

# Protective Effect of *Aerva javanica* Against Ethanol Induced Hepatic Stress in Rats: A Randomized Control Report

Rahmat Ali Khan

Department of Biotechnology University of Science and Technology Bannu, KPK, PAKISTAN.

## ABSTRACT

**Background:** *Aerva javanica* (AJME) possess antioxidant activity and hepatoprotective effects, and traditionally in Pakistan for liver diseases. **Methods:** 24 male albino rats were divided into five groups of 4 chicks of each, having free access to food and water. Group I (control) while group II, III and IV were treated with ethanol 24 hrs for 2 weeks. Rats group II received only ethanol group III received silymarin at a dose of 50 mg/kg while group IV was given AJME (200 mg/kg). The activities of alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and were determined in serum. Catalase (CAT), peroxidase (POD), glutathione-S transferase (GST), glutathione peroxidase (GSH-Px), glutathione reductase (GSR) activity was measured in liver homogenates. Thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) concentration were also evaluated in liver homogenates. **Results:** Hepatotoxicity induced with ethanol was evidenced by significant increase in lipid peroxidation (TBARS) and serum, ALT, ALP, LDH. Level of GSH determined in liver was significantly reduced, as were the activities of antioxidant enzymes; CAT, POD, GSH-Px, GSR, GST treated with ethanol. AJME (200 mg/kg bw) and silymarin (50 mg/kg bw) co-treatment prevented all the changes observed with ethanol-treated rats. **Conclusion:** The results of present work indicate that AJME has a significant protective effect against ethanol induced hepatotoxicity in chicks, which may be due to their antioxidant potential.

**Key words:** *Aerva Javanica*, Ethanol, Liver, Antioxidant Enzymes, Lipid Peroxidation.

## BACKGROUND

During metabolism process reactive oxygen species (ROS) are constantly produced to control various physiological disorders.<sup>1</sup> These reactive species oxidases the macro molecules like protein, carbohydrate, lipids and fats to maintain balance and stability of natural coordination. When the creation of reactive species is increased as a result the ability of endogenous antioxidant system to reclaim these reactive species, an imbalance is produced called oxidative stress<sup>2</sup> which leads to cause damages of membrane, protein, lipid peroxidation, aberration of chromosome, DNA mutations and deactivation of numerous antioxidant defense enzymes in living organism.<sup>3</sup> Ethanol is

a natural product that has been available for human consumption for thousands of year's causes' oxidative stress and in slighter breathing toxicities. Ethanol consumption causes various disease of other tissue but among them hepatotoxicity is on the top.<sup>4</sup> Ethanol causes lipid peroxidation of lipids, hepatitis, cirrhosis and its constant use cause fibrosis. Inside the living cells ethanol are converted into acetaldehyde and free radicals which further extract electron and causes oxidative stress<sup>5</sup> viz; intestinal brush-border membrane, serum electrolytes and haematology. Metabolism of alcohol in liver generates excessive free radicals and increased peroxisomal oxidation of fatty

Submission Date: 29-01-2017;

Revision Date: 18-03-2017;

Accepted Date: 10-04-2017

DOI: 10.5530/ijper.51.2s.56

**Correspondence:**

**Rahmat Ali Khan,**  
Assistant Professor,  
Department of Biotechnology,  
Faculty of Biological  
Sciences, University of  
Science and Technology  
Bannu, KPK, PAKISTAN.  
Tele.No: +92 51 90643086  
Fax No: +92 51 9205753  
E-mail: Rahmatgul\_81@  
yahoo.com



www.ijper.org

acid, which would ultimately affect functionality of the antioxidant systems to eliminate ROS in the body.<sup>5</sup> Therefore, the mechanism to restore hepatic injuries caused by alcoholic oxidative stress is tightly regulated by the antioxidant status of a living system. To maintain this balance medicinal flora plays a crucial role in maintaining human health in addition shelter and foodstuff. Medicinal plants are traditionally used for treatment of much human disease with low side effect and easily viability as compare to synthetic medicine due to presence of secondary metabolites viz; phenolic compounds, essential oil, saponin and tannins.<sup>6</sup> *Arva javanica* (Burm.f.) Juss. ex Schult., locally known as Khar Buta, is found over a broad range of sandy sediments. Phytochemistry reveals the presence of bioactive constituent<sup>7</sup> which play a crucial role in removing kidney stones,<sup>8</sup> dysentery<sup>9</sup> relief of hepatotoxicity<sup>10</sup> and enzymes inhibition.<sup>11</sup>

## Methodology

### Plant collection and formation of Extracts:

*Arva javanica* at maturity was collected from district Bannu, identified by Professor Dr. Fizan Ullah Khan, Department of Botany, University of Science and Technology Bannu and a specimen was submitted in the herbarium of the University for future Correspondence via voucher no (RA-116). The plant was shed dried, chopped, ground mechanically into small fine powder and extracted with 70% methanol to obtain methanol crude extract (AJME). The methanol extract was filtered and concentrated under reduced pressure through rotary evaporator at room temperature by using rotary evaporator.

### Experimental plan

24 male albino rats were randomly divided into five groups. Study protocol was approved by the ethical committee of the University. The rats were kept for seven days in the animal house at room temperature and given 12 hr dark/light environment before the preceding of experiment. Group I (control) was free access of food and water while group II was induced with ethanol (0.25 ml/100 g) orally after 24 hrs for 2 weeks. Rats of group III and IV were received 50 mg/kg silymarin and 200 mg/kg AJME along with ethanol for assessment of hepatoprotective effects. At the end of experiment all the rats were given anesthesia and blood was collected through cardiac puncture. Blood was centrifuged at 6000 rpm and separate serum and kept at -20°C for further analysis.

## Serum analysis

Serum bilirubin, aspartate amino transferase (AST), triglyceride (GT), high density lipoprotein (HDL), low density lipoprotein LDL, alanine amino transferase (ALT) and cholesterol.

## Endogenous enzymatic analysis

Various endogenous enzymes including protein,<sup>12</sup> CAT and POD,<sup>13</sup> GST<sup>14</sup> and GPx<sup>15</sup> were measured respectively for assessment of antioxidative capability.

## Lipid peroxidation assessment

Lipid per-oxidation was analyzed by the determination of thiobarbituric acid reactive substances (TBARS)<sup>16</sup> with some modifications<sup>17</sup> and GSH contents liver tissue homogenate by using the method of.<sup>18</sup>

## Statistical analysis

Data are expressed as means  $\pm$  SE (n=6) and significant difference between the groups was statistically analyzed by Duncan's multiple range test (Statistica Software, 1990). Level of significance among the various treatments was determined at  $p < 0.05$ .

## RESULTS

### Effect of extract on serum biochemistry

ALT, ALP and serum bilirubin level are significantly increased in ethanol treated group as shown in Table 1 showing injuries of hepatic cell and hepatic degeneration. These parameters were significantly restored by co-administration of 200 mg/kg b.w., AJME and 50 mg/kg b.w., silymarin closely towards the non-treated control group.

### Effect of extract on protein and antioxidant enzymes

Protein, CAT and POD play a crucial play in assessment of antioxidant profile in rats as presented in Table 2. Ethanol administration caused reduction in protein contents and activities of antioxidant enzymes viz; CAT and POD as compare to control groups. Co-administration of silymarin and 200 mg/kg b.w., AJME significantly improved these parameters near to control level showing the presence of bioactive compounds.

Alterations in different enzyme activity of liver of glutathione peroxidase and glutathione reductase are presented in Table 2. Ethanol treatment caused significant reduction in GST and GSH-Px activity as compared to the control group. Treatment with 200 mg/kg AJME and silymarin significantly increased the levels of GSH-Px, and GST up to normal level.

**Table 1: Effect of AJME on serum biochemistry of rats**

Treatment	ALT (U/L)	ALP (U/L)	Serumbilirubin (mg/dl)
Control	93±6.9	146±17.5	2.15±1.06
Ethanol	185±16	278±24.7	8.20±3.19
50 mg/kg Silymarin	112±21	140±18.4	3.65±0.92
200 mg/kg AJME	133±9.8	177±20.0	6.60±2.40

Mean ±SE (n=6 number)

\*, \*\* indicate significance from the control group at  $P<0.05$  and  $P<0.01$  probability level++ indicate significance from the ethanol group at  $P<0.01$  probability level**Table 2: Effect of AJME on tissue homogenate protein and antioxidant enzymes**

Treatment	Protein ( $\mu\text{g}/\text{mg}$ tissue)	CAT (U/min)	GST (nmol/min/g protein)	GPx (mol/g tissue)	POD (U/min)
Control	83±3.1++	0.70±0.03++	42.5±1.3++	197.5±4.3++	0.50±0.02++
Ethanol	35±4.2**	0.29±0.01**	10.9±2.0**	63.3±5.1**	0.22±0.04**
50 mg/kg Silymarin	70±5.3++	0.43±0.04++	37.0±2.1++	190.1±1.7++	0.46±0.05++
200 mg/kg AJME	56±2.2++	0.35±0.03++	29.4±3.3++	143.0±9.0++	0.30±0.02++

Mean ±SE (n=6 number)

\*, \*\* indicate significance from the control group at  $P<0.05$  and  $P<0.01$  probability level++ indicate significance from the ethanol group at  $P<0.01$  probability level**Table 3: Effects of AJME on liver TBARS and GSH**

Treatment	GSH (mol/g tissue)	TBARS (nmol/ min/ mg protein)
Control	26.7±3.0++	13.1±3.6++
Ethanol	15.1±1.6**	26.0±2.5**
50 mg/kg Silymarin	26.1±8.3++	13.3±3.8++
200 mg/kg AJME	23.6±4.7++	14.6±2.7++

Mean ±SE (n=6 number)

\*, \*\* indicate significance from the control group at  $P<0.05$  and  $P<0.01$  probability level++ indicate significance from the ethanol group at  $P<0.01$  probability level

### Effects of extract on lipid peroxidation contents

Lipid peroxidation assessments are very important in antioxidant profile. Ethanol treatment caused significant reduction in the contents of Glutathione reduced and thio-berbitoric acid reactive substances (TBARS) as shown in Table 3. Supplementation of 50 mg/kg silymarin and 200 mg/kg AJME reversed these contents and protects cells from lipid peroxidation.

### DISCUSSION

Excessive amounts of alcohol and ethanol drinking causes hepatotoxicity and liver degeneration. Metabolism of ethanol occurs inside the liver in the presence of an enzyme known as acetaldehyde which is further converted into by dehydrogenase to acetate into aldehyde in microsomal ethanol oxidizing system (MEOS). This pathway requires P450 2E1 or CYP2E1

that strips hydrogen away from alcohol to produce acetaldehyde. In addition inside the peroxisomes ethanol is catalyzed via catalase enzyme into more toxic radical called acetaldehyde.<sup>19</sup> These free radicals attach to unsaturated fatty acid, producing of lipidperoxide and elevated levels of serum marker enzymes, reduction of GSH, decreased protein synthesis, triglyceride accumulation, increased lipid per-oxidation, and thus damage liver cells.<sup>20</sup> Due to free toxicity resulted into fatty liver and the damage of liver cells there by the fat metabolism in the liver is affected leading to increased levels of serum triglycerides, HDL and LDL.<sup>21-22</sup> The levels of serum cholesterol, triglycerides, HDL and in ALT, ALP were found to be significantly increased in ethanol intoxicated group as compared to control group. The elevated level is reduced by AJME towards silymarine group. The result found has resemblance with other study.<sup>23</sup>

Free radical produced by the metabolism of ethanol causes oxidative damages and hepatic necrosis due imbalance of oxidative system. To overcome the toxicity of free radicals induced by the metabolism of ethanol a defense system protects the body called antioxidant. Ethanol causes elevation in the level of NADH in mitochondria which further causes an increase in number of superoxide free radicals leaked from oxidative phosphorylation leading to formation of hydroxyl radicals and depleted activities of antioxidant enzymes.<sup>24-25</sup> Antioxidant enzymes viz; CAT, POD, GST, GPx was significantly ( $P<0.05$ ) decreased in ethanol treated

rats group which were recovered by co-administration of 200 mg/kg b.w., AJME significantly ( $P < 0.05$ ) and increased the activity to normal level. Similar reports were obtained by other study.<sup>26</sup> Free radicals of ethanol also causes lipid peroxidation depleted GSH contents and increasing TBARS. In our study TBARS contents in ethanol treated group were increased while GSH contents are reduced from the normal range. This was significantly altered by administration of AJME toward the normal rat as well as standard hepatoprotective silymarine rats. This finding has closely resemblance with the results of.<sup>27</sup>

## CONCLUSION

It is inferred from the present study that ethanol caused hepatotoxicity which were significantly reversed by co-administration of AJME. This shows the presence of bioactive ingredients which probably inhibit ethanol uptake and protect it.

## ACKNOWLEDGEMENT

We are thankful for Vice Chancellor for provision of Research facilities.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

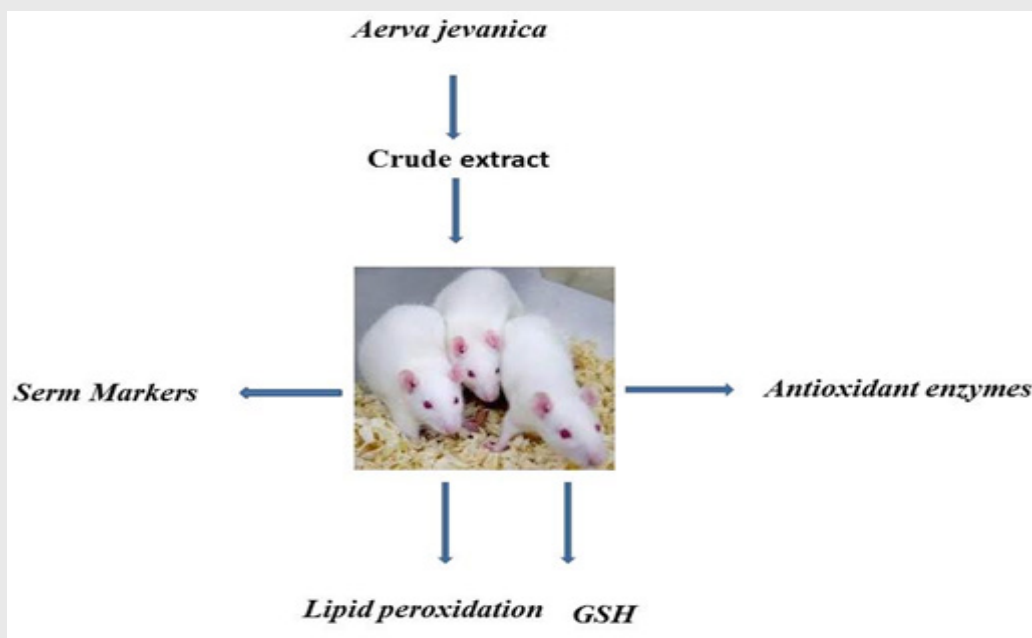
## ABBREVIATION USED

**AJME:** *Aerva javanica*; **TBARS:** Thio-barbituric acid reactive substances; **MEOS:** Microsomal ethanol oxidizing system; **ALT:** Alanine transaminase; **ALP:** Alkaline phosphatase; **LDH:** Lactate dehydrogenase and were determined in serum; **CAT:** Catalase; **POD:** Peroxidase; **GST:** Glutathione-S transferase; **GSH-Px:** Glutathione peroxidase; **GSR:** Glutathione reductase.

## REFERENCES

- Arulmozhi V, Mani K, Kandhan K, Ganesan D, Sankaran M. Antioxidant and anti-hyperlipidemic effect of *Solanum nigrum* fruit extract on the experimental model against chronic ethanol toxicity. *Pharmacogn Mag*. 2010;6(21):42-50. <https://doi.org/10.4103/0973-1296.59965>; PMID:20548935 PMCID:PMC2881655.
- Arun R, Praveen KV, Sheeba V, Mukkadan JK, Joseph PK. Biochemical effects of feeding soft drink and ethanol. *Indian J Exp Bio*. 2009;47:333-7.
- Ashakumary L, Vijayammal PL. Additive effect of alcohol and nicotine on lipid metabolism in rats. *Indian J Exp Biol*. 1993;31(3):270-4. PMID:8500841.
- Bashir M, Javed MT. Effects of ethanol on brain and pancreas weights, serum sodium and potassium and haematological parameters in quail (*Coturnix japonica*). *Avian Pathol*. 2005;34(2):96-100. <https://doi.org/10.1080/03079450500059222>; PMID:16191688.
- Blanco CD, Gentil S. Alterations of glucose metabolism in chronic liver disease. *Diab Res Clin Prac*. 1990;8(1):29-36. [https://doi.org/10.1016/0168-8227\(90\)90093-9](https://doi.org/10.1016/0168-8227(90)90093-9).
- Mongi S, Samira J, Kamel J, Ghada BS, Choumou K, Abdelfattah EF. Haematological and biochemical toxicity induced by methanol in rats: Ameliorative effects of *Opuntia vulgaris* fruit extract. *Hum Exp Toxicol*. 2011; 30(12):1963-71. <https://doi.org/10.1177/0960327111403175>; PMID:21422078
- Emam SS. Phytochemical studies on the herb *Aerva javanica* growing in Egypt. *Cairo University Faculty of Agriculture Bulletin*. 1999;50:488-514.
- Khan UG, Nazir T, Ahmed VU. Chemical Constituents of *Aerva javanica*. *Fitoterapia*. 1982;53:75-7.
- Deshmukh T, Yadav BV, Badole SL, Bodhankar SL, Dhaneshwar SR. Antihyperglycaemic activity of alcoholic extract of *Aerva lanata* (L.) A. L. Juss. Ex J. A. Shultes leaves in alloxan induced diabetic mice. *J App Biomed*. 2008;6(2):81-7.
- Garg SP, Bhushan R, Kapoor RC. Aervanone, a new flavanone from *Aerva persica*. *Phytochemistry*. 1980;19(6):1265-10. 1016/0031-9422(80)83110-4.
- Khan AW, Jan S, Parveen S, Khan RA, Saeed A, Jabbar A, Shad AA. Phytochemical analysis and enzyme inhibition assay of *Aervajavanica* for ulcer. *Chemistry Central Journal*. 2016;6:76. DOI: 10.1186/1752-153X-6-76. <https://doi.org/10.1186/1752-153X-6-76>.
- Lowry OH, Rosenberg NJ, Farr AL, Randall RJ. Protein measurement with Folin Phenol reagent. *Journal of Biological Chemistry*. 1951;193(1):265-75. PMID:14907713.
- Chance B, Maehly AC. Assay of catalase and peroxidases. *Methods in Enzymol*. 1955;11:764-75.
- Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferases: the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*. 1974;249(22):7130-9.
- Mohandas J, Marshal JJ, Duggin GG, Horvath JS, Tiller DJ. Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney. Possible implications in analgesic nephropathy. *Biochemical Pharmacology*; 1984;33(11):1801-7. [https://doi.org/10.1016/0006-2952\(84\)90353-8](https://doi.org/10.1016/0006-2952(84)90353-8).
- Wright JR, Colby HD, Miles PR. Cytosolic factors that affect microsomal lipid peroxidation in lung and liver. *Archives of Biochemistry and Biophysics*. 1981;206(2):296-304. [https://doi.org/10.1016/0003-9861\(81\)90095-3](https://doi.org/10.1016/0003-9861(81)90095-3).
- Iqbal M, Sharma SD, Zadeh HR, Hasan N, Abdulla M, Athar M. Glutathione metabolizing enzymes and oxidative stress in ferric nitrilotriacetate (Fe-NTA) mediated hepatic injury. *Redox Reports*. 1996;2(6):385-91. <https://doi.org/10.1080/13510002.1996.11747079>; PMID:27406673.
- Carlberg I, Mannervik EB. Glutathione level in rat brain. *Journal of Biological Chemistry*. 1975;250(14):4475-80.
- Kurose I, Higuchi H, Kato S, Miura S, Ishii H. Ethanol induced oxidative stress in the liver. *Alcohol Clinical Experimental Research*. 1996;20(S1):77a-85a. <https://doi.org/10.1111/j.1530-0277.1996.tb01736.x>; PMID:8659698.
- Manna P, Sinha M, Sil PC. Aqueous extract of *Terminalia arjuna* prevents carbon tetrachloride induced hepatic and renal disorders. *BMC Complementry and Alternative Medicine*. 2006;6(1):33. <https://doi.org/10.1186/1472-6882-6-33>; PMID:17010209 PMCID:PMC1599753.
- Hegde K, Arun BJ. Hepatoprotective effect of *Carissa carandas* Linn root extract against CC1<sub>4</sub> and paracetamol induced hepatic oxidative stress. *Industrial Journal of Experimental Biology*. 2009;47:660-7.
- Karmia Moawad M: Possible prophylactic effects of vitamin E or Lycopene treatment on renal toxicity induced by CCl<sub>4</sub> administration in albino rats. *World J. Zool*. 2007;2(2):19-28.
- Ogbera AO, Dada O, Adeyeye F, Jewo PI. Complementary and alternative medicine use in diabetes mellitus. *West African Journal of Medicine*. 2010;29(3):158-62. PMID:20665458.
- Ostrowska J, Skrzydlewska E. The comparison of effect of catechins and green tea extract on oxidative modification of LDL *in vitro*. *Advance Medical Science*. 2006;5:1298-303.
- Ozars R, Tahan V, Aydin S, Uzun H, Kaya S, Senturk H. N-acetylcysteine attenuates alcohol-induced oxidative stress in rats. *World Journal of Gastroenterology*. 2003;9(1):791-4. PMID:12679934.
- Ray G, Hussain SH. Oxidants, antioxidants and carcinogenesis. *Industrial Journal of Experimental Biology*. 2002;40:1213-32. PMID:13677623.
- Sadrzadeh SM, Nanji AA, Meydani M. Effect of chronic ethanol feeding on plasmal normal and vitamin E-deficient rats. Relationship to lipid peroxidation. *Biochemistry and Pharmacology*. 1994;47(11):2005-10. [https://doi.org/10.1016/0006-2952\(94\)90075-2](https://doi.org/10.1016/0006-2952(94)90075-2).

## PICTORIAL ABSTRACT



## SUMMARY

- *Aerva jevanica* (AJME) possess antioxidant activity and hepatoprotective effects, and traditionally in Pakistan for liver diseases. 24 male albino rats were divided into five groups of 4 chicks of each, having free access to food and water. Group I (control) while group II, III and IV were treated with ethanol 24 hrs for 2 weeks. Rats group II received only ethanol group III received silymarin at a dose of 50 mg/kg while group IV was given AJME (200 mg/kg). The activities of alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and were determined in serum. Catalase (CAT), peroxidase (POD), glutathione-S transferase (GST), glutathione peroxidase (GSH-Px), glutathione reductase (GSR) activity was measured in liver homogenates. Thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) concentration were also evaluated in liver homogenates. Hepatotoxicity induced with ethanol was evidenced by significant increase in lipid peroxidation (TBARS) and serum, ALT, ALP, LDH. Level of GSH determined in liver was significantly reduced, as were the activities of antioxidant enzymes; CAT, POD, GSH-Px, GSR, GST treated with ethanol. AJME (200 mg/kg bw) and silymarin (50 mg/kg bw) co-treatment prevented all the changes observed with ethanol-treated rats. The results of present work indicate that AJME has a significant protective effect against ethanol induced hepatotoxicity in chicks, which may be due to their antioxidant potential

## About Authors



**Dr. Rahmat Ali Khan**, main area of research Pharmacology and Toxicology. Dr. Khan is also focusing on the Pharmacological potentials of medicinal plants

**Cite this article:** Khan RA. Protective effect of *Aerva jevanica* against ethanol induced hepatic stress in rats: A randomized control report. Indian J of Pharmaceutical Education and Research. 2017;51(2s):110-4.