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EFFECT OF ETHYL ALCOHOL CONSUMPTION ON PREGNANCY IN SWISS ALBINO LABORATORY MOUSE

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ABSTRACT : This study was attempt to identify the effect of ethyl alcohol on weight change, maternal death, pregnancy termination, Percentage of Survival rate, developmental toxicity and pre-post implantation loss. Fifteen female Swiss strain mice, 10 weeks old (20-30 gm) were used in this study. Mice were housed in group of 4-5. Animal were sustained on pelleted food as diet and water ad libitum. All the treatments were given intraperitonealy at the volume 0.040ml in concentration of 40%, 60% and 80% with controls from 6th to 18th day of gestation and mice were sacrificed by cervical dislocation. The animal model based research result showed that the significant decrease in the body weight of mice treated with 60% and 80% of ethyl alcohol. While the mice treated with 40% was not showed significant change in the body weight with respect to controls. The persistent result revealed that high dose (80%) of ethyl alcohol treatment caused death of 60% mice on gestational day 14-15 while in other 40% treated mice the pregnancy were terminated. The result identified that number of live blastocysts were significantly decreased in 60% ethyl alcohol treated mice compared with the controls. The study indicated that alcohol consumption can decrease the uterine receptivity and effect the pre-post implantation loss resulting in pregnancy failure also can be the chiefest underlying cause of infertility. Therefore the study is suggestive about the persistency of alcohol which is detrimental during pregnancy.

Key words : Ethyl alcohol, implantation, mice, resorption, toxicity.

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INTRODUCTION

Currently the numerous myth has developed to consuming alcohol can prevent SARS-COV-2. Several studies reported from different countries (Below) (Sunday Times, 2020). Also in India, the myths spread among many people about drinking of alcohol may destroy the virus in human body. Chronic consumption of heavy alcohol can decrease the immunity to viral as well as bacterial diseases (Szabo and Saha, 2015; Barr *et al*, 2016).

Pregnancy is the state of carrying a developing embryo or fetus inside a woman and just over the period of 40 weeks. Pregnancy can occur by sexual intercourse of a male and female. There are lot of factors can affect the chances of conception such as age, timing, and frequency of intercourse, distance of time trying to conceive, body weight and diet pattern, Medication such as antianxiety drug, antitoxins, pain reliever and other drugs used to treat chronic disorders may cause sometimes infertility. Other causes of infertility are Excessive smoking, drinking and recreational drugs, Exposure of chemicals, heavy metals, pesticides, etc.

Higher than moderate amounts of alcohol consumption during gestation is associated with the infertility, malformation, birth abnormalities, miscarriage and abortion especially, identifying conception period and intercourse within that time can considerably become greater chances of conception probabilities (Hilgers *et al*, 1992; Gnoth *et al*, 2005).

Consumption of excessive amount of alcohol throughout the gestational periods is injurious and severely harmful for healthy development of embryo with continued growth and development after birth (Shrestha and Singh, 2013; Ding *et al*, 2015). Intake of huge amounts of alcohol for the duration of gestation often results are malformations, birth defects including mental retardation

in offspring called as fetal alcohol syndrome shortly known as FAS (Clarren and Smith, 1978).

Presently, alcoholic beverages are very well-known and recognized teratogen for the embryo resulting in growth delays, birth defects, implantation loss, physical and facial anomalies, neurological defects including intellectual disabilities and behavioral problems referred as fetal alcohol spectrum disorders (FASD) (Coriale *et al*, 2013). Toxic effect of alcohol during progesterone or pregnancy can causes fetal alcohol spectrum disorder including mental and behavior disabilities, nervous abnormalities and developmental retardation during prenatal and postnatal development of fetus (Mattson *et al*, 2011). Teratogenicity is a condition when any synthesized drug which produce variations and abnormalities in the development of embryo. The transfer of drug occurs through the placenta during pregnancy through which the essentials nutrients required for the normal development of fetus.

Ethanol or ethyl alcohol also well known as alcohol is produced through the fermentation of yeast, starches, and sugar. A large amount of alcohol use during pregnancy is associated with the malformed birth defects, as well as increased the chances of miscarriage and infertility (Armstrong *et al*, 1992). Heavy consumption of alcohol during gestation is also linked with low birth weight, preterm birth, implantation loss, stillbirth and infant mortality (Strandberg-Larsen *et al*, 2009; O'Leary *et al*, 2012; Tai *et al*, 2016). In 2014, World Health Organization (WHO) is released a report on alcohol and health the report stated that 38.3% of the global population consumed alcohol and in India the amount of alcohol intake is rise between the periods of 2008 - 2012 (World Health Organization, 2014). In India, the majority of the population especially 20-50% of men in comparison to 5% women in most region of the country consume alcohol. This may cause the adverse effect on their reproductive capability as well as pregnancy. Toxicological effect of ethyl alcohol during developmental period of gestation depends upon the time of exposure and the level of dose (Mattson *et al*, 2001).

Based on such harmful effects, the effort was made in present study deals with the ethyl alcohol-induced adverse effects on pregnancy of the experimental animal model that is on the laboratory mouse carried out with the objectives of effect of ethyl alcohol on maternal death, pregnancy termination and percentage of survival rate. Studied bodyweight, developmental toxicity of alcohol in female mice also pre- and post implantation loss Resorption site count, Corpus luteum count, live and dead blastocyst count, in the uteri of the control and alcohol treated mice.

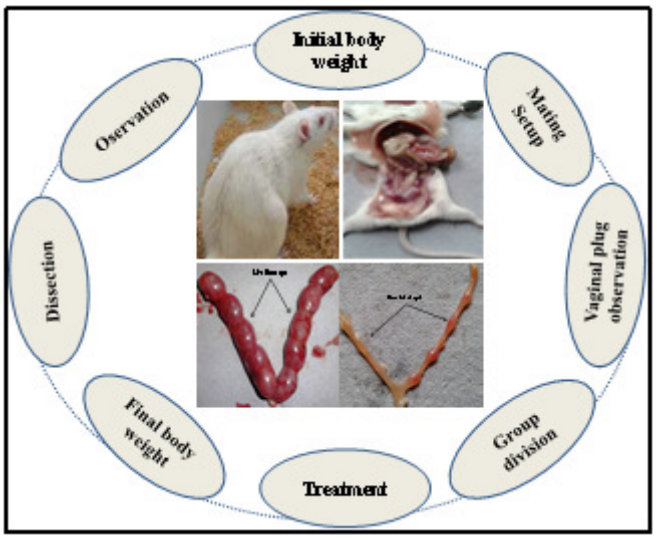

Fig. : Schematic representation of toxicity evaluation of ethyl alcohol on pregnancy.

MATERIALS AND METHODS

Female laboratory mice of Swiss strain were procured from IMS, BHU, at Varanasi and housed in polypropylene cages while rice husk used as the bedding stuff and were kept in the animal house under controlled environment at 22±2ÚC. A 12 h light and 12 h dark cycle was ensured during which they were allowed to acclimatize under optimum feeds and water access for a period of 2 weeks before the commencement of the experiment. Animals were given pelleted food as diet and water ad libitum. Then the experiments were started by using adult female mice of 10 weeks old with initial body weight ranging from 20 to 30 gm.

Mating was placed in an individual cage by taking two virgin females and one male for one week. Following morning the presence of a vaginal plug and sperm in collected vaginal smear was confirmed the conception, and the day was designated as gestation day zero (GD_0).

The second day was calculated as day one of the gestation then the dose of ethanol was started from the 6th (GD6) day of gestation to 18th (GD18) day of gestation period. On the 19th (GD19) day the animals were sacrificed and the abdomen was checked to examine the uterine horn. Animals of GD0 were randomly divided into five different groups and three female mice in each one (n-3) and treated as followed –

Group and percentage of doses

Group (n=3)	**Dose (From 6th to 18th day of gestation)**
G **I**	Control - Untreated
G **II**	Vehicle - treated control
G **III**	40% alcohol-
G **IV**	60% alcohol-
G **V**	80% alcohol-

All the treatments were given intraperitonealy at the volume 0.040 ml. After completion given treatment, the bodyweight of the mice in each group was regularly recorded and sacrificed by cervical dislocation and collected their uterine to study various parameters. Observations of the implantation loss in the Excised uteri were checked in ammonium sulfide solution.

In this study, following parameters were studied: Mating confirmation by assessing presence of vaginal plug, Bodyweight observation, observation of Mother mortality rate, Number of pregnant female, implantation site, dead and live blastocyst will be observed, Number of resorption count, Pre–implantation and post-implantation losses were calculated by using following formulae: 1) Pre–implantation loss = Number of corpus luteum – (number of resorbed implants + number of live implants + number of dead implants) 2) Calculation of Post implantation loss was = Total number of resorbed implants and dead implants.

Statistical calculation

According to the variables the value of mean and standard error of mean were calculated for all and expressed as mean±SD. Comparisons between the initial or final bodyweight were calculated by using Student's t-test while other data were analyzed by using ANOVA followed by Student-Newman-Keul's method. Statistically differences of value at $p<0.05$ were considered significant.

RESULTS

Body weight analysis

Non-significant increases in the body weight were found in control, vehicle treated control and low dose (40%) alcohol-treated animals. However, significant decrease in the body weight were found in 60% and 80% alcohol-treated animals.

Live, dead and resorbed Implantation loss

Intraperitoneal administration of low dose of 40% ethyl alcohol caused non-significant decrease in live and dead implants, compared with controls. While significant decrease in the number of live blastocyst along with significant increase in the dead blastocysts were found in high dose (60%) of ethyl alcohol-treated animals, compared with the controls. There was significant decreased in the number of resorbed implants in both doses (40% and 60%) of ethyl alcohol-treated animals.

Implantation loss

Intraperitoneal administration of high dose (60%) of ethyl alcohol in pregnant mice caused significantly increased number of the pre– implantation loss whereas post–implantation loss was significantly increased at both doses (40% & 60%) of ethyl alcohol, compared with the controls.

DISCUSSION

The present study was carried out to investigate the adverse effects of ethyl alcohol on pregnancy of the laboratory mouse by examining different parameters such as bodyweight, number of dead implants and live implants, pre-implantation loss and post-implantation loss in the uteri of the control and alcohol treated mice.

In the present study, we have found that intraperitoneal administration of ethyl alcohol from GD6 to GD18 caused significant decrease in body weight of mice treated with 60% and 80% of ethyl alcohol, while the mice treated with 40% ethyl alcohol did not show

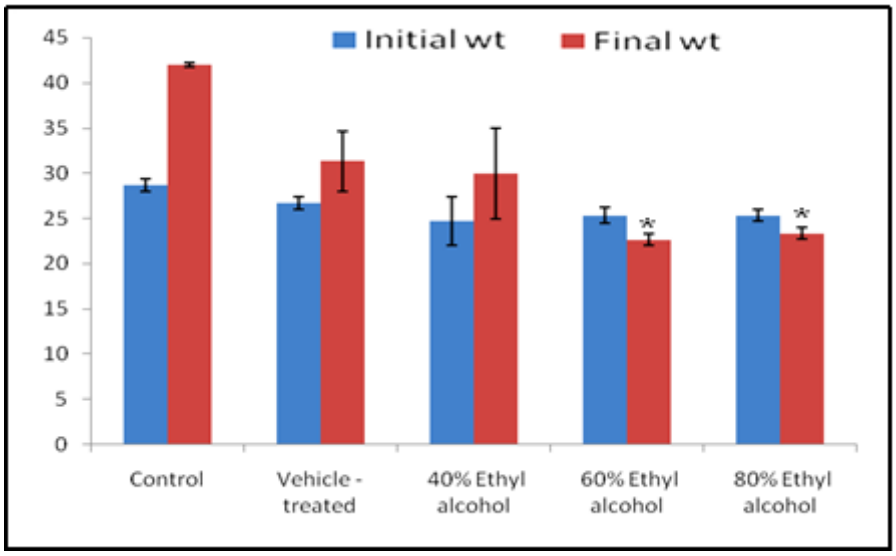

Fig. 1 : Effect of Intraperitoneal administration of alcohol on the body weights. (Values are mean ± SEM), *significantly different from controls (P< 0.05) through Student's t-test.

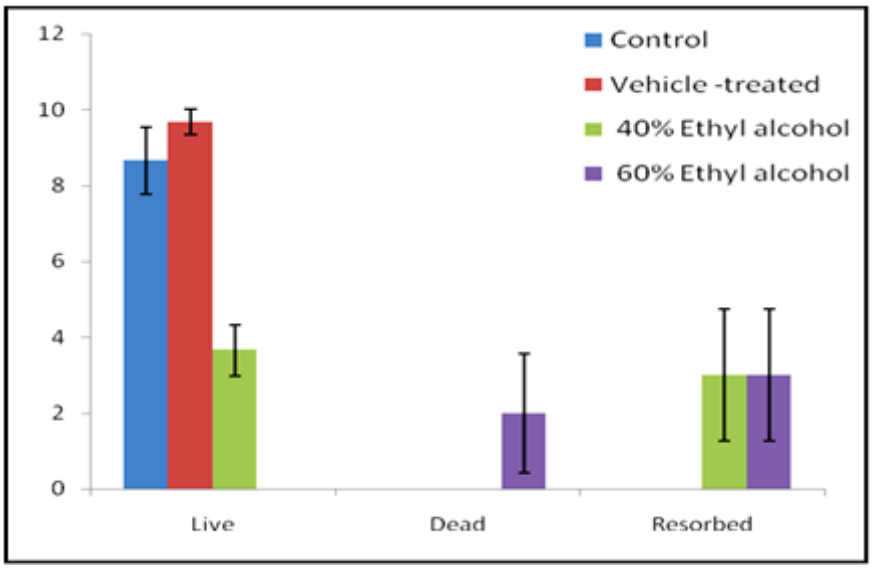

Fig. 2 : Effect of Intraperitoneal administration of alcohol on the live, dead and resorbed implants (Values are mean ± SEM), * significantly different from control and vehicle treated group (P< 0.05) test ANOVA followed via Student Newman-Keul's multiple range method.

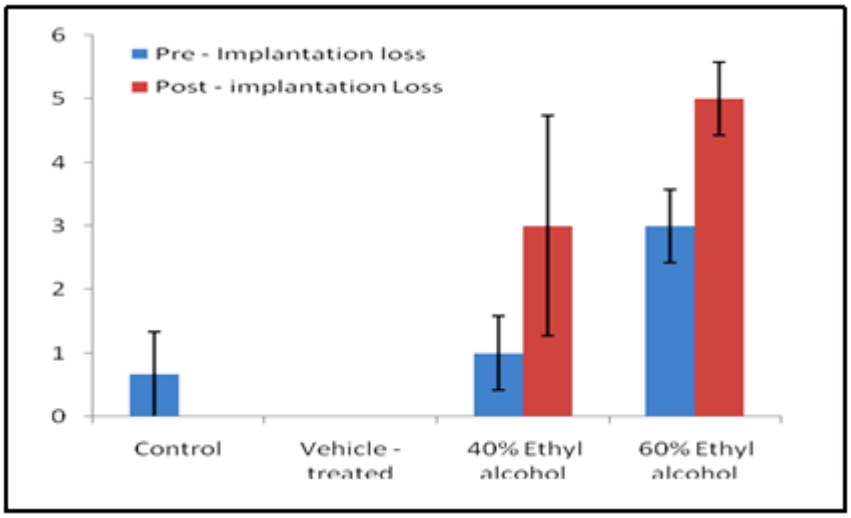

Fig. 3 : Effect of Intraperitoneal administration of alcohol on the pre – implantation and post implantation losses (Values are mean ± SEM), * significantly different from control and vehicle treated group (P<0.05) used by the ANOVA followed by student Newman-Keul's multiple range method.

significant change in the body weight with respect to controls. This supports the observation of researchers, Abel and Dintcheff (1978), who reported that the significant decrease in the bodyweight of the pregnant rats, intubated with alcohol daily throughout gestation with 4.0 or 6.0 g/kg of ethanol. Scientists Gundogan *et al* (2015) also observed that the high-dose of (37%) ethanol the dams exposed to significantly reduced weight gain relative to other groups. Krawczyk *et al* (2016) reported similar finding regarding the body weight of prenatally alcohol exposed animals, showing significant decreases in the bodyweight during first trimester of the pregnancy from GD 0.5 to GD 7.5 treated to 10% (v/v) alcohol with 262.1 mg/day of alcohol.

In our study, the high dose (80%) of ethyl alcohol treatment caused death of 60% mice on (GD 14 to 15) while in other 40% treated mice the pregnancy was terminated. Consistent findings were reported by Wang LL *et al* (2009), who reported that 38.01% v/v ethanol in mice from period GD 6 to GD 15 caused maternal death and spontaneous miscarriage.

In this study the number of live blastocyst were significantly decreased in 60% ethyl alcohol-treated mice compared with the controls.

In 60% ethyl alcohol-treated pregnant mice there were significance increase in dead, resorbed and pre-implantation loss along with significant increase in post-implantation loss. Another research in animal model revealed that ethanol or ethyl alcohol may enhance the chances of resorptions and embryo death in animal model

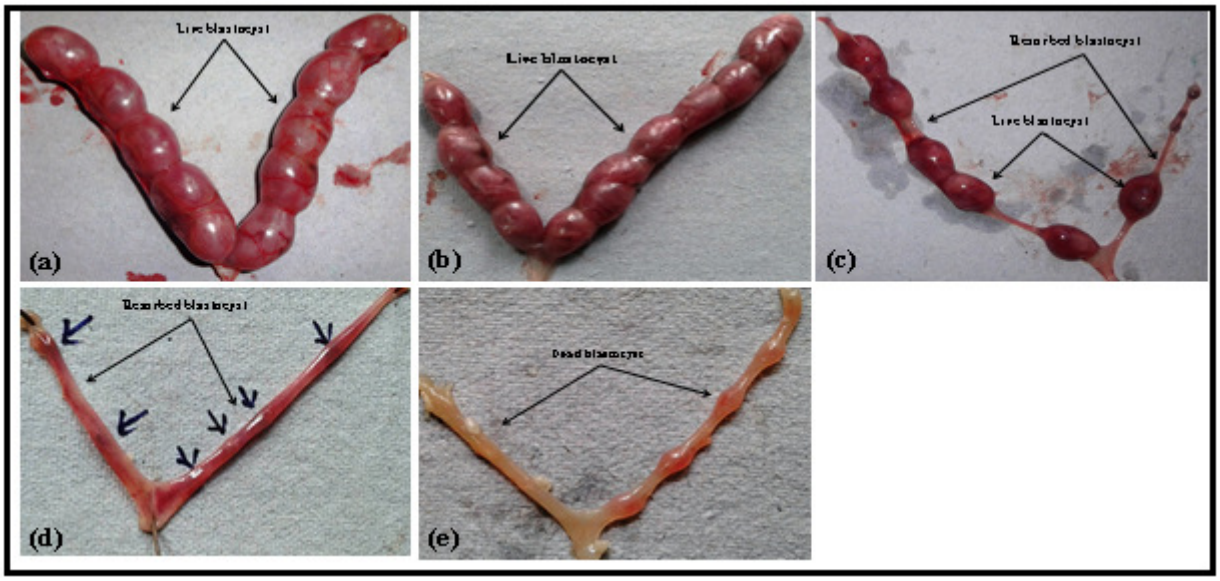

Fig. 4 : Shows Uterine horn of (a) untreated control, (b) vehicle treated control, (c) ethyl alcohol (40%) – treated, (d) ethyl alcohol (60%)-treated, (e) ethyl alcohol (60%)-treated.

Table 1 : Summary of results of the studies.

Group	**Control group**		**Low dose group**	**High dose group**
Treatment material	**Control untreated**	**Distilled water treated**	**40% Ethyl alcohol**	**60% Ethyl alcohol**
Dose per day	**0.040 ml**	**0.040ml**	**0.040ml**	**0.040ml**
Day of gestation when treated	6-18th day	6-18th day	6-18th day	6-18th day
No. of pregnant mice (n=3)	3	3	3	3
Av. range gain in body wt. during pregnancy (gm)	28-50	26-38	22-40	26-22
No. of corpora lutea (Mean)	9.33	9.67	7.67	8
No. of resorptions (Mean)	0 ± 0	0 ± 0	3 ± 1.73*	3 ± 1.73*
No. of live implants (Mean)	8.67 ± 0.82	9.67 ± 0.33	3.67 ± 3.67	0 ± 0*
No. of dead implants (Mean)	0 ± 0	0 ± 0	0± 0	2 ± 1.15*
Pre-implantation loss (Mean)	0.67 ± 0.667	0 ± 0	1 ± 0.577	3 ± 0.578*
Post-implantation loss (Mean)	0 ± 0	0 ± 0	3± 1.73*	5 ± 0.578*

* Significantly different from control and vehicle treated group ($P < 0.05$) used by the ANOVA followed by the Student Newman-Keul's multiple range method.

(Henderson, 1982; Webster *et al*, 1983; Padmanabhan and Hameed, 1988). Another study was also reported that the pre- implantations as well as post- implantation loss were higher when alcohol was administered during gestation period (Mishra and Singh, 2013). If an excessive amount of alcohol is consumed before implantation of embryo in uterine wall, the impact can be severe resulting in miscarriage.

The results thus indicate that alcohol consumption might have decreased the uterine receptivity (endometrial wall), which is essential for normal implantation. Further imbalance in the levels of ovarian hormones (estrogen and progesterone) may be the causative factor in termination of pregnancy in alcohol- treated mice. Progesterone plays a critical role in the establishment and the maintenance of pregnancy, both by its endocrine and immunological effects. It is well reported that progesterone is essential for stimulating of the production the range of endometrial secretion because of it was essential for successful development of fetus during pregnancy (Geisert *et al*, 1993, Muggli *et al*, 2017; Elmazoudy and Attia, 2018). Presently in this study, though the level of progesterone has not been measured, even then it can be assumed that alcohol consumption might have decreased the level of progesterone resulting in pregnancy failure.

CONCLUSION

Alcohol consumption during gestation causes adverse effect on pregnancy. The study further indicates that alcohol consumption affect the pre-and post-implantation loss resulting in pregnancy failure. Alcohol consumption can be an underlying cause of infertility and missed abortions in females. Alcohol may also raise a chance of pregnancy termination in females. Therefore, on the basis of this study it is suggested that the use of alcoholic beverage should be avoided by women during pregnancy.

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