Effect of Physalis alkekengi Extract on the Histology of the Liver in Male Albino Rats

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Summary

Herbal drugs are getting into use with the notion that these are relatively harmless; the practice has shown that many of them also have toxic effects. The therapeutic value of Physalis alkekengi, has been recognized in different system of traditional medicine for the treatment of various conditions. Since hardly any work is available on the toxic aspect of Physalis alkekengi, the present study was planned to see the effect of ethanolic extract of Physalis alkekengi on liver using albino rats as an experimental model. The animals were divided into three groups A, B and C. Group A served as a control and received only distilled water comparable to the experimental animals calculated according to their body weight, where as B and C served as experimental groups. 100 and 200 mg of ethanolic extract of Physalis alkekengi was dissolved in one ml of distilled water each and was given orally for 30 days/kg body weight. Liver enzyme ALT and gamma GT were significantly raised when compared to the control group, p-value being <0.05. Histological studies showed ballooning degeneration of hepatocytes, focal areas of hepatocytes necrosis with lymphocytic infiltration, providing supportive evidence for biochemical findings indicative of functional derangement. The effect of the extract was not dose dependent. Statistical analysis using ANOVA and chi-square showed statistically significant difference when the values from experimental animals were compared with those from the control, indicating that the ethanolic extract of Physalis alkekengi possesses hepatotoxic effect. The ethanolic extract of Physalis alkekengi extract is toxic to liver as evident by derangement in liver enzyme levels and disturbed liver histology.

Keywords: Physalis alkekengi, Hepatotoxicity, Liver, Hisopathogical Study.

Introduction

Nowadays, medicinal plants receive attention to research centers because of their special importance in safety of communities. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occur as secondary metabolites (1,2). Medicinal and aromatic plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals, perfumery, flavor and cosmetic industries. Physalis alkekengi, belongs to the family Solanaceae and it has been distributed in Asia (Iran, India, Japan and China) and Europe (Spain, Italy and Turkey). It has a large history of herbal use, and an interesting chemistry but it is seldom used in modern practices (3). Chemical studies have demonstrated the presence of physalin, citric acid and vit C as the major components of P. alkekengi extract. Physalin is the most chemical compound with various pharmacological characteristics including, anti bacterial, anti leishmanial and anti tumor (4-8). The whole plant is anti phlogistic, anti pyretic, anti tussive and expectorant (9-11).

It is used in treatment of urinary and skin diseases (12). Its extract has been used for treatment of wide range of diseases, including kidney and bladder stone, febrile diseases, inflammation, general edema, arthritis and rheumatism (11, 12). The anti-fertility properties of P. alkekengi have been vastly described in the Persian traditional medicine and it has been studied by some researchers (10, 13-16). Since there is hardly any work reported on the effect of Physalis alkekengi on liver, the present study was planned to evaluate effects of Physalis alkekengi on liver using rats as an experimental animals.

Materials and Methods

Physalis alkekengi was collected from Guilan province, and then was identified by a botanist. Fresh plant was thoroughly washed using deionized water, separated into leaves and fruits, and mopped with tissue paper and air-dried in shade so as to prevent the decomposition of chemical constituents. One gram of the material was ground into fine powder using blender. Hundred gm of powder was suspended in 250 ml of distilled water and allowed it to stand overnight in refrigerator; it was then sieved through several layers of muslin cloth. The filtrate (water extract) was discarded. The residue was extracted with 95% ethanol using sox halation wherein ethanol was evaporated in a rotatory evaporator at 40–50 ºC. The yield was 3.2 g/100 g of powder. Eighteen male albino rats of 6–8 weeks age, 150–200 gm of weight were procured from Razi Institute, (Karaj, Iran). Animals were maintained under a 12:12-hour light/dark photoperiod, under controlled room temperature (23–25 ºC), humidity (60%). They were fed on standard rat diet and water ad libitum, allowed to acclimatize for one week, weighed before the start of experiment and subsequently at weekly interval. The animals were randomly divided into three groups A, B and C, having 6 rats each. Group A served as control, received distilled water orally for thirty days comparable to that given to the experimental animals, and experimental groups B and C were given ethanolic extract of Physalis alkekengi orally at doses of 100 and 200 mg/kg respectively, daily each dissolved in 1 ml distilled water. Blood samples from each group were collected by cardiac puncture in vacuum tubes and allowed to stand, for one hour to separate the serum, using test tube stand. The tubes were centrifuged at the speed of 3000 revolution/min, the clear serum was collected with the help of a clean dropper in plastic tubes and stored in freezer at -20 ºC for testing on a later date; the tubes were properly labeled. Serum Alanine amino transferase and Gamma glutamyl transferase levels were measured by using commercially available kits. The histological preparations of liver were stained using Eosin & Haematoxylin for general histological study, Periodic Acid Schiff and Diastase techniques for the demonstration of glycogen in the liver sections. The information from three groups was entered into computer software Statistical Package for Social Sciences (SPSS) version 15 and analyzed through it. The qualitative and quantitative measurements were compared between the groups for differences. Any difference in the qualitative measurements was tested by fisher exact test and for quantitative value the ANOVA test was applied. The p-value of 0.05 or less was considered as statistically significant.

Results

The mean value of glutamyl transferase (Gamma GT) of group A, B and C were 71.74±2.07, 95.87±5.19 and 93.89±3.73 U/l respectively. Statistically significant difference was observed when group A was compared with groups B and C, p-value in both cases being <0.001); there

was, however, no statistically significant differences were observed between groups B and C ($p>0.92$). The mean values of Alanine amino transferase of group A, B and C were 47.76±3.54, 54.98±4.34 and 108.5±16.76 U/l respectively. The liver of animals in the control group showed typical hepatolobular architecture, consisting of central vein with radiating cords of hepatocytes separated by sinusoids; portal areas composed of portal vein, hepatic artery and bile duct were situated at the periphery. The hepatocytes were polygonal in shape, with central, lightly stained nucleus and clear nucleolus, few binucleated cells were also present, and the cytoplasm was regularly distributed without vaculations (Figure-1). In group B the general hepatolobular architecture of the liver was deranged, there was a loss of radial arrangement of hepatocytes and sinusoids, number of binucleated cells was increased (Figure-2), Inflamantory cells, especially lymphocytes, were found infiltrating around portal track, i.e., periportal inflammation (Figure-3), areas of necrosis were found around the central vein, centrilobular necrosis. Examination of preparations obtained from the animals of group C, showed comparable changes to group B; it, therefore, appeared that the effects of Physalis alkekengi extract on the liver were not dose dependent.

Table-1: Mean value of serum enzymes (U/L) in groups (Mean ± SE).

Groups	Group A	Group B	Group C	p
Alanine amino transferase	47.76±3.54	54.98±4.34	108.5±16.76	0.002*
Gamma glutamyl transferase	71.74±2.07	95.87±5.19	93.89±3.73	0.001*

*p<0.05 is statistically significant

Figure 1: liver section from group A, received distilled water. Central vein (CV) in the centre of hepatic lobule filled with blood (B). Hepatocytes (H), arranged in form of cords, are rounded to polyhedral in shape and radiate peripherally; they show nucleus (N) with clear nuclear membrane and nucleolus (No), cords are separated by sinusoids (s) with Kupffer cells (k), H&E stain, ×200.

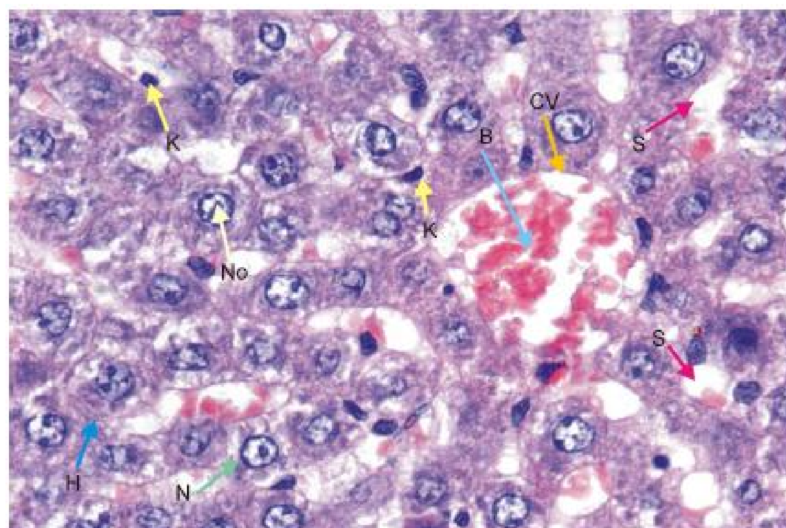


Figure 2: liver section from group B, treated with 100 mg/kg ethanolic extract of Physalis alkekengi. Deranged architecture of liver, ballooning of hepatocytes (H) with hyperchromatic nuclei (N), nucleoli are not clearly seen, multiple binucleated cells (BN), sinusoidal (S) arrangement are also seems to have been deranged, central vein (CV) filled with blood H&E stain ×200.

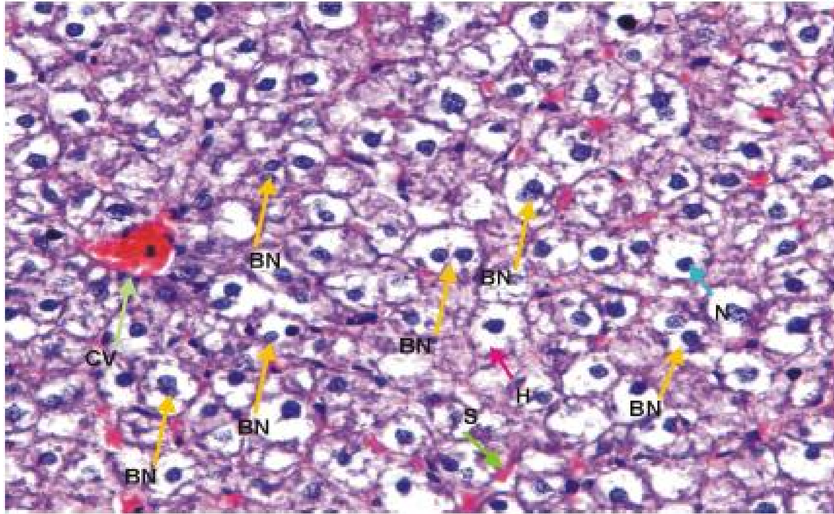
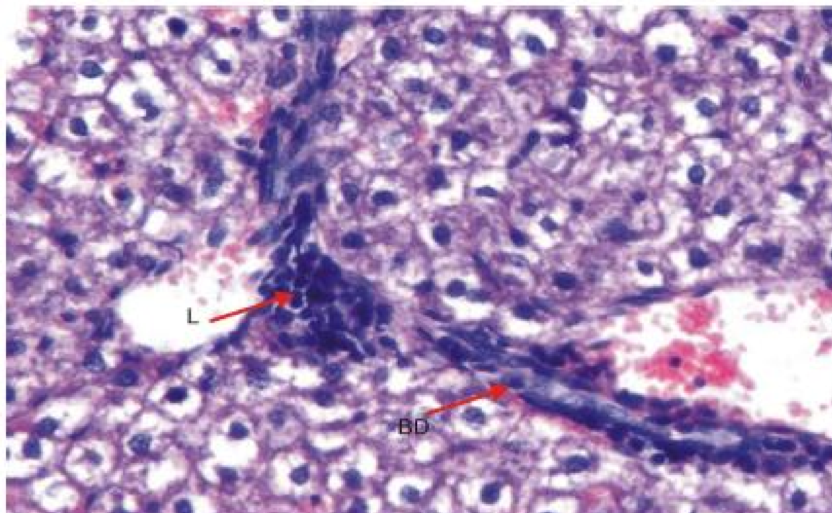


Figure 3: liver section from group C, treated with 100 mg/kg ethanolic extract of Physalis alkekengi. Periportal inflammation consisting of collection of lymphocytes (L) around the bile duct (BD), H&E stain, ×200.



Discussion

Liver enzymes are the primary markers of liver damage and were observed to have increased at the end of the experimental period indicating deleterious effect on the function of the liver which also showed histological changes. Physalis alkekengi extract, increased the liver enzymes, ALT and gamma GT, which was statistically significant, when group A was compared with that of groups B and C respectively, indicating toxic effect on the liver functions. In our study it was observed that the general architecture of liver in the experimental groups was damaged, possibly on account of hepatocytic swelling. The size of hepatocytes after the use of Physalis alkekengi extract was significantly increased, when the size of hepatocytes from the control was compared with those of the experimental groups, the difference was statically significant, ($p<0.000$). Physalis alkeekngi extract caused statistically significant necrosis in the experimental animals and when the findings of the control were compared with those in the experimental animals, the difference was found to be statistically significant, ($p<0.000$). The histological preparations from the Rats of the experimental groups showed lymphocytic infiltration around the bile duct, periportal inflammation. This finding was although, statistically not significan.

Conclusion

The ethanolic extract of Eugenia jambolana seed extract is toxic to liver as evident by derangement in liver enzyme levels and disturbed liver histology.

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