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A STUDY TO EVALUATE HEPATO AND UTERO PROTECTIVE ROLE OF VITAMIN E IN ETHANOL FED OVARIECTOMIZED RATS

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ABSTRACT
Vitamin E has been attributed with a plethora of health- promoting actions. The purpose of this study was to reveal the hepato and utero protective role of vitamin E in ethanol treated bilateral ovariectomized rats. Experiments were carried out in – different group, as - A) Sham-operated control,B) Control+EtOH[ethanol], C)Bilateral Ovariectomized, D) Ovariectomized+EtOH,E) Ovariectomized+ Sesame oil and F) Ovariectomized + EtOH+ VitaminE. In this study of consicutive 30 days, significant hepatic damage was observed in bilateral ovariectomized rats treated with ethanol (0.5ml of 15% ethanol/100gm b.wt. /day) as reflected in their altered serum AST (SGOT), ALT (SGPT) and serum Alkaline phosphatase activity. Also, uterine regression was confirmed in this group of rats by significant lower uterine protein content and decreased activity of uterine AST, ALT and Alkaline phosphatase activity. Vitamin E supplementation (10mg/100gm b.wt.), apart from its unique influence in preventing hepatic damage, also could prevent ethanol induced uterine regression in ethanol fed bilateral ovariectomized rats.

KEYWORDS: Vitamin E, Ethanol, Ovariectomized, Hepatic, Uterus.

INTRODUCTION
Alcohol abuse and alcoholism represents one of the major health, social and economic issues facing the world. Chronic hepatitis is profoundly associated with alcohol ingestion. In chronic hepatitis hepatocellular damage occurs, where impairment is most prominent within liver cells[1] Regular ingestion of moderate amounts of alcohol leads to increased accumulation of acetaldehyde, in part due to reduced activity of alcohol dehydrogenase [ADH]. Acetaldehyde dehydrogenase [ALDH] then catalyzes conversion of acetaldehyde to acetate.[2] It is also reported that less gastric metabolism of ethanol occurs in women than in men, which may explain in part the greater susceptibility of women to ethanol.[3] Additionally it has also been consistently observed that the incidence of alcohol induced liver injury is higher and progresses faster among men with similar history.[4] Menopause is associated with a number of physiological problems (hot flashes, osteoporosis etc.) due to estrogen insufficiency and may be linked with the higher susceptibility to ethanol induced hepatic and uterine damage.[5] The present study was designed to investigate the extent of hepatic damage by ethanol in bilateral ovariectomized rats as well as the uterine status and prevention of the damages by antioxidant vitamin E supplementation.

MATERIAL AND METHODS
A) Experiment Design
Experiments were carried out with adult virgin female albino rats weighting 110-120g. They were divided into 6 groups having 6 animals in each -

Groups	Treatments	No. of rats
A	Sham-operated control	6
B	Sham-operated control+ EtoH	6
C	Bilateral ovariectomized (OVX)	6
D	Bilateral ovariectomized (OVX) + EtoH	6
E	Bilateral ovariectomized(OVX) + sesame oil	6
F	Bilateral ovariectomized(OVX) + EtoH + vitamin E	6

All animals were pair-fed and the composition of diet was same (as available standard rat diet). Water was given *ad libitum.* Under light ether anesthesia, bilateral (dorsolateral) ovariectomized were performed in rats of C, D, Eand F, whereas the rats of group A andB were sham-operated. After the surgical convalescence, rats of group B, D and F were fed 0.5 ml 15% (v/v) ethanol/ day for consecutive 30 days by gavage.[6] Group F rats were supplemented with a single dose of vitamin E dissolved in sesame oil (Vit E – 10mg/0.5ml/100gm body weight) for 30 days.[7] Group E rats were supplemented with same volume (0.5ml/100gm b.wt.) of only sesame oil as placebo.

B) Collection Of Blood

At the end of 30 days blood from rats of different groups were taken by syringe directly through cardiac puncture and kept for serum collection. All sampling was performed between 1 pm to 3 pm in order to avoid diurnal variation on the parameter observed in the study.[8]

C) Preparation of Tissue Extracts

The abdomen was opened, uterus was quickly removed, weighed and placed in a beaker containing ice-cold 10mM Phosphate Buffered Saline (PBS). It was cut into small pieces and homogenized with 10mM PBS. The homogenate was processed according to Koyama et. al, 1983[9] for estimation of protein and activities of uterine alkaline phosphatase, AST and ALT.

D) Measurment Of Different Parameters

SGPT, SGOT and ALP were measured by standard kits where protein content was measured by the method of Lowry et. al, 1951.[10]

E) Statistical Calculations

Data were expressed as mean+_ SE. Statistical significance was determined using Student's t test. SPSS-10 software was used for statistical analysis. Differences were considered significant if P<0.05.

RESULTS

a) Serum SGPT/ALT profile

The Group A animals showed normal value of SGPT but Group C rats showed increased SGPT activity when compared with Group A. SGPT activity is markedly increased in ethanol fed ovariectomized rats. The Vitamin E treatment in Group F rats restored the SGPT value towards normal when compared with Group D rats. Finally, the results showed that on ethanol administration there was a significant increase in the activity of serum SGPT. This effect of ethanol was blunted significantly by vitamin E in ovariectomized rats (ethanol treated).

Table1: Serum SGPT Level (U/ml).

A	11.75 ± 1.01
B	54 ± 5.57
C	16 ± 0.57
D	56.75 ± 1.88
E	39 ± 1.46
F	18.25 ± 0.6

Values are expressed as means ±SEM, N=6

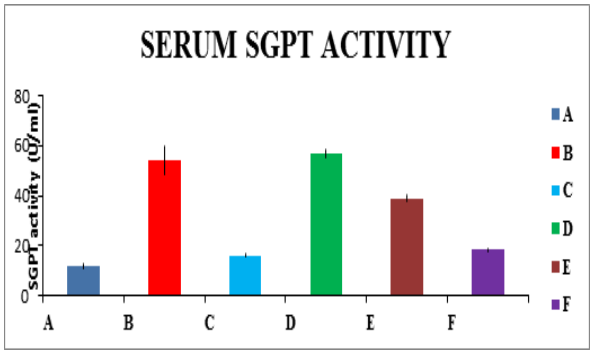

Fig 1: Graphical Representation of Relative changes of Serum SGPT Level of different groups.

b) Serum SGOT/AST profile

The Group A animals showed normal value of SGOT, where Group C rats showed increased SGOT activity when compared with Group A rats. SGOT activity is markedly increased in ethanol fed ovariectomized rats. Vitamin E treatment in Group F rats restored the value towards normal level when compared with Group D rats. Ultimately the results showed that on ethanol administration there was a significant increase in the activity of SGOT; which was blunted significantly by vitamin E in ovariectomized rat (ethanol treated).

Table2: Serum SGOT Level (U/ml).

A	51.87± 2.9
B	90± 2.88
C	63.75±2.39
D	101±4.49
E	81.25±2.39
F	75±2.88

Values are expressed as means ±SEM, N=6

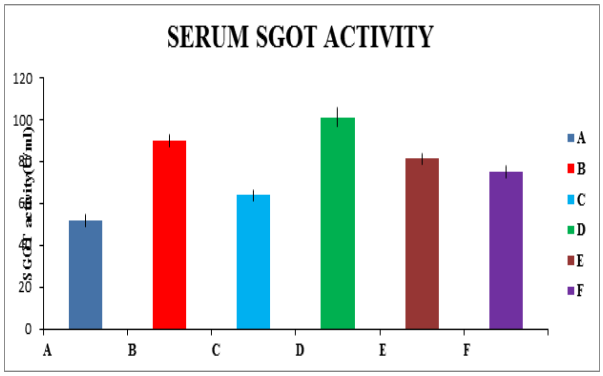

Fig 2: Graphical Representation of Relative changes of Serum SGOT Level of different groups.

c) Serum Alkaline Phosphatase (ALP)profile

In this study, Group A animals showed normal value of serum ALP but Group C rats showed increased ALP activity when compared with Group A rats. ALP activity is markedly increased in ethanol fed ovariectomized rats but Vitamin E treatment in Group F rats restored the ALP value towards normal level when compared with Group D rats.Truly, the results showed that on ethanol administration there was a significant increase in the activity of ALP and this effect of ethanol was blunted significantly by vitamin E inovariectomized rat (ethanol treated).

Table 3: Serum ALP Level (KA Unit/mg of tissue).

A	30.36 ± 1.06
B	54.60 ± 0.93
C	39.05 ± 0.75
D	64.70 ± 2.97
E	45.16 ± 2.34
F	44.22 ± 0.34

Values are expressed as means ±SEM, N=6

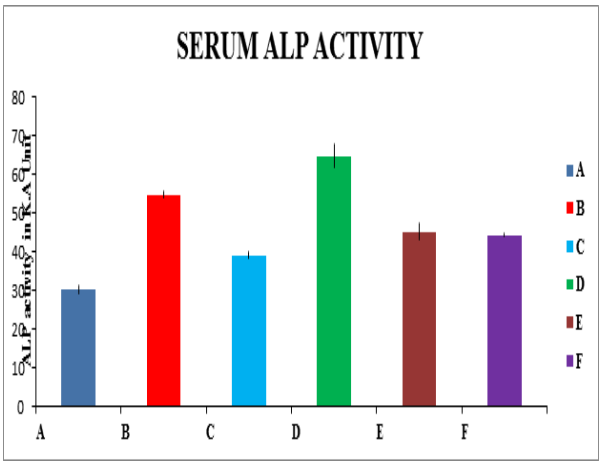

Fig 3: Graphical Representation of Relative changes of Serum ALP Level of different groups.

d) Uterine Protein Content

In the present experiment, ethanol administration caused damage of uterus both in control and ovariectomized rat. Group B and C animals showed decreased protein content in the uterine tissue when compared with Group A. The Group D animals showed significant($p<0.001$) decrease protein content of uterine tissue when compared with Group C animals. Vitamin E treatment in Group F restored the value towards normal level when compared with Group D rats.

Table 4: Uterine Protein Content (mg/gm tissue).

A	16.60 ± 1.35
B	7.97 ± 0.22
C	9.49 ± 0.94
D	1.4 ± 0.35
E	8.28 ± 0.77
F	8.48 ± 0.98

Values are expressed as means ±SEM, N=6

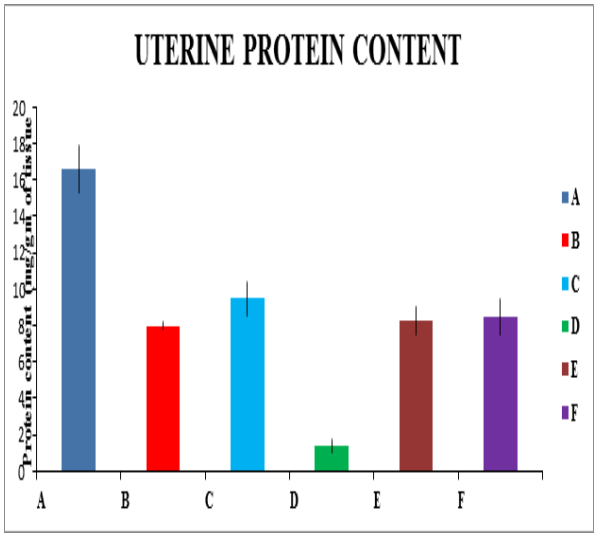

Fig 4: Graphical Representation of Relative changes of Uterine Protein Level of different groups.

e) Uterine GPT Content

In the present experiment, ethanol administration caused damage of uterine protein and decrease in uterine enzyme activity both in normal and ovariectomized rats. Group C rats showed significant decrease in GPT activities of the uterine tissue than Group A again Group D showed significant decrease in GPT activities of the uterine tissues than Group C.Vitamin E treatment in group Frestored the GPT value towards normal when compared with Group D animals.

Table 5: Uterine GPT Content(U/mg of tissue).

A	7.71± 0.66
B	3.01± 0.44
C	4.11± 0.11
D	1.66± 0.16
E	2.33± 0.16
F	3.68± 0.23

Values are expressed as means ±SEM, N=6

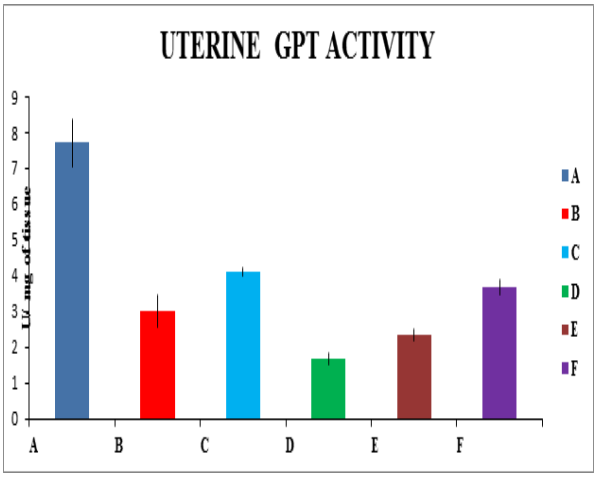

Fig 5: Graphical Representation of Relative changes of Uterine GPT Level of different groups.

f) Uterine GOT Content

Group C rats showed significant decrease in GOT activities of the uterine tissue than Group A and Group D rats showed significant decrease in GOT activities in the uterine tissues in compare to Group C animals. Vitamin E treatment restored the value towards normal when compared with Group D rats.

Table 5: Uterine GOT Content Level(U/mg of tissue).

A	57.41 ± 3.1
B	37.01 ± 1.52
C	49.81 ±1.29
D	29.71±1.58
E	41.18±0.85
F	46.68 ± 0.95

Values are expressed as means ±SEM, N=6

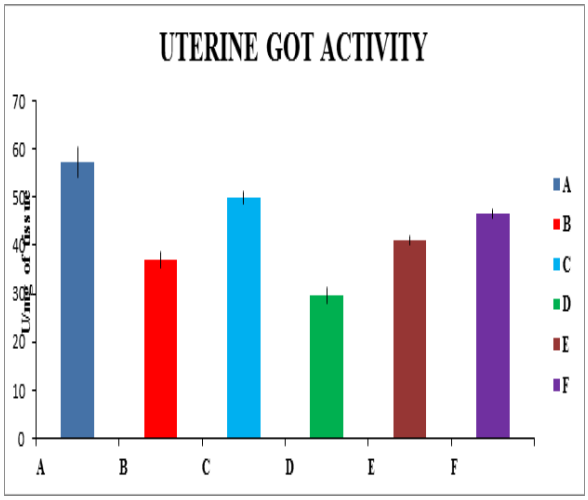

Fig 6: Graphical Representation of Relative changes of Uterine GOT Level of different groups.

g) Uterine ALP content

Here, the Group C rats showed significant decreased in ALP activities in uterine tissue than Group A.Again, Group D rats also showedsame significant decrease in ALP activities than Group C, Where, Vitamin E supplement restored the values towards normal in compare to Group D animals.

Table 5: Uterine ALP Content Level(KA Units/mg of tissue)

A	58.50± 1.63
B	18.25± 0.85
C	44.31 ± 1.07
D	11.68±0.60
E	22.80± 0.97
F	35.66±1.96

Values are expressed as means ±SEM, N=6

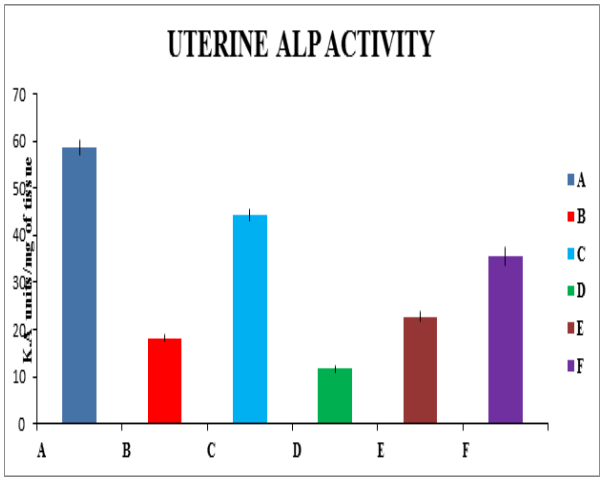

Fig. 7: Graphical Representation of Relative changes of Uterine ALP Level of different groups.

DISCUSSION

The most significant observation of the study was that vitamin E could prevent ethanol induced biochemical changes of liver toxicity in bilateral ovariectomized female rats by altering activities of different marker enzymes of hepatocellular injury i.e. AST,ALT and Serum Alkaline phosphatase activity. Earlier it has been well documented that both enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are considered among the most sensitive markers of hepatocellular injury.[11]

Alkaline phosphatases are a group of enzymes found primarily in the liver (isoenzyme ALP-1) and bone (isoenzyme ALP- 2). There are also small amounts produced by cells lining in the intestine (isoenzyme ALP -3), the placenta and the kidney (in proximal convoluted tubule).[12] It is a marker enzyme for plasma membrane and endoplasmic reticulum of the tissue studied.[13] It is often employed to assess the integrity of plasma membrane, since it is localized predominantly in the microvilli of the bile canaliculi located in the plasma membrane of hepatocytesALP hydrolyses phosphate monoesters, the hyperproduction of the enzymecould constitute a threat to the life of the cells that are dependent on a variety of phosphate esters for their vital processes as it may lead to indiscriminate hydrolysis of phosphate ester metabolite of the liver, an important biochemical symptom of cytolysis.[14,15] Thus, the serum ALP is a measure of the integrity of the hepatobiliary system and the flow of bile into the small intestine.[16] Bilateral Ovariectomized rats treated with ethanol shoed more pronounced hepatic damage than bilateral ovariectomized rats alone. Results indicate that Vitamin E supplementation could blunt ethanol induced increase in different hepatic enzymes studied, suggesting that Vitamin E has protective influence against ethanol induced hepatocellular damage and degenerative changes.

To ascertain whether ethanol apart from causing hepatic toxicity parallel progresses more regression ofuterine

protein content and reduction of different enzyme activitiesi.e. uterine alkaline phosphatases, uterine Ala – AT and Asp-AT activity in bilateral ovariectomized rats. Possibly this is due to estrogen deficiency in the bilateral ovariectomized rats.[17] Ethanol fed ovariectomized rats showed more uterine regression when compared with ovariectomized rats alone. Uterus is an active site of protein biosynthesis and estrogen metabolism whose function is modulated by ovarian hormones i.e. estradiol and progesterone.[18] Texture of uterine epithelium and uterine stroma as well as number of uterine glands decrease in ovariectomized rats due to insufficiency of estrogen titer.[19] Reference suggests that estrogen probably decreases gluconeogenesis and proteolysis by decreasing the activities of Ala-AT and Asp-AT in uterus of ageing female rats.[20] Ethanol fed ovariectomized rat showed much deterioration of uterine tissue metabolism as reflected in their different enzyme activities in the present study. Significant uterine recovery was observed in Vitamin E supplemented rats which established that vitamin E could protect ethanol induced uterine damage in ovariectomized rats.

Taken together, this study suggests that vitamin E has hepato protective as well as utero protective role in ethanol fed ovariectomized rats.

CONCLUSION

Ethanol increases SGPT, SGOT and Serum ALP activity in non-ovariectomized and ovariectomized rats but decreases uterine GPT, GOT, ALP activity along withtissue protein content in thoseanimals. The Vitamin E supplementation restored the enzyme activities and uterine protein content towards normal level in ethanol fed alone andethanol fed ovariectomized rats. It is indicative that vitamin E has hepato protective as well as utero protective role in ethanol fed ovariectomized rats.

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