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Effect of Kalmegh Leaf Extract on alcohol induced toxicity in rat liver and ovary.

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ABSTRACT:

Alcohol is a drug which acts as depressant. It is a liquid produced by fermentation of glucose in the presence of yeast, which slows down vital functions of the body.To relate the ancient therapies to an ancient problem in this study we will rely on a much-known herbal medicine Kalmegh extract (*Andrographispaniculata*). The present study observed the effect of ethanol on liver and ovary as well as the role of Kalmegh as therapeutic agent against alcohol induced rat model.Urea and Creatinine has increased in ethanol treated group according to our result. Urea and Creatinine has increased in Kalmegh group according to our result.

INTRODUCTION:

Alcoholisadrugwhichactsasdepressant.It isaliquidproducedbyfermentationofglucoseinthe presenceofyeast,whichslowsdownvitalfunctionsofthebody. It isapsychoactive substance that is the active ingredient in drinks such as beer, wine, and distilled spirits (hard liquor).Alcoholic beveragesshavebeenusedinhumansocietiessincethebeginningofrecordedhistory.Thepatterns of alcohol intake around the world are constantly evolving. The fastest growth has been in developing countries in the Asian subcontinent like India where per capita alcohol consumption hasmorethandoubledfrom2005to2016,accordingtoareportbytheWHO.Theconsumptionof alcoholhasincreasedfrom2.4litresin2005to5.7litresin2016with4.2litresbeingconsumedby men and 1.5 litre by women, it said. The pattern of drinking in India has changed from occasional and ritualistic use to social use. The legal age of alcohol consumption in Chhattisgarhis 21 according to the Chhattisgarh Excise Act, 1915. Many reasons have been reported for the increase in alcohol consumption in Chhattisgarh, some of them are formaloccasions,partyorcelebrations,influenceofcolleagues,friendsandadults,status symbol, peer pressure and many more. Alcohol consumption is often portrayed as a response to poverty andmisery,butglobalpatternssuggest thatdrinkingisalsoassociatedwithrelativeaffluence.The GovernmentofIndiahastakenmanypreventivemeasurestocheckalcoholconsumptioninIndia.

Alcohol is a psychoactive substance that is the active ingredient in drinks such as beer, wine, and distilled spirits (hard liquor). Alcohol has a variety of short-term and long-term adverse effects. Short-term effects include impairment of neuro-cognitive functions, dizziness, nausea, vomiting like symptoms and allergy like reactions. Long-term effects include brain damage (Wernicke–Korsakoffsyndrome),cardiomyopathy,arrhythmias(Irregularheartbeat),stroke,high blood pressure, Steatosis (fatty liver), Alcoholic hepatitis Fibrosis, liver Cirrhosis, pancreatitis, birthdefectsasalcoholisateratogenandcancer (Klingemann*etal*.,2001).Deathfromethanol consumption is possible when blood alcohol levels reach 0.4%. Alcohol also affects female reproductive system. Alcohol is a gonadal toxin in females and disrupts the ovarian structure and function. Follicle maturation doesnot occur properly. Plasma estradiol concentrations decreases while plasma estrogen level increased (Abraham *et al*., 1971). Researchers, clinicians and public health officials are attempting to develop effective prevention and treatment approaches must consider the population's attitudes and expectations regarding alcohol consumption and its effects. To reduce the effect of alcohol in human physiology many preventive drugs has been introduced to the global market. However, as those drugs are effective to the physiology it leaves severe side effects on the same. In ancient India herbal remedies were main functional therapy as well as in Indian mythology it depicts the reports of alcohol consumption. To relate the ancient therapies to an ancient problem in this study we will rely on a much-known herbal medicine Kalmegh extract (*Andrographispaniculata*).

Kalmeghplant(*Andrographispaniculata*)isanimportantmedicinalplantandwidelyusedaround theworld.Thechemicalcompoundfoundintheaerialpartsoftheplantareandrographolidewhich is the reason behind its anti-inflammatory and anti-oxidant property (Jarukamjorn et al., 2008) Andrographolide and neoandrographolideis

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also responsible for increase in bile flow which facilitates digestion. It also cures hepatitis and has an hepato-protective effect. It cures diarrhea. Thepresentstudywasintendedtoseetheeffectofethanolon the liverandovaryaswellastheroleof Kalmegh as therapeutic agent against alcohol induced ratmodel.

MATERIALS AND METHOD:

Adult female albino rats weighing 100-150 g were maintained in the animal house as per the recommendation from the institutional ethical committee for the care and use of laboratory animals. They were maintained in animal house of facility of SLT Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya under standard conditions (25 ± 2^0C, 55-60% RH and 12:12 hour light and dark cycling). They have been providing with standard condition, and were allowed free access to commercial pelleted rat feed. All experimental protocols and procedures were in accordance of Institutional regulations and national criteria for animal experimentation and due approval was taken from the Institution Animal Ethics Committee (IAEC).

CHEMICALS AND REAGENTS:

Biochemical Estimation:

Allchemicalsusedwereofanalyticalgradeorofthebestqualitysuppliedby SRL, Sigmachemical co. and high media (INDIA), as well as from SigmaCo, USA. Distilled water, ethanol thiobarbituric acid (TBA), Tris-hydrochloric acid (Tris-HCl), Phosphorous acid and Butylated hydroxyl-toluene(BHT),Sodium hydroxide (NaOH), Sodium potassium tartarate, Sodium bicarbonate(Na_2CO_3), Copper Sulphate (Cu_2SO_4), Phenazine methosulphate (PMS), Reduced Glutathione(GSH), Glucose-6- phosphate, Tris Buffer, Tris HCl, Sodium chloride (NaCl),Dihydrogen potassium phosphate (NaH_2PO_4), Dipotassium hydrogen phosphate (Na_2HPO4),Sodium pyrophosphate,Nitrobluetertazolium (NBT),Nicotinamide Adenosine Dihydride (NADH), Sulphosalicylic acid,5,5'Dithio-bis2-nitrobenzoic acid (DTNB), Ethylene diamine tetra acetic-acid(EDTA)and Potassium chloride, Picric acid, Folin'sCiocalteau reagent, Hydrogen per oxide (H_2O_2), Hydrochloric acid (HCl),Glacial-aceticacid, Chloroform andEosin.

Serum Biochemistry Kits:

Separate kits for liver function tests include alanine amino transaminases (ALT), aspartate amino transaminases (AST) and alkaline phosphatase (ALP), Kidney function tests creatinine urea, and uric acid, Lipid profile kits include; triglyceride (TG), cholesterol (CHOL), low- and high-density lipoprotein kits (LDL, HDL). Total protein content, albumin, bilirubin (BUN) and electrolyte kits sodium (Na^+), potassium (K^+) were purchased from ErbaTransasia Biomedical Ltd, Solan (HP), India.

EXPERIMENTAL DESIGN:

Albino rats are used as experimental model. 16 rats were divided into 4 groups namely control group, ethanol group, ethanol with kalmegh group and drug (kalmegh) group. Each group of rats wereput inseparatecagesandweremarked.Allratsareacclimatizedfor7dayswithnormalfood. Once rats are acclimatized, control group was subjected to oral dosing of normal saline, ethanol groupwassubjectedtooraldosing of42.5%ethanol,kalmeghgroupwassubjectedtooraldosing kalmegh extract for 14 days while ethanol with kalmegh group was subjected to oral dosing of ethanol for 7 days followed by kalmegh for the next 7 days. After 14 days of dosing, rats are sacrificed and tissues arestored.

DOSAGE:

a. Control - Normal saline is given through oral dosing, 0.5 ml per day.

b. Ethanol: 42.5% ethanol (country liquor) was prepared and given by oral dosing. 1 ml ethanol was given to rats through oral dosing two times per day (total 2 ml perday).

c. Ethanol with kalmegh: Ethanol was given 1 ml per day throughoral dosing for 7 days. After completion of 7 days, 0.5 ml of kalmegh extract was given for 7days.

d. Kalmegh: Kalmegh extract was introduced in rats by oral dose. The dosages are done at concentration of 500 mg/ Kg weight of body weight. Accordingly, dose is made by dissolving kalmeghpowderindistilledwater.0.5mlofkalmeghextractwasgiventoratsthroughoraldosing perday.

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RESULTS

In case of liver, in comparison to control, protein content is highest in Kalmegh group, low in ethanol treated groups, and lowest in ethanol with Kalmegh group.

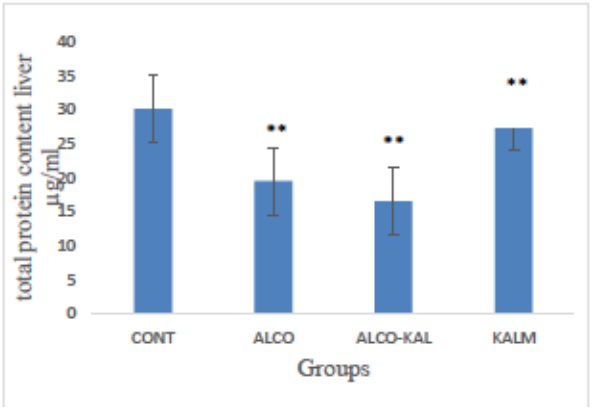

Fig 1 (a)

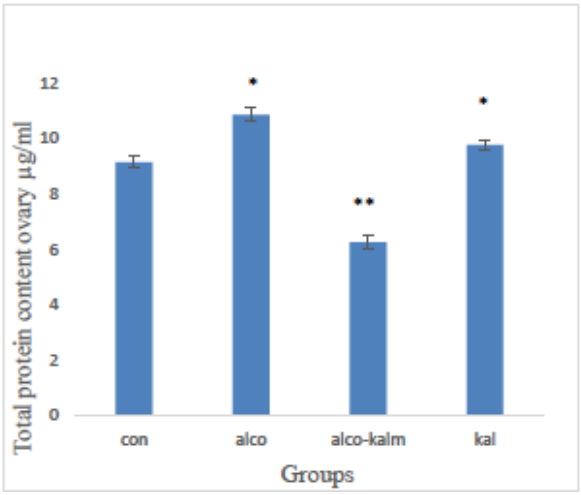

Fig 1(b)

Fig:1 Histogram represents the level of total protein content in (a) liver and (b) ovary of different groups of rats; * represents level of significance ($p<0.05$=*; $p<0.01$=**)

In case of liver, in comparison to control, GSH activity is highest in ethanol with Kalmegh group, high in Kalmegh group and lowest in ethanol treated group. In case of ovary, in comparison to control, GSH activity is highest in ethanol treated group, high in Kalmegh group and lowest in ethanol with Kalmegh group.

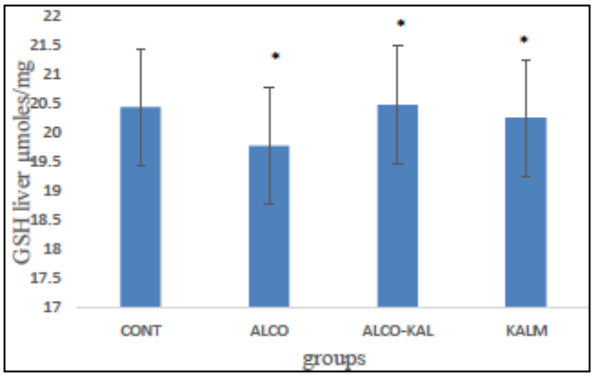

Fig 2 (a)

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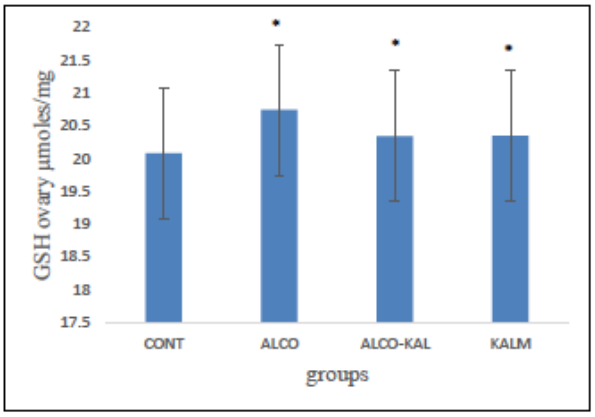

Fig 2 (b)

Lipid Profile Tests To Show The Effect Of Kalmegh Extract On Ethanol Treated Rats:

1. Cholesterol Test :

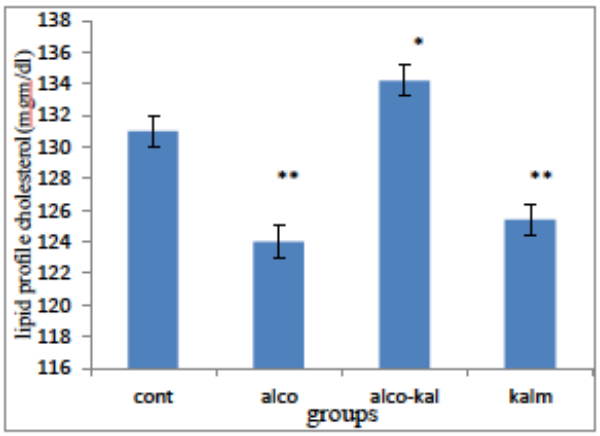

Fig 3 (a)

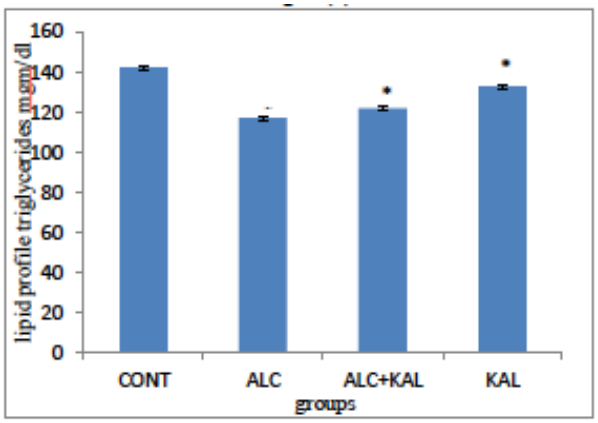

Fig 3(b)

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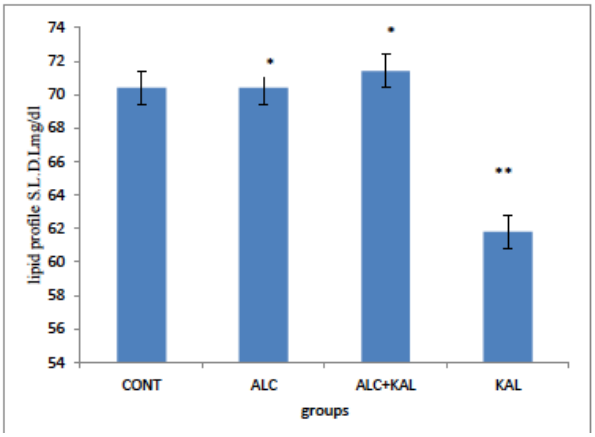

Fig 3(c)

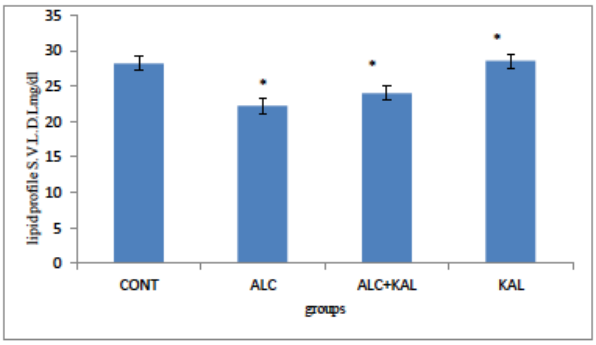

Fig 3(d)

Fig:5 Histogram represents Lipid Profile Test of (a) cholesterol and (b) triglycerides (c) S.L.D.L (d) S.V.L.D.L of different groups of rats; * represents level of significance ($p<0.05$=*; $p<0.01$=**)

In case of cholesterol, in comparison to control group, lipid profile test of cholesterol is highest in ethanol with Kalmegh group, low in Kalmegh group and lowest in ethanol treated group. In case of triglycerides, in comparison to control group, lipid profile test of triglycerides is low in Kalmegh group, lower in ethanol with Kalmegh group and lowest in ethanol treated group.

In case of S.L.D.L, in comparison to control group, lipid profile test of S.L.D.L, highest in ethanol with Kalmegh group, almost similar in ethanol treated group and lowest in Kalmegh group. In case of S.V.L.D.L, in comparison to control group, lipid profile test of V.L.D.L, highest in Kalmegh group, lower in ethanol with Kalmegh group and lowest in ethanol treated group.In case of S.Bilirubin, in comparison to control group, liver function test of S.Bilirubin is low in ethanol with Kalmegh group, lower in Kalmegh group and lowest in ethanol treated group.

In case of Alkaline Phosphatase, in comparison to control group, liver function test of Alkaline Phosphatase is highest in ethanol group, higher in ethanol with Kalmegh group and high in Kalmegh group.In case of S.Protein (albumin,globulin), in comparison to control group, liver function test of S.Protein (albumin,globulin) is highest in Kalmegh group and higher in ethanol treated and ethanol with Kalmegh group.In case of Urea, in comparison to control group, renal function test of Urea is highest in ethanol treated group, higher in Kalmegh group and high in ethanol with Kalmegh group.

In case of Creatinine, in comparison to control group, renal function test of Creatinine highest in ethanol treated group, higher in Kalmegh group and high in ethanol with Kalmegh group.

DISCUSSION:

The liver is the primary site of alcohol metabolism (Gao*et al*., 2011). When alcohol is metabolized by the liver cells, free radicals are produced. They are highly reactive and destroys the cell membrane. Chronic alcohol consumption

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decreases the levels of these antioxidants and makes the liver cells more susceptible to free radical-induced injury.
Livers generate low levels of reactive oxygen species (ROS), especially superoxide in the mitochondria. The large number of mitochondria and their capacity to leak electrons from complex I and III of the electron transport chain make them quantitatively the most important intracellular source of ROS (Jaeschke*et al*., 2010). Each liver cell expresses superoxide dismutases (SOD1 in the cytosol; SOD2 in mitochondria), glutathione peroxidases (cytosol and mitochondria), catalase (peroxisomes), thioredoxins (Trx1 in cytosol; Trx2 in mitochondria) and peroxiredoxins (Prx-I, -II, -VI in the cytosol; Prx-III, -V in mitochondria). In addition, liver cells contain mMconcentrations of glutathione in all cellular compartments, have radical chain-breaking antioxidants (vitamin E) in cell membranes, and keep redox-active iron tightly boundtostorageortransportproteins.BecauseofthisdefensesystemagainstROS,livercellsand especially hepatocytes, have a substantial capacity to metabolize and effectively detoxify ROS and repair oxidant damage. An example where the combination of increased ROS formation and impaired defense systems causes cell death is excessive drinking of alcohol, which depletes GSH in liver and causes oxidative stress (Jaeschke*et al*., 2011). Due to their special chemical characteristics, ROS causes lipid peroxidation, DNA strand breaks, and oxidize virtually all molecules in biological membranes and tissues, resulting in liver injury (Mittler*et al*., 2002). Liver is a major organ attacked by ROS (Sanchez-Valle*et al*., 2012). Alcohol increases the level of plasma triglyceride and hepatic TBARS significantly. Expression of antioxidant defense genes, such as GSH-Px-1, Cu/Zn-SOD, and paraoxonase enzymes, were significantly lowered in the liver. TNF, a group of cytotoxic pro-inflammatory cytokines, is thought to play a vital role in initiation of liver damage (Feagins*et al*., 2015). Cholesterol metabolism is affected due to ethanol,bloodcholesterollevelisincreasedwhilebileleveldecreasesduetoexcessivedrinkingof alcohol (Lefevre*et al*., 1972). Blood triglyceride level is also increased (Chait A *et al*., 1972) Thusfromtheexperimentperformedbymeitisconcludedthatconcentrationofproteindecreases in ethanol treated liver and increases in ethanol treated ovary. Concentration of protein has increased in kalmegh group in both liver and ovary according to ourresult.

Lipid Peroxidation should increase in ethanol treated liver which is parallel to my result. Lipid Peroxidation has decreased in ethanol treated ovary in our result. LPO has increased in kalmegh group in liver while it has decreased in ovary according to our result.
GSH activity should decrease in ethanol treated liver which is parallel to my result. GSH activity has increased in ethanol treated ovary according to our result. GSH activity has increased in kalmegh group in both liver and ovary according to our result.
SOD activity should decrease in ethanol treated liver and ovary which is parallel to our result. SOD activity has decreased in kalmegh group in liver while increased in ovary according to our result.
Cholesterol, triglycerides, V.L.D.L and S.L.D.L has decreased in ethanol treated groupaccording to our experimental result. Cholesterol, triglycerides, V.L.D.L and S.L.D.L has decreased in kalmegh group according to ourresult.
S.Bilirubin should decrease in ethanol treated group which is parallel to my result. Alkaline PhosphataseandS.Protein(albumin,globulin)hasincreasedinourexperimentalresult.S.Bilirubin and alkaline phosphatase has decreased in kalmegh group while S.Protein has increased in kalmegh group according to ourresult.

Urea and Creatinin has increased in ethanol treated group according to our result. Urea and Creatinin has increased in kalmegh group according to our result.

CONCLUSION:

The administration of ethanol causes deleterious effects on rat liver. These effects measured by changes in concentration of antioxidant enzymes in liver. SOD level significantly decreased in liver.LPO levelsignificantlyincreased inwholeliver,whileGSHactivitydecreased inliver.

The treatment of the drug kalmeghresults in increase of both SOD and GSH level in liver, while LPO level decreased. From these observationsit is seen that kalmegh effects significantly in maintainingtheproperlevelofantioxidantenzymesinliver.Astheseenzymesarecrucialforliver functioning, so, it can be concluded that kalmegh therapy is effective against alcoholism. Alcohol has also deleterious effects on ovary. For detailed study, more experiment isrequired.

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