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Effect of glycyrrhiza glabra on the liver of growing embryo of mice

M Pandey*, S.K Pandey, Y.B.Tripathi[1]

Department of Anatomy and [1]Department of Medicinal chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi - 221005.UP, India.

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Abstract

In the present study the Swiss white female mice were obtained from the department of Anatomy and the central animal house, Institute of medical sciences, Banaras Hindu University. Each group of experimental animal received, orally, 400mg/kg body weight of the drug with 0.5mlof distilled water, whereas the control mice of each group received the same volume of distilled water. The first group was treated with daily single oral dose of the Glycyrrhiza glabra for 1-3 days of gestation, the second group with daily dose of the same drug for 1-7days, whereas the third treated group received single dose from 1-17 days of gestation. The embryos of each group were collected on 18th day of gestation. The liver of each experimental fetuses were observed, but there were no any external abnormalities found in any group of liver as compared to their corresponding control. The first and second group of liver revealed no any significant changes in the weight but it was found significantly increased in third group of fetuses. The microscopic examination of the liver appeared to be normal in the first experimental group, as compared with its control group, whereas the second group revealed dilatation of central vein and sinusoids, destruction of central vein and distortion of endothelial lining along with increase in number of hepatocytes, leading to hepatocytosis. The histological feature of the third treated liver revealed almost the same histological findings as seen in second group along with pericentral necrosiss.The finding showed that the drug had no antioxidant activities in higher dose and duration.

INTRODUCTION

Glycyrrhiza glabra (Gg) is an important medicinal plant and also known as licorice, sweetwood, mulethi and yastimadhu. It is found mainly in Mediterranean and certain areas of Asia [1]. Historically, the dried rhizomes were employed by the Egyptian, Chinese, Indian and Roman civilization as an expectorant and carminative. In Modern medicine, licorice extracts are often used as flavoring agents to mask the bitter test of the preparation and as an expectorant in cough and cold preparation [1]. The licorice shrub is a member of the pea family and attains the height up to five feet. Glycyrrhiza is derived from the Greek word glykos, means sweet, and rhiza, means root. In the traditional system of medicine, the roots and rhizomes of Glycyrrhiza glabra have been clinically employed since long as anti inflammatory [2] , antiulcer [3], expectorant, anti microbial activity and many gynaecological disorders [4]. Most of the drug used in pregnancy, crosses placental barrier to effect the growth of the growing embryos. Therefore, the teratogenic effect of the drug should be tested. The drug cannot be tested in human thus the experimental animals are used to observe its teratogenic effect. In the present study the effect of Glycyrrhiza glabra is to be observed (macro and microscopically) in the growing liver of the mice.

MATERIALS AND METHOD

Swiss albino female mice weighing 20-25 gm of an average age of about fifty days were used for the present study. These experimental animals were obtained from the Department of Anatomy and the central animal house, Institute of Medical Sciences, Banaras Hindu University, India. The roots of Glycyrrhiza glabra (Gg) was purchased from the local market after its proper identification and authencity. The specimen was preserved as dried powder. Fine powder of Glycyrrhiza glabra was prepared with the help of grinder and sieved and then stored in air tight container.

The animals were divided into three experimental groups along with their corresponding control. Each experimental mouse received a single dose of the drug dissolved in 0.5ml of distilled water, whereas each group of the control mice received same amount of distilled water. The drug was administered orally, in empty stomach, in the morning. First group received the drug daily from 1-3 days of gestation; second group received daily from 1-7 days, whereas the third group- received from 1-17days of gestation.

The animals were housed individually in noise free, air conditioned animal house with temperature maintained at 75ºF and on a light dark cycle of 12-12 hours. The mice were fed on diet pellets and drinking water was provided ad libitum. The female mice during their proestrus phase of estrus cycle were caged over night with the males of same stock (female: male = 2:1). The vaginal smear was examined next morning at 8 am to confirm the presence of spermatozoa. The smear positive day was taken as day 'zero' of gestation.

The pregnant mice of each group were sacrificed on day 18th of gestation by giving excess amount of ether anesthesia, followed by laparotomy. The uterine horns were exposed to collect the fetuses. The liver of both treated and control fetuses were taken out and observed for gross anomalies, if any and then weight was taken and recorded. Finally, the liver of each experimental group and the corresponding control groups were processed for histological study. The students `t' test was used to observe the statistical value of the weight of all groups of liver.

RESULT

Effect of the Glycyrrhiza glabra has been observed in the liver of growing embryos, treated with a single, oral dose of the drug at different gestational days. Macroscopically the experimental liver of all the groups showed no any gross abnormality as compare to their corresponding control group. In the first and second group the weight of the growing liver showed no any significant changes whereas the weight of the liver was found

Table 1: Effect of Glycyrrhiza glabra on the weight of growing liver of different groups

Group		Number	mean	SD	SE	t' value	P value
1	Control	25	0.0536	0.0480	0.00096	0.867	0.389
	treated	50	0.0547	0.0049	0.0007		
2	control	25	0.0583	0.0654	0.0113	1.566	0.122
	treated	50	0.0614	0.0088	0.0013		
3	control	25	0.0607	0.00733	0.00147	2.344	0.022*
	treated	50	0.0654	0.00862	0.00122		

increased in the second group. The weight of the third group of liver was significantly increased ($p<0.05$) as compared to the corresponding controls (table -1).

On the Microscopic examination the effect of Glycyrrhiza glabra on the first group of the liver revealed no any remarkable changes, but the size and the number of kuffer,s cells and hepatocytes were increased as compare to its control group (fig 1B&D). The second group of liver revealed dilatation of the central vein and the endothelial lining of the central vein and sinusoids were distorted. The number of hepatocytes was significantly increased leading to hepatocytosis (fig 2 B & D). The histological feature of the third group treated liver revealed almost the same findings as seen in second group i.e. distortion of central vein, broken endothelial lining and hepatocyosis (fig3 B&D). The pericentral necrosis was also observed in third treated group of liver (fig 3B &D) as compared to their corresponding control groups.

DISCUSSION

Although, acceptance for herbal remedies has gradually increased [5] especially for chronic diseases, but the consumers are still ignorant about the adverse effect of these preparations. They are also not aware of the effect of these herbal drugs when these are taken as food suppliments [6, 7]These herbal drugs are now also used in gynaecological and pregnancy related complications therefore they should also be tested for teratogenicity. Glycyrrhiza glabra having multifacet pharmacological action thus was selected for the present study, but very little is known about its teratological effects on growing liver.

As reported earlier the drug is hepatoprotective [8, 9, 10, 11]; the present study highlights the ability of high dose of the drug(400mg /kg body weight) to induce histopathological changes in the growing liver, indicating the need to be cautiously used in pregnancy in low doses. The changes in the histological features are suggestive of damage to hepatocytes. Considering the fact that Glycyrrhiza glabra has been an integral part of our traditional medicine for centuries, it is taken for granted that it is safe in wide ranges of doses; even in pregnancy. However earlier few reports highlighted its toxic effects. In previous study hypertension, hypokalemia and peripheral odema was observed with higher doses of Glycyrrhiza [12] Elevation of blood pressure associated with headache, and hypokalemia was also reported as another commonest side effect of this drug by Stomer [13]. It exhibited estrogenic activity and has reputed abortifacient effects [14, 15]. Excessive consumption may lead to preterm delivery

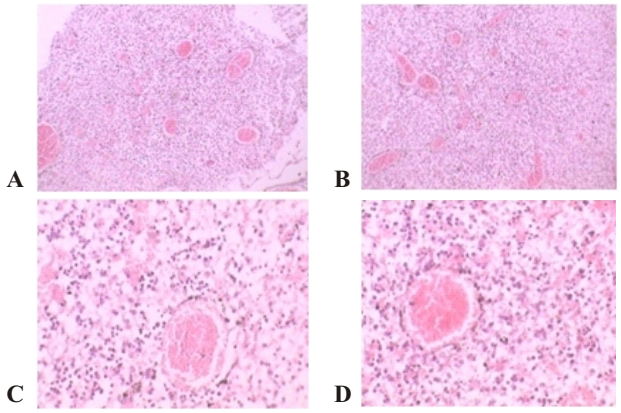

Fig 1

Fig A: Photograph of control liver showing normal architecture and features H&E; 100x
Fig B: Photograph of first group treated liver showing no any significant pathological changes in the architecture as compared to control group, except the increased in the number of hepatocytes. H&E; 100X
Fig C: Higher magnification of A, H&E;400x
Fig D: Higher magnification of B, H&E;400x

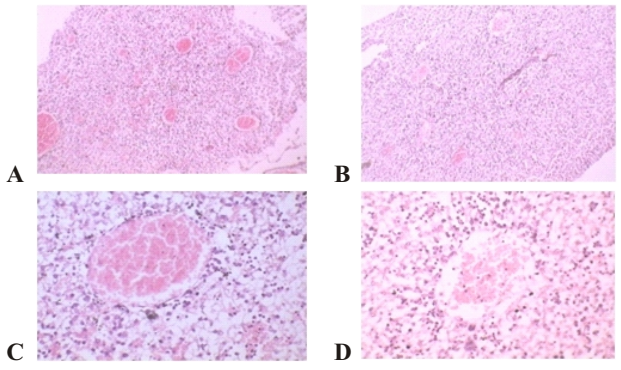

Fig 2

Fig A: Photograph of control liver showing normal architecture and features H&E; 100x
FigB: Photograph of second group treated liver showing dilatation of central vein and hepatocytosis.
Fig C: Higher magnification of A, H&E;400x
FigD: Higher magnification of A, H&E;400x

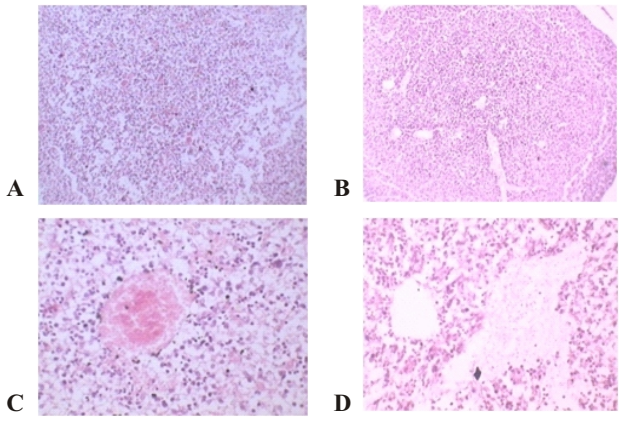

Fig 3

Fig A: Photograph of control liver showing normal features. H &E; 100x;
FigB: Photograph of third group treated liver showing dilatation, distortion of central vein along with pericentral necrosis. Hepatocytosis is also seen. H &E; 100x;
Fig C: Higher magnification of A, H&E; 400x
FigD: Higher magnification of A, H&E; 400x

[16]. Thus it could be suggested that Glycyrrhiza glabra is safe at lower doses i.e. recommended dose for human being and should not be used in higher doses in pregnancy.

The antioxidant activity of Glycyrrhiza glabra has been reported earlier [11, 17].This antioxidant activity is due to glabridine, isoliquiritigenin, lincochalcones [1]. Microscopically at higher dose of Gg, dialatation, destruction of central vein, broken endothelial lining, hepatocytosis and pericentral necrosis was observed in this present study. These findings were suggestive of damage to growing hepatocytes, which revealed that the Glycyrrhiza glabra has no any antioxidant activity with higher dose and prolonged duration. Destruction of endothelial lining and dialatation of central vein and sinusoids along with pericentral necrosis in the third group revealed that this drug has teratogenic effect in higher dose for long duration.

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