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

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## ABSTRACT

Vitamin E and Aloe vera have been attributed with a plethora of health promoting actions. The purpose of this study was to evaluate the hepatic and utero protective role of Aloe vera and vitamin E in ethanol fed bilateral ovariectomized rats. Experiments were carried out in different groups – a. Sham operated control (SOC), b. Control (c) + E (Ethanol), c. Ovariectomized (O), d. O+E, e. O + E + Vit-E, and f. O + E + A (Aloe Vera). In this study of consicutive 30 days, significant hepatic damage was observed in bilateral ovariectomized rats treated with ethanol (0.5ml, 15% v/v /100g b.wt. /day) as reflected in their altered serum AST(SGOT), ALT(SGPT) and ALKP activity. Also, uterine regression was confirmed in these group of rats by significant lowered uterine protein content and decreased activity of uterine AST, ALT, and ALKP activity. Vitamin E supplementation (10mg in 0.5ml olive oil /100g b. wt. /day), apart from it's unique influence in preventing hepatic damage, also could prevent ethanol induced uterine regression in ethanol fed bilateral ovariectomized rats. Comparatively Aloe Vera supplementation (30mg in 0.5ml distilled water /100g b. wt. /day) also prevents hepatic damage lesser than vitamin E but could prevent ethanol induced uterine regression in ethanol fed bilateral ovariectomized rats quite greater than Vitamin E.

**KEYWORDS:** Aloe vera, Ethanol, Ovariectomy, Vit-E.

## 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 more than moderate amounts of alcohol leads to increased accumulation of acetaldehyde, in part due to reduced activity of alcohol dehdrogenase (ADH). Acetaldehyde dehydrogenase (ALDH) then catalyzes the 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 suscetibility 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 the prevention of the damages by anti-inflammatory and antioxidant compounds of Aloe vera and its comparison with vitamin E following the studies of Serum AST, ALT & ALKP activities and total protein content, ALKP, AST & ALT activities in uterine tissues.

## MATERIAL AND METHODS

### A) Experimental Design

Experiments were carried out with adult female albino rats weighing 120-130g. They were kept into 6 groups having 6 animal each as follows.

| Group | Treatments | No. of Female 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) + EtOH+ Vit-E | 6 |
| F | Bilateral ovariectomized (OVX) + EtOH+ Aloe vera | 6 |

All animals were pair-fed and the composition of diet was same (as available standard rat diet).[6] Water was given *ad libitum*. Animals were maitained under standard laboratory conditions (temp. 25+- 2'C, 12/12 hr darkandlight, relative humidity 40 - 60 %). Under light ether anesthesia, bilateral (dorsolateral) ovariectomy were performed in rats of group C, D, E and F whereas the rats of group A and B were sham-operated. After the surgical convalescence, rats of group B, D, E and F were fed 0.5ml15% (v/v) ethanol/100g body weight/day for 30 days by gavage technique.[7] Group E rats were supplied with vitamin E dissolved in olive oil (10mg/0.5ml /100g body weight) for 30 days.[8] The Group F rats were supplemented with lyophilized Aloe vera extract (dissolved in absolute alcohol first and after evaporation with distilled water) at a dose of 30mg /0.5ml /100g body weight/day for 30 days consicutively [9]. Animal experiments were performed following the ethical guidelines under IAEC, The University of Burdwan, Burdwan, West Bengal, India.

### B) Collection of Blood

At the end of 30 days, blood from rats of different groups were taken by syringe directly through Cardiac puncture. All sampling were performed between 1 pm to 3 pm in order to avoid diurnal variation on the parameter observed in the study.[10]

### C) Preparation of Tissue Extracts

The abdomen was opened, uterus was quickly removed, weighted 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, for estimation of protein and activities of uterine alkaline phosphatase, AST and ALT.[11]

### D) Measurement of Different Parameters

SGPT, SGOT and ALKP were measured by standard kits,[12,13] where protein content was measured by Lowry et. al, 1951.[14]

### 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

In the present study, the results showed that on ethanol administration there was a significant increase in the activity of SGPT in ovariectomized rats than the control group. This effect of ethanol was blunted significantly better by Aloe vera than vitamin E supplementation. In group A and C animals showed normal values though group C animals have showed increased SGPT activity. SGPT activity of Group D animals were markedly increased among other groups and was significantly reduced in group E and group F animals both, though group F showed more protection than group E, indicating the better protection in SGPT by Aloe vera (Table 1 next, Figure 1).

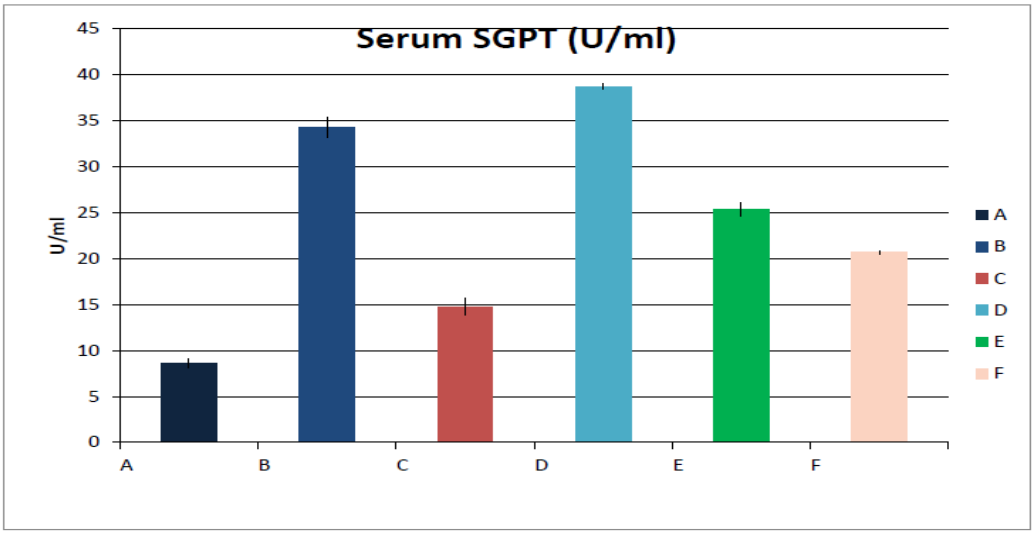


**Fig. 1: Graphical representation of serum SGPT level of different groups of rats.**

**Serum SGOT (AST) profile**

In the present study, the results of SGOT also showed the similar pattern more or less alike the SGPT. Here, the hepato toxic effect of ethanol was blunted significantly by vitamin E and Aloe vera supplementation both. But the Aloe vera supplementation showed also the better protection in SGOT activities than in Vit-E. like SGPT (Table 1 next, Figure 2).

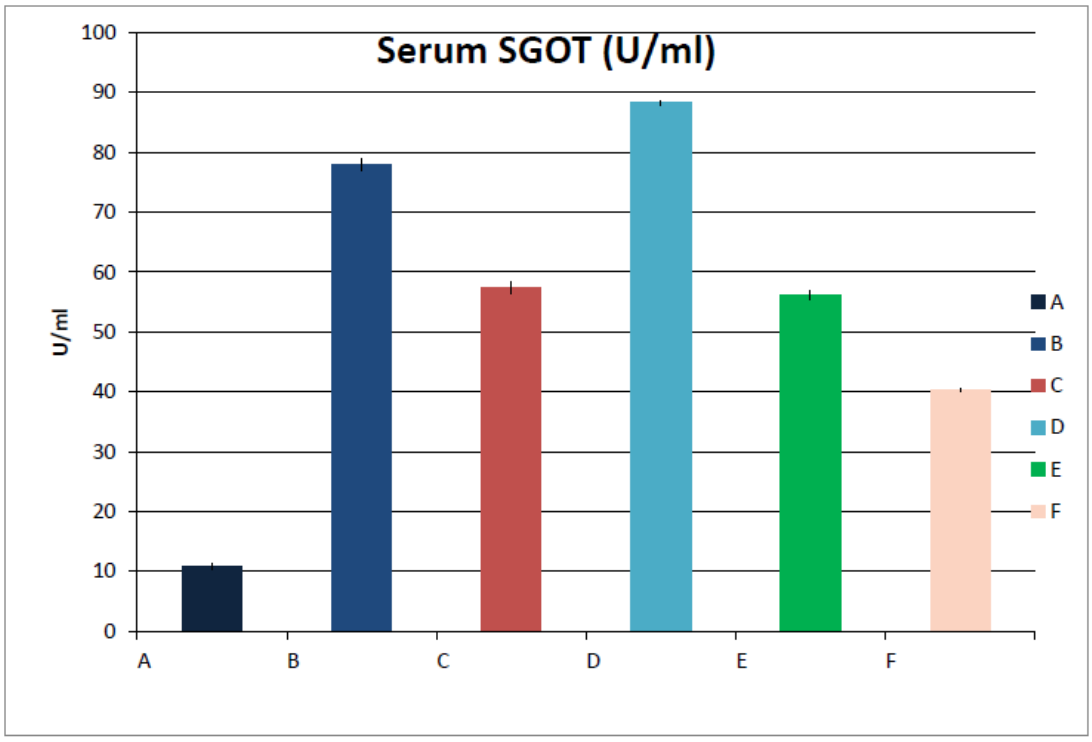


**Fig. 2: Graphical representation of serum SGOT level of different groups of rats.**

**C) Serum ALKP/ALP profile**

The studies of serum ALKP/ALP inthe present work, showed that on ethanol administration, there was a significant increase in the activity of ALKP in ovariectomized rats than the control and bilateral ovariectomized alone groups. The marked significant rise of serum ALKP level was noticed in group D, which was ethanol fed bilateral ovariectomized group. This effect of ethanol was blunted significantly by vitamin E better than Aloe vera group. Inspite, ALKP values were higher in group E and F when compared with control and overiectomized alne group (Table 1 next, Figure 3).

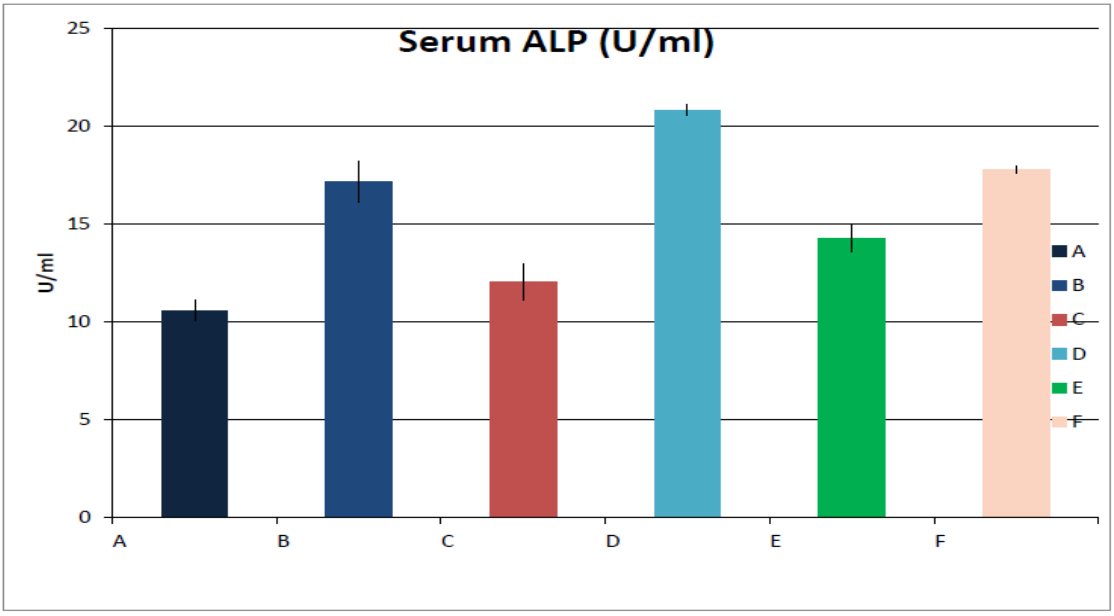


**Fig. 3: Graphical representation of serum ALP level of different groups of rats.**

**Table 1: Values of SGPT, SGOT and Serum ALKP in different Groups of rats. P<0.05 was considered significant.**

| GROUP | SGPT (U/ml) | SGOT(U/ml) | Serum ALP (K.A. unites) |
|---|---|---|---|
| A | 8.63 ± 0.5 | 10.79 ± 0.2 | 10.57 ± 0.2 |
| B | 34.26 ± 1.06 | 78.03 ± 0.5 | 17.16 ± 0.5 |
| C | 14.75 ± 0.93 | 57.37 ± 2.7 | 12.02 ± 0.2 |
| D | 38.71 ± 0.3 | 88.28 ± 1.3 | 20.82 ± 0.1 |
| E | 25.35 ± 0.7 | 56.21 ± 0.2 | 14.27 ± 0.5 |
| F | 20.69 ± 0.2 | 40.27 ± 0.5 | 17.78 ± 0.4 |
| A vs B | P < 0.02 | P < 0.001 | P < 0.001 |
| A vs C | P < 0.001 | P < 0.001 | P < 0.02 |
| C vs D | P < 0.001 | P < 0.001 | P < 0.001 |
| C vs E | P < 0.05 | P > 0.05 | P < 0.02 |
| C vs F | P < 0.01 | P < 0.01 | P < 0.001 |
| D vs E | P < 0.001 | P < 0.001 | P < 0.001 |
| D vs F | P < 0.001 | P < 0.001 | P < 0.01 |
| E vs F | P < 0.001 | P < 0.001 | P < 0.01 |

Data are mean ± SEM (n=6)
P value indicate significance level The numbers indicate:
Group A = Control
Group B = Control + Alcohol Group C = Ovx
Group D = Ovx + EtOH
Group E = Ovx + EtOH + Vitamin E Group F = Ovx + EtOH + Aloe vera

**D) Uterine GPT Profile**

In present study ethanol administration causes decrease in uterine GPT enzyme activity both in Group B and Group C animals. The effect of ethanol was cured significantly by Aloe vera supplementation, though was better than vit-E in this dose and duration. Group C animals have showed decreased GPT activity as compared with Group A where GPT activity of Group D animals showed markedly decreased among all the animal groups. Group F animals showed more restoration of the value than Group E (Table 2 next, Figure 4).

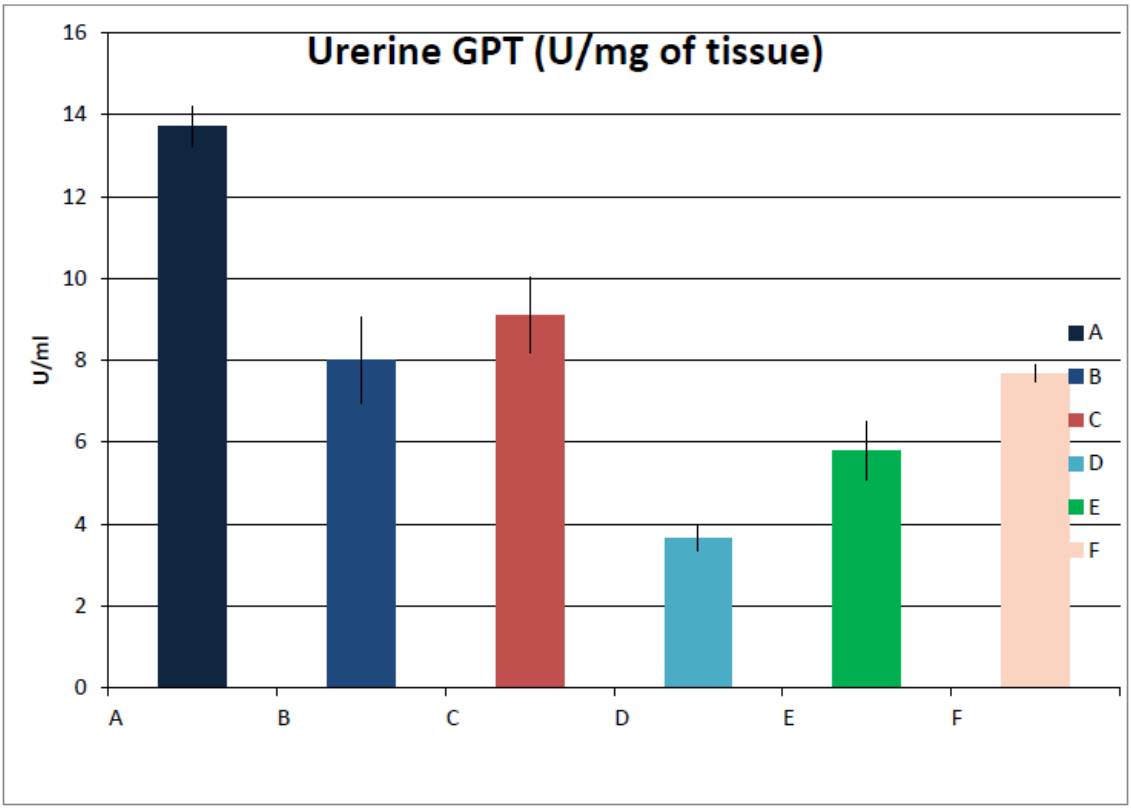


**Fig. 4: Graphical representation of Uterine GPT level of different groups of rats.**

**E) Uterine GOT Profile**

In present study ethanol administration causes decrease in uterine GOT enzyme activity both in Group B and Group C animals. The effect of ethanol was cured

significantly by Aloe vera ; comparatively which is higher than vit-E. Group C animals have showed decreased GOT activity as compared with Group A. GOT activity of Group D animals showed markedly decreased. Where, Group F animals showed more restoration of the value than Group E; towards normal level when compared with Group D animals. (Table 2 next, Figure 5).

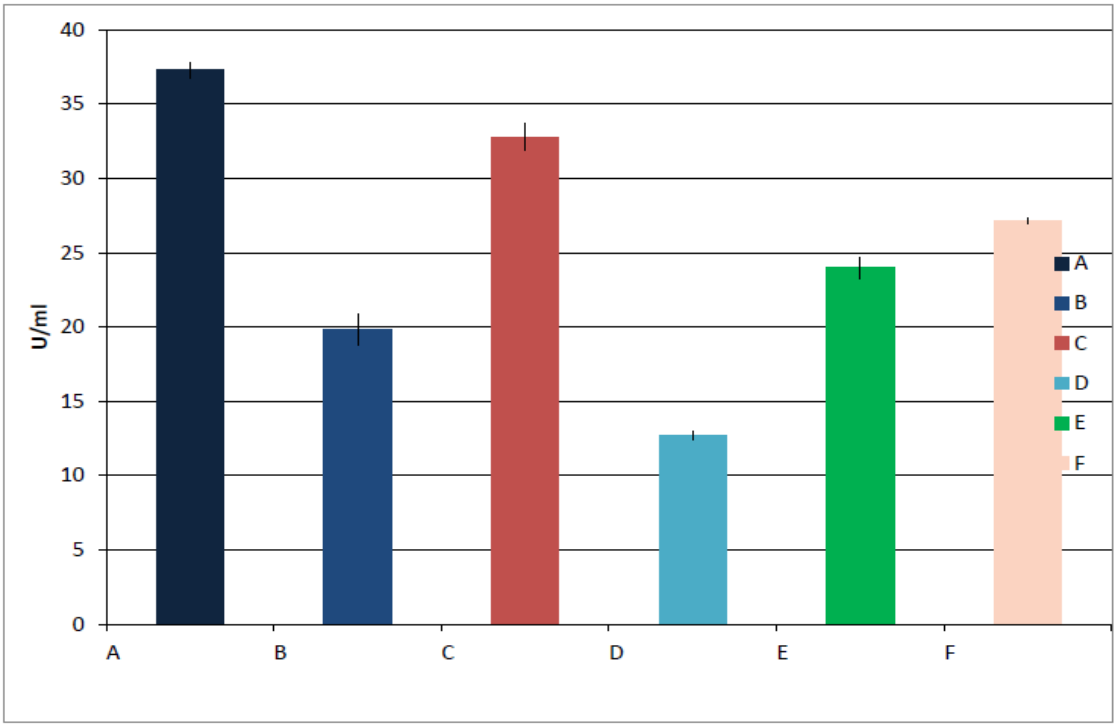


**Fig. 5: Graphical representation of Uterine GOT level of different groups of rats.**

**F) Uterine Alkaline Phosphatase (ALKP/ALP) profile**

Alike the above parameters in uterine tissues, ethanol administration caused decrease in uterine ALKP enzyme activity both in Group B and Group C animals. The effect of ethanol was cured significantly by Aloe vera, comparatively which is higher than vit-E supplemented group. Group C animals have showed decreased ALKP activity when compared with Group A. The ALP activity of Group D animals showed markedly decreased thanall other groups. Group F animals showed more restoration of the value than Group E ; towards normal level when compared with Group D animals. (Table 2 next, Figure 6).

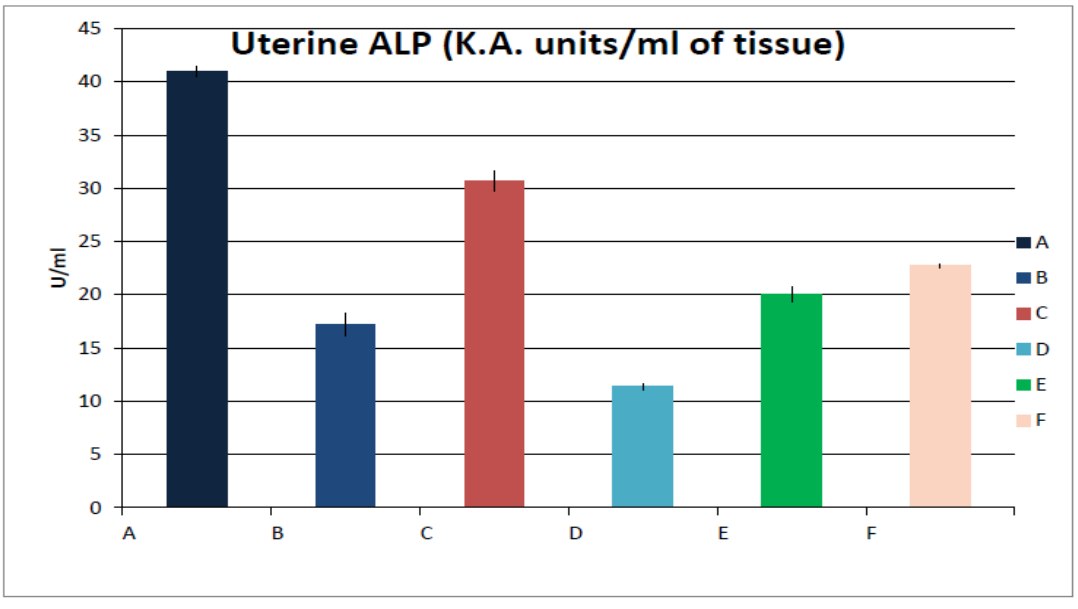


**Fig. 6: Graphical representation of Uterine ALP level of different groups of rats.**

**Table 2: Value of Uterine GPT, GOT and ALKP in different groups of rats. P<0.05 was considered significant.**

| GROUP | Uterine GPT (U/ml) | Uterine GOT(U/ml) | Uterine ALP (K.A. unites) |
|---|---|---|---|
| A | 13.71 ± 0.6 | 37.28 ± 0.5 | 41.01 ± 0.9 |
| B | 8.01 ± 0.2 | 19.83 ± 0.3 | 17.25 ± 0.8 |
| C | 9.11 ± 0.5 | 32.81 ± 1.3 | 30.68 ± 0.3 |
| D | 3.66 ± 0.6 | 12.68 ± 0.2 | 11.37 ± 0.1 |
| E | 5.79 ± 0.3 | 23.97 ± 0.3 | 20.03 ± 0.5 |
| F | 7.68 ± 0.5 | 27.18 ± 0.7 | 22.72 ± 0.2 |
| A vs B | P < 0.001 | P < 0.001 | P < 0.001 |
| A vs C | P < 0.001 | P < 0.001 | P < 0.001 |
| C vs D | P < 0.001 | P < 0.001 | P < 0.02 |
| C vs E | P < 0.001 | P < 0.001 | P < 0.001 |
| C vs F | P < 0.001 | P < 0.001 | P < 0.001 |
| D vs E | P < 0.02 | P < 0.02 | P < 0.001 |
| D vs F | P < 0.001 | P < 0.001 | P < 0.001 |
| E vs F | P < 0.05 | P < 0.01 | P < 0.01 |

Data are mean = SEM (n=6)
P value indicate significance level The numbers indicate:
Group A - Control
Group B = Control + Alcohol Group C = Ovx
Group D = Ovx + EtOH
Group E = Ovx + EtOH + Vitamin E Group F = Ovx + EtOH + Aloe vera

**G) Uterine Protein Content**

In the present study, ethanol administration caused significant decrease in uterine protein content than control and ovariectomized group alone. The effect of ethanol was cured significantly by Aloe vera, comparatively which is higher protective than vit-E. Group C animals have showed decreased protein content as compared with Group A. Where, the protein content of Group D animals showed markedly decrease than all other animals. The Group F animals showed more restoration of the value than Group E ; towards normal level when compared with Group D animals. ( Table 3, Figure 7).

**Table 3: Value of Uterine Protein (mg/gm of tissue) in different groups of rats. P<0.05 was considered significant.**

| GROUP | Uterine Protein (mg/gm of tissue) |
|---|---|
| A | 18.01± 0.2 |
| B | 9.81 ± 0.4 |
| C | 10.4 ± 0.2 |
| D | 3.33 ± 0.5 |
| E | 7.01 ± 0.7 |
| F | 8.98 ± 0.3 |
| A vs B | P < 0.001 |
| A vs C | P < 0.001 |
| C vs D | P < 0.001 |
| C vs E | P < 0.001 |
| C vs F | P < 0.05 |
| D vs E | P < 0.001 |
| D vs F | P < 0.001 |
| E vs F | P < 0.02 |

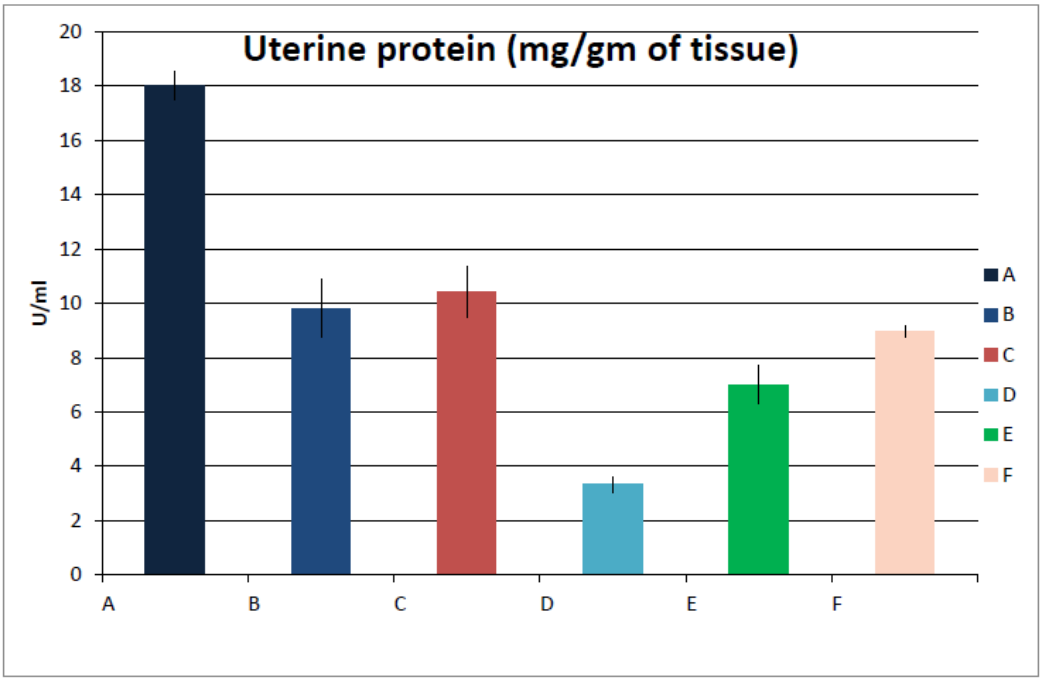


**Fig 7: Graphical representation of Uterine Protein level of different groups of rats.**

Data are mean + SEM (n=6) The numbers indicate:
Group A= Control
Group B = Control + Alcohol Group C = Ovx
Group D= Ovx + EtOH
Group E = Ovx + EtOH + Vitamin E Group F = Ovx + EtOH + Aloe vera

## DISCUSSION

Vitamin E and Aloe vera have been attributed with health promoting actions. The purpose of this comparative study was to evaluate the hepatic and utero protective role of Aloe vera and vitamin E in ethanol fed bilateral ovariectomized rats. The most significant observation of the present study was that Vitamin E and Aloe vera could prevent ethanol induced biochemical changes of liver toxicity in bilateral ovariectomized female rats by altering activities of different marker enzymes of hepatocellular injury viz. AST, ALT and serum alkaline phosphatase (ALKP/ALP) activity. Earlier it has been well documented that both the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are considered among the most sensitive markers of hepatocellular injury.[15]

Again, the ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum of the tissue studied.[16] It is often employed to asses the integrity of plasma membrane, since it is localized predominantly in the microvilli of the bile canaliculi, located in the hepatocellular plasma membrane.[17] ALP hydrolyses phosphate monoesters, the enzyme hyperproduction could constitute a threat to the life of the cells that are dependent on a variety of phosphate esters for their vital process, as it may lead to indiscriminate hydrolyses of phosphate ester metabolite of the liver , an important biochemical symptom of cytolysis.[18] In this study, bilateral ovariectomised rats treated with ethanol showed more pronounced hepatic damage than bilateral ovariectomized rats only. Results indicate that Vitamin E and Aloe vera supplementation could blunt Ethanol-induced increase in different hepatic enzymes, suggesting that both Vitamin E and Aloe vera have protective influence against ethanol induced hepatocellular damage and degenerative changes. Earlier we have studied the protective role of Vit E against hepatocellular damage in ethanol fed bilateral ovariectomized rats.[19]

To ascertain, whether ethanol apart from causing hepatic toxicity could parallelly progresses more uterine regression in bilateral ovariectomized rats, uterine protein content, reduced uterine ALP, ALT and AST activity were measured in different groups of rats. In this study as expected bilateral ovariectomized rats showed uterine regression following reduced uterine protein content and decreased enzyme activities possibly due to estrogen insufficiency.[20] though, ethanol fed ovariectomized rats showed more uterine regression when compared with the ovariectomized rats only.

It is very welknown that uterus is an active site of protein biosynthesis and estrogen metabolism whose function is modulated by ovarian hormones estradiol and progesterone [21]. Texture of uterine epithelium and uterine stroma as well as number of uterine glands are decreased in ovariectomized rats due to insufficiency of estrogen titer. The previous studies suggest that estrogen probably regulates gluconeogenesis and proteolysis by modulating the activities of ALT and AST in uterus of aging female rats.[20] As in this study, ehanol fed ovariectomized rats

showed much deterioration of uterine tissue metabolism as reflected in their different enzyme activities and significant uterine recovery was observed in Vitamin E and Aloe vera supplemented rats which established that both Vitamin E and Aloe vera could protect ethanol induced uterine damage in ovariectomized rats. Taken together this study suggest that both Aloe vera and Vitamin E have hepato protective as well as utero protective role in ethanol fed ovariectomized rats, though the Aloe vera showed better protection in a dose and duration dependent manner.

## CONCLUSION

Ethanol increases SGPT, SGOT and serum ALP activity where it decreases uterine GPT, GOT, ALP activity and protein content both in control and ovaiectomised group of rats. Both vitamin E and Aloe vera restores the enzyme activity and uterine protein content towards normal level in ethanol fed ovariectomized rats. Comparatively, in this study the effect of Vitamin-E is better in serum enzymes level (hepatic markers), whereas Aloe vera could restore both the uterine enzyme and protein contents much better than vitamin E. Taken together this study suggest that both Aloe vera and vitamin E have hepato protective as well as utero protective role in ethanol fed ovariectomized rats.

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## REFERENCES

1. Dolganiuc A. Alcohol & Viral Hepatitis: Role of Lipid Rafts. Pub Reader, 2015; 37(2): 299-309.
2. Cederbaum I.A. Alcohol Metabolism. Pub Reader, 2012 Nov; 16(4): 667- 685. doi:10.1016/j.cld.2012.08.002.
3. Seitz H.K., Egerer G., Simanowski U.A., Waldherr R., Eckey R., Agarwal D.P., Goedde H.W., Von Warburg J.P.Human gastric alcohol dehydrogenase activity: effect of age, gender and alcoholism. Gut, 1993; 34: 1433-1437.
4. Baraona E., Abittan C.S., Dohmen K., Moretti M., Pozzato G., Chayes Z.W., Schaefer C. Lieber C.S.Gender differences in pharmacokinetics of alcohol. Alcohol Clin Exp Res, 2001; 25: 502-507.
5. Sejal B. Doshi, Ashok Agarwal. The role of oxidative stress in menopause. J. Midlife Health, 2013 Jul-Sep; 4(3): 140–146. doi: 10.4103/0976-7800.118990.
6. Mathur J N. ICMR Bulletin, Natinal Centre for Laboratory Animal Sciences (NCLAS).A Profile, Published By The Indian Council of Medical Research, New Delhi, 2004; 34 (4): 21-28.
7. Luma P and Vorne M. Changes in the hepatic microsomal enzyme activity during long- term ethanol feeding in rat. Acta Pharmacol. Toxicol, 1876; 138: 260-266.
8. Singh M. Alteration in Calcium homeostasis in rat erythrocytes with atrazine treatment: positive modulation by Vitamin E. Molecular and Cellular Biochemistry, 2010; 340(1-2): 231-238.
9. Rajasekaran S, Ravi K, Sivagnanam K, Subramanian S. Beneficial effects of aloe vera leaf gel extract on lipid profile status in rats with streptozotocin diabetes. Clin. Exp. Pharmacol. Physiol, 2006; 33(3): 232-7.
10. Ida B. Andersen, Claus L. Brasen, Henry Christensen, Lene Noehr-Jensen, Dorthe E. Nielsen, Ivan Brandslund, Jonna S. Madsen. Standardised Resting Time Prior to Blood Sampling and Diurnal Variation Associated with Risk of Patient Misclassification: Results from Selected Biochemical Components. PLOS ONE, 2015; 10(10): 1-9.
11. Koyama, L., Tsugikazu, K., Yoshikatsu, S., Munetsugu , K. A possible mechanism for the changes in hepatic and intestinal alkaline phosphatase activities in bile duct ligated rats and guinea-pigs. Biochemical Biophysical Acta, 1983; 760: 169-174.
12. Tietz NW. Electrolytes. In: Tietz NW, editor. Fundamentals of Clinical Chemistry. Philadephia: W.B. Saunders, 1970; 630-631.
13. King EJ and Jegatheesan KA. A method for the determination of tartrate labile, prostatic acid phosphatase in serum.J. Clin. Pathol, 1959; 12(1): 85 - 89.
14. Lowry OH. Rosebrough, NJ. Farr AL and Randall R.J. Protein measurement with the Folin phenol reagent. J. Biol., 1951 Nov; 193(1): 265-75.
15. Mitchell R. McGill. The past and present of serum aminotransferases and the future of liver injury biomarkers. EXCLI J., 2016; 15: 817–828.
16. Wright PJ and Plummer DT. The use of urinary enzyme measurement to detect renal damages caused by nephrotoxic compounds. Biochem. Pharmacol, 1974 Jan 1; 23(1): 65-73.
17. Akanji MA., Olagoke OA and Oloyede OB. Effect of chronic consumption of metabisulphite on the integrity of the kidney cellular system. Toxicol, 1993; 81: 173 - 179.
18. Butterworth P J and Moss DW. The effect of urea on human kidney alkaline phosphatase, Biochem. J., 1966; 99: 9-10.
19. Banerjee M, Roy A, Poley P, Pradhan N. A Study To Evaluate Hepato and Utero Protective Role of

Vitamin E in Ethanol fed Overiectomized Rats. EJBPS, 2020; 7(6).
20. Moorthy K, Sharma d, Basir S.F, baquer N.Z. Administration of estradiol and progesterone modulate the activity of antioxidant enzyme and amino transferase in naturally menopausal rats. Experimental Gerontology, 2005; 40(4): 295-302.
21. Mukherjee S, Nag M,, Nayyar T, Maitra I, Chakrabarti P and Dasgupta P . Hydrogen peroxide formation and lipid peroxidation in rat uterus effect of hormones and vitamin E. Journal of Biosciences, 1986; 10: 311–317.