon tetrachloride induces lipid peroxidation which produces changes in biological membranes resulting in serious hepatocellular injury. Concentrated Prune juice extract significantly reduced effects of carbon tetrachloride induced hepatocellular damage and it was evidenced by the decreased level of liver enzymes and restoration of hepatocellular architecture. The present study reveals that the *Concentrated Prune Juice extract* showed hepatoprotective effect against oxidative damages caused by carbon tetrachloride. The *Concentrated Prune Juice extract* may be used as an effective protector against chemical induced liver damages.

Conclusion

The present study concludes that *Concentrated Prune Juice extract* shows hepatoprotective potential against oxidative damages caused by carbon tetrachloride. The *Concentrated Prune Juice extract* may be used as an effective protector against chemical induced liver damages.

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Table 1. Liver enzyme levels in controls, *CCl_4 and CCl_4+ CPJ† extract groups

Groups	ALT (IU/L)	AST (IU/L)	LDH (IU/L)	ALP (IU/L)
Group. I (Controls)	48.5±3.19	92.5±15.61	723.5±45.8	96.9±7.88
Group. II (*CCl_4)	184.7±10.77	473.7 ±13.9	2758.9±127.6	179.1±6.02
Group. III (*CCl_4 + CPJ†)	86.9±16.98	167.9±20.3	2138.6±131.3	133.8±16.15

*Carbon tetrachloride † Black garlic extract

Table 2. Histology of liver injury of controls, *CCl_4 and CCl_4+ CPJ† extract groups

Groups	Congestion	Fatty Change	Vacuolar degeneration	Necrosis
Group. I (Controls)	**0**	**0**	**0**	**0**
Group. II (*CCl_4)	++++	+++	++++	++++
Group. III (*CCl_4 + CPJ†)	++	++	+++	++

*Carbon tetrachloride † Concentrated Prune juice extract

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