

Intermittent Alcoholic Binge Induced Liver Injury in Wistar Albino Rats: De Ritis ratio and Histological Correlates

Raymond Akpobome Vhritherhire^{1*}, Ahmed Mohammed Sabo²

1. Department of Morbid Anatomy, College of Health Sciences, Bingham University, Jos, Nigeria
 2. Department of Human Physiology, Faculty of Medical Sciences, University of Jos, Jos, Nigeria
- * E-mail of the corresponding author: akp4ray@yahoo.com

Abstract

The pattern, frequency and quantity of alcohol consumption are important in the development of alcoholic liver disease. It has been shown epidemiologically that binge drinking augments liver injury. This study was undertaken to examine the sequential changes in serum markers of liver function and their relationship to quantifiable histological features following intermittent binge administration of ethanol to Wistar albino rats. Groups of male and female rats were given 30% (w/v) ethanol at a dose of 5g/kg on alternate days for six weeks. The serum activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were measured as indicators of liver function. Liver biopsy specimens were collected and subjected to histological analysis. In the ethanol administered groups, there was no significant gender difference in the enzymes assay value (P- value, 0.65; α , 0.01) and grades of histological features (P- value, 0.11; α , 0.01). There was 175% increase in AST/ALT ratio from 1.2 to 3.3. The severity of histopathological features (steatosis, necrosis and lobular inflammation) observed increased (216%) from a score of 3 to 9.5. The AST/ALT ratio positively correlated moderately strongly with lobular inflammatory foci (Pearson coefficient, $r = 0.661$). A strong correlation is obtained when AST/ALT ratio multiplied by the values of alkaline phosphatase (ALP) is plotted against the hepatocyte injury severity scores giving a Pearson's correlation coefficient of 0.74 and coefficient of determination of 0.55. Intermittent binge administration of ethanol has profound damaging effects on the liver cells probably resulting from acute induction of inflammation. Combination of the serum markers of liver function significantly correlate with histological changes.

Keywords: liver; alcohol; De Ritis ratio

1. Introduction

Ethanol is a constituent of alcoholic beverages commonly consumed because of the psychological effects it induces. Excessive consumption of ethanol causes behavioural changes and derangement of physiological functions of various organs in the body including the liver. Ethanol induced liver disease is a leading cause of chronic liver disease worldwide.

The pattern of drinking (daily versus binge) and total alcohol intake (quantity and frequency) are factors which contribute to the alcoholic liver disease process (Zakhari and Li, 2007).

Emerging evidence suggests that the pattern, frequency and quantity of ethanol consumption are important in the development of alcoholic liver disease (Zakhari and Li, 2007; Reuben, 2007).

Data from animal studies have also demonstrated that binge ethanol consumption pattern augments liver damage during chronic ethanol intake in rats. In the study conducted by Aroor et al (2011), rats were chronically treated with ethanol in liquid diet for four weeks followed by binge doses of 5mg/kg body weight. Chronic ethanol treatment resulted in mild steatosis and necrosis. However, marked steatosis and significant increase in necrosis were noted in the animals that were given binge doses (Aroor et al, 2011).

Binge consumption has been arbitrarily grouped into single binge; intermittent repeat binge and chronic ethanol consumption followed by episodes of binge, and animal models have been used to study the injury induced by these different patterns (Shukla et al, 2013).

There have been efforts by several authors to evolve a simple scoring system to evaluate the severity of liver injury due to a variety of conditions. Such tests include De Ritis ratio (AST/ALT). The diagnostic usefulness of the AST/ALT ratio has been studied and observed to be a more accurate indicator of deranged liver function than absolute elevations in the serum assay values of the enzymes (Kim et al, 2007). Its diagnostic and prognostic relevance and accuracy has been reported by several workers (Kim et al, 2007; Sorbi et al, 1999; Das et al, 2005; Nyblom et al, 2004; Pujar et al, 2010; Siddiqi et al, 2007 and Ehsan et al, 2008). Following this path of seeking a simple predictive model, this study examined the relationship between the De Ritis ratio and each major pathological feature observed following intermittent binge administration of ethanol to Wistar albino rats.

2. Materials and Methods

Groups of male and female Wistar rats obtained from the Animal House of the University of Jos, Jos, Nigeria, were used for this study. These animals were housed in temperature and light controlled animal facilities with adequate feeding on standard pellet chow and water until they were about eight weeks old and had attained the

weight of between 145 - 200g.

The recommendations from the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals for Experimentation were followed in the course of this research.

The one hundred and twenty male and female Wistar albino used in this study were randomly divided into two groups, the control and ethanol administered groups. Each group consisted of 60 rats made up of 30 males and 30 females.

Those in the experimental group were each given ethanol (BDH Chemicals Ltd, Poole, England) at alternate day dose of 5g/kg body weight at a concentration of 30% weight /volume. This is a modification of the technique of studying ethanol induced liver injury employed by Amanvermez et al, 2009 and Zahr et al, 2010. The rats in the control group were given plain drinking water of equivalent volume as the alcohol given to other rats in the other groups. All the treatments were orally administered via a gavage tube on alternate days for six weeks.

Specimens were collected from the rats in each of the groups weekly. On each day of specimen collection, five male and five female rats were randomly selected from each group. The animals were initially anaesthetized with ether and then euthanized.

About 2ml of intra-cardiac blood was collected from each animal and the serum levels of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were determined using commercially available kits.

The liver was dissected out and a wedge biopsy specimen collected was fixed in 10% formalin. The tissue specimen was processed and 3 – 5µ sections were made from each paraffin wax tissue block and stained with haematoxylin and eosin. The microscope slides were each assessed for pathological changes. The degree of these changes was semiquantitatively determined using a modified scoring system employed by Nanji et al, 2001 and Tadic et al, 2002. The histological parameters used in the evaluation of the degree of liver injury include steatosis (fatty liver), necrosis and inflammation. Steatosis was assessed as the percentage of liver cells containing fat and graded as 1+, ≤25% of cells; 2+, 26-50% of cells; 3+, 51-75% of cells; 4+, >75% of cells. Lobular inflammation was graded as 0 (none), 1 (<2 foci per high power field, hpf), 2 (2-4 foci/hpf), 3 (4-6 foci/hpf), 4 (>6 foci/hpf). Necrosis was graded as 0 (absence), 1 (minimal), 2 (mild), 3 (moderate), 4 (severe).

3. Results

Analysis of variance on the values of enzymes assayed in the control and ethanol administered groups gave a probability value (P-value) of 1.57×10^{-7} which is less than the alpha significance level (α) of 0.05. The F ratio of 28.63 is greater than the critical F value of 3.87. Thus, the null hypothesis, $H_0: \mu$ Control group = μ Ethanol group was not accepted. Hence, the enzyme assay values of the ethanol administered group were statistically significantly different from those of the control groups depicting the impact of the treatment given.

Similarly, a comparison of the grades of histological changes (steatosis, necrosis and inflammation) in both control and ethanol groups yielded a significant difference at an alpha level of 0.05 and P-value of 5.56×10^{-34} . There were functional and structural effects on the liver induced by the administration of ethanol during the six weeks period.

Further analysis of the values of enzyme assays in the ethanol group failed to yield any gender difference (α , 0.01; P-value, 0.65; F ratio, 0.21; critical F, 6.78). Similarly, the histological grades did not show any statistically significant gender difference (α , 0.01; P-value 0.11; F, 2.53; critical F, 6.78).

As shown in Table 1 below, there was a 17.2% increase in the activity of aspartase transaminase (AST) from the first week with an average range of 75.5 IU/L in the first week to 88.5 IU/L at the end of the sixth week. Meanwhile, there was an initial average spike of alanine transaminase (ALT) during the first week followed by a decrease of 12% from the second to the sixth week. The mean AST/ALT ratio (De Ritis ratio) increased from 1.2 to 3.3, a 175% increase during this period.

Table 2: Mean weekly male and female AST/ALT ratios

Week	AST		ALT		AST:ALT ratio		
	Male	Female	Male	Female	Male	Female	Average
1	65	86	43	88	1.5	1.0	1.2
2	92	89	34	28	2.7	3.2	2.9
3	99	90	33	27	3.0	3.3	3.2
4	99	69	43	30	2.3	2.3	2.3
5	99	68	34	29	2.9	2.3	2.6
6	89	88	26	28	3.4	3.1	3.3

Hepatocyte injury severity score, the calculated total of the histological grades, increased from 3 to 9.5 (216% increase) from the first to the sixth week (Table 2). Thus, the proportion of overall histological changes during the study period is about 1.3 times more than the changes observed in the De Ritis ratio.

Table 3: Mean distribution of grades of histological features and hepatocyte injury severity scores (HISS) of the ethanol administered group.

Week	Steatosis			Necrosis			Inflammation			HISS
	Male	Female	Mean	Male	Female	Mean	Male	Female	Mean	
1	1	1	1	1	1	1	1	1	1	3
2	0.2	0.6	0.4	0	0.6	0.3	1	1	1	1.7
3	3.2	1.2	2.2	0.8	0.6	0.7	2.8	2.8	2.8	5.7
4	2.8	1.8	2.3	1	1.8	1.4	2	1.2	1.6	5.3
5	3	2.8	2.9	3.8	3	3.4	3	2.2	2.6	8.9
6	4	4	4	3.2	2	2.6	2.8	3	2.9	9.5

Necrosis correlated very poorly with enzyme ratios, giving Pearson r , 0.145, R^2 value of 0.02 when plotted against AST/ALT ratio. The De Ritis ratio correlated better with the grades of steatosis and inflammation giving Pearson r , 0.457; R^2 values of 0.21 and Pearson r , 0.661; R^2 , 0.44 respectively (Figure 1). The relationship between the De Ritis ratio and the liver injury severity score is shown on Figure 2.

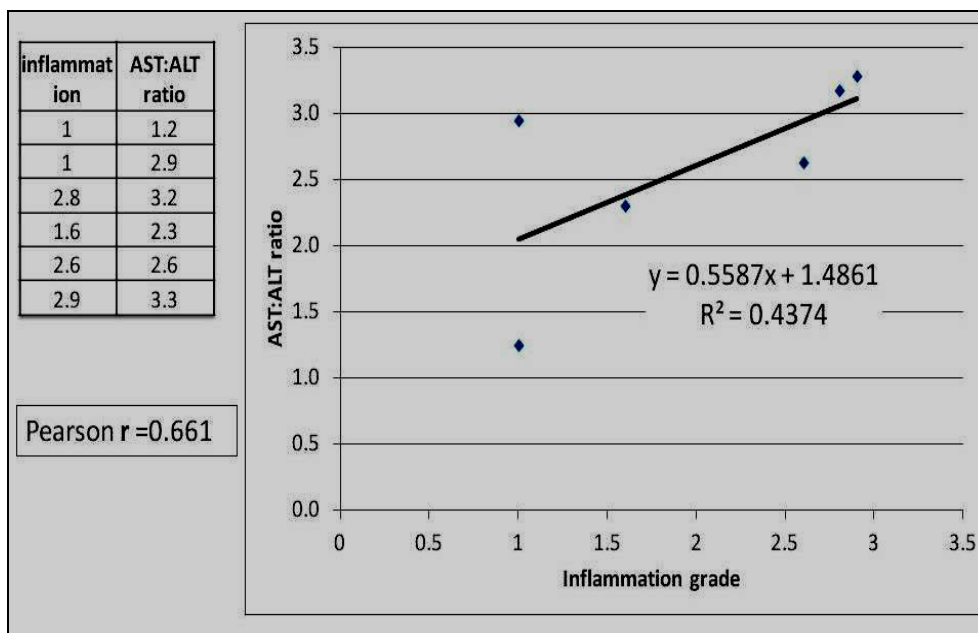


Figure 1. Scatter chart showing the relationship between the AST:ALT ratio and the grade of inflammation.

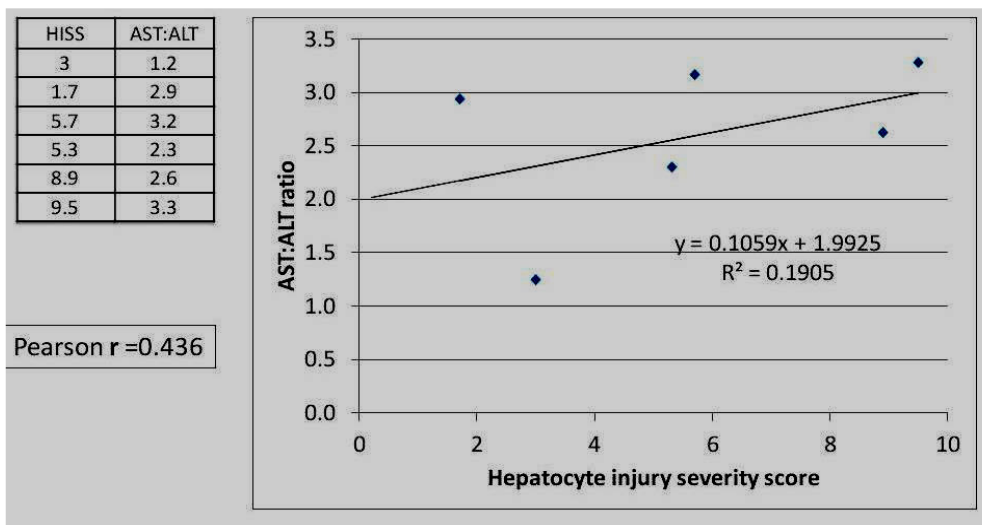


Figure 2. Chart showing the relationship between the AST:ALT ratio and the grade of hepatocyte injury severity score.

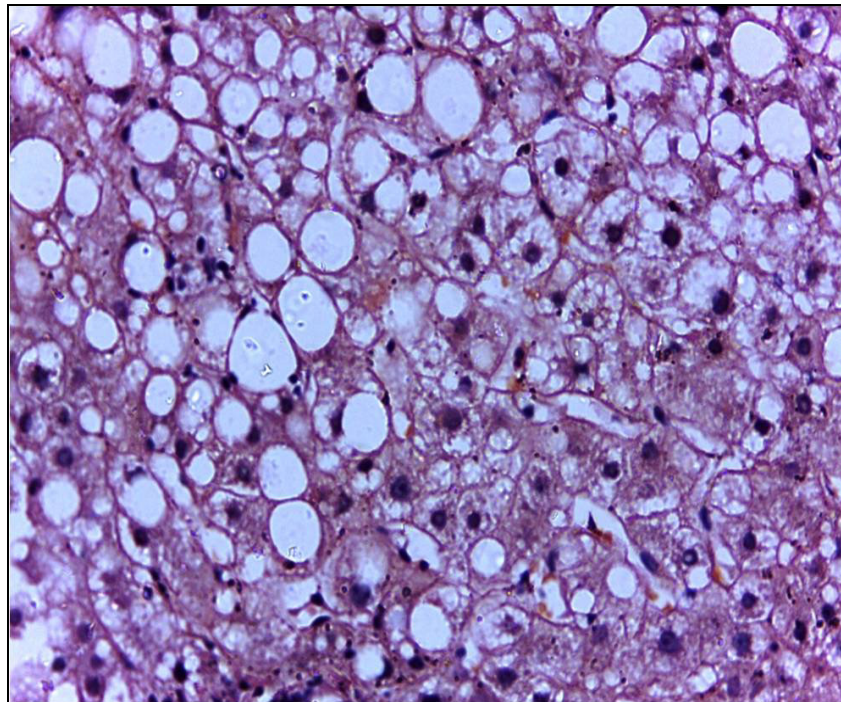


Plate I: Photomicrograph of liver showing steatosis with intracytoplasmic microvesicles which have coalesced in some of the liver cells to form large vacuoles with peripheral displacement of the nuclei. H & E, x 40 objective.

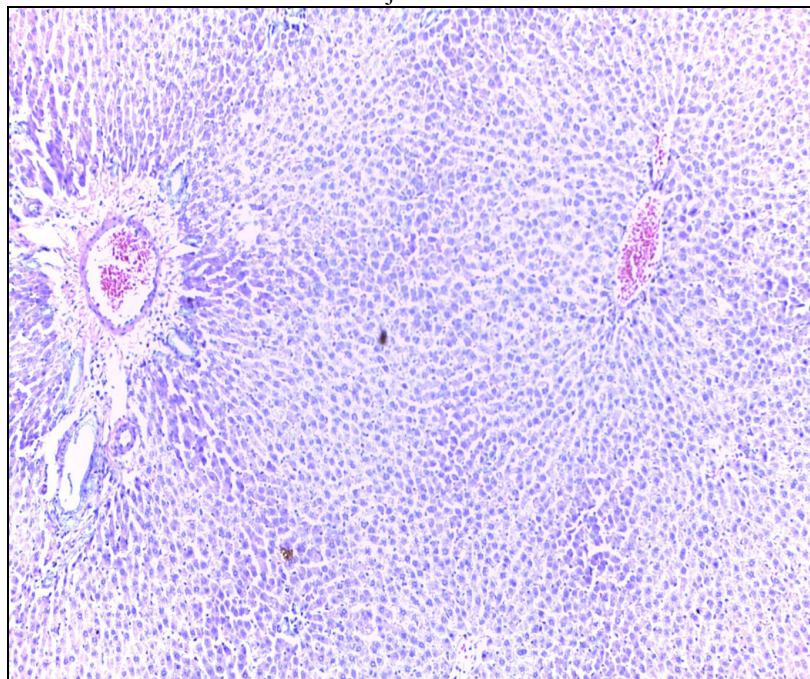


Plate II: Photomicrograph of liver showing inflammatory and necrotic foci. H & E, x 10 objective.

4. Discussion

The pattern of drinking (daily versus binge) and total alcohol intake (quantity and frequency) are factors which contribute to development of alcoholic liver disease (Zakhari and Li, 2007).

Binge consumption of alcohol is on the rise at an alarming rate worldwide. A binge is defined by the National Institute on Alcohol Abuse and Alcoholism as a consumption of five and four drinks for men and women, respectively, in two hours to produce a blood level of ethanol of 80 mg/dl (Shukla et al, 2013). Binge drinking has been suggested to be a major trigger for the development and progression of steatohepatitis (Bertola et al, 2013A; Bertola et al, 2013B).

The high blood concentration of ethanol occasioned by binge consumption rapidly accelerates the onset and

severity of injury. This was showed in the study conducted by Aroor et al, during which rats were chronically treated with ethanol in liquid diet for four weeks followed by binge doses of 5mg/kg body weight (Aroor et al, 2011). Chronic ethanol treatment resulted in mild steatosis and necrosis. However, marked steatosis and significant increase in necrosis were noted in the animals that were given binge doses (Aroor et al, 2011).

It is notable that the grade of inflammation, measured as the number inflammatory foci per high power field, has the strongest correlation with liver injury following alcoholic binges. The liver has minimal constitutive production of inflammatory cytokines but may be induced to produce these effector molecules when the cells are activated by a variety of physiologic and pathologic stimuli including exposure to ethanol (Tilg et al, 2000). Nanji et al (2001) reported a rise in tumour necrosis factor alpha in rats exposed to alcohol. However, in a later study, Zahr et al (2010) noted that there was no significant change in the level of tumour necrosis factor alpha and interferon gamma following a four day binge administration of ethanol to Wistar rats. Perhaps there is a slow onset of rise of these cytokines to detectable levels in the serum.

Ehsan et al (2008) demonstrated a strong correlation between De Ritis ratio and fibrosis which is the end stage of damaging conditions of the liver including alcohol. Fibrosis was not observed in this study probably due to the short period of experimentation. A ratio of more than one in heavy drinkers has been observed to be indicative of severe liver disease in humans (Nyblom et al, 2004).

The AST/ALT ratio had a lower correlation with the hepatocyte injury severity score (HISS) (Pearson $r = 0.436$, $R^2 = 0.1905$) presumably because this is the resultant vector of the three combined histological parameters.

The mathematical correlation between the combination of serum enzymes and cellular changes was significantly strengthened when the values of ALP were factored into the equation by the multiplication of AST/ALT ratio with the corresponding values of ALP. The AST/ALT*ALP product correlated strongly with the hepatocyte injury severity score (Pearson's r , 0.74; R^2 , 0.55). The significance of this combination AST/ALT*ALP is that the resultant composite model represented by the linear regression, $y=35x + 197$, may be used to derive the hepatocyte severity score once the enzyme values are known.

The coefficient of determination, such that, $0 \leq R^2 \leq 1$, gives the proportion of the variance (fluctuation) of one variable that is predictable from the other variable (Correlation coefficient, 2013). Hence, it is a useful tool to evaluate the certainty of predictions made from the regression plot. The R^2 value 0.55 means that least 55% of the total variations in AST/ALT*ALP product (y axis) can be explained by the relationship between this product and the liver injury severity score (x axis). The other 45% of the total variation cannot be explained by the plot. This may be due to some of the confounding factors affecting the serum level of the liver enzymes such production by other tissues, individual rat's genetic variation, immunity and nutritional status, etc.

In a review of binge alcohol consumption, Shukla et al (2013) stated that the approximately four years life span of well fed rats is equivalent to about 80 years of a healthy human and chronic alcohol abuse of ten years will relate approximately to 28 weeks in rats. On this basis, the observations of this six weeks study may be expected to occur in approximately over two years of consistent binge alcohol consumption in humans. However, this should be interpreted with caution because sensitivity in human, rat, mice and other animal models is different because of genetic variation in different species and strains.

5. Conclusion

Intermittent binge administration of ethanol has been shown to have profound damaging effects on the liver cells probably as a result of rapid induction of inflammation which may be modelled by a correlation of the De Ritis ratio to histological parameters. When validated and applied to human subjects, the usefulness of this composite predictive model is underscored by the fact that the enzyme assay values are obtained by a minimally invasive procedure of venepuncture. The model lends itself to the clinician as an easily accessible semi-quantitative construct of cellular changes for rapid assessment of the alcoholic liver disease patient. This may obviate the need for more invasive procedures. Furthermore, it will assist in monitoring of therapy by serving as a measure of rate and extent of restoration of cellular functions and structure.

References

- Amanvermez R, Tuncel OK, Tomak L and Alvur M (2009). Effects of chronic high dose alcohol consumption on the general biochemical parameters. *Turk Journal of Biochemistry*, 34(3): 113-120.
- Aroor AR, Jackson DE and Shukla SD (2011). Elevated activation of ERK1 and ERK2 accompany enhanced liver injury following alcohol binge in chronically ethanol fed rats. *Alcoholism: clinical and experimental research*; 35(12): 2128 — 2138.
- Bertola A, Mathews S, Ki SH, Wang H and Gao B (2013). Mouse model of chronic and binge ethanol feeding (the NIAAA model). *Nature protocols*, 8(3):627—637.
- Bertola A, Park O and Gao B (2013). Chronic plus binge ethanol feeding synergistically induces neutrophil infiltration and liver injury: A critical role for E-selectin. *Hepatology*. doi: 10.1002/hep.26419.
- Correlation coefficient (2013). Available from:

- <http://mathbits.com/MathBits/TIsection/statistics2/correlation.htm>. Assessed on 20/1/2013, 5:23pm.
- Das SK and Vasudevan DM (2005). Biochemical diagnosis of alcoholism. *Indian journal of clinical biochemistry*, 20(1): 35-42.
- Ehsan N, Badr MT, Raouf AA and Badra G (2008). Correlation between liver biopsy findings and different serum biochemical tests in staging fibrosis in Egyptian patients with chronic hepatitis C virus infection. *Arab Journal of Gastroenterology*, 9(1): 7-12.
- Kim BK, Kim SA, Park YN, Cheong JY, Kim HS, Park JY et al (2007). Non-invasive models to predict liver cirrhosis in patients with chronic hepatitis B. *Liver international*, 969-976.
- Nanji AA, Jokelainen K, Fotouhinia M, Rahemtulla A, Thomas P, Tipoe GL, Su GL and Dannenberg AJ (2001). Increased severity of alcoholic liver injury in female rats: role of oxidative stress, endotoxin and chemokines. *American Journal of Physiology and Gastrointestinal liver physiology*, 281: 1348-1358.
- Nyblom H, Berggren U, J. Balldin J and Olsson R (2004). High AST/ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking. *Alcohol & Alcoholism*, 39(4): 336-339.
- Pujar S, Kashinakunti SV, Kallaganad GS, Dambal A and Doddamani GB (2010). Evaluation of De Ritis in alcoholic and non alcoholic liver disease - a case control study. *Journal of clinical and diagnostic research*, 4:2463 – 2466.
- Reuben A. (2007). Alcohol and the liver *Curr Opin Gastroenterol*, 23: 283 – 291.
- Shukla SD, Pruett SB, Szabo G and Arteel GE (2013). Binge ethanol and liver: new molecular developments. *Alcoholism: Clinical and Experimental Research*, 37(4): 550—557.
- Siddiqi AI, Siddiqeh M, Mehmood A and Siddiqi AM (2007). Alanine aminotransferase/aspartase aminotransferase ratio reversal and prolonged prothrombin time: a specific indicator of hepatic cirrhosis. *Journal of Ayub Medical College*, 19(3): 22 – 24.
- Sorbi D, Boynton J and Lindor KD (1999). The ratio of aspartase aminotransferase to alanine aminotransferase: potential value in differentiating non-alcoholic steatohepatitis from alcoholic liver disease. *American journal of Gastroenterology*, 94(4): 1018 – 1022.
- Tadic SD, Elm MS, Li HS, Londen GJ, Subbotin VM, Whitcomb DC and Eagon P (2002). Sex differences in hepatic gene expression in a rat model of ethanol induced liver injury. *Journal Applied Physiology*, 93: 1057 - 1068.
- Tilg H and Diehl A M (2000). Cytokines in alcoholic and non-alcoholic steatohepatitis. *New England Journal of Medicine*, 343:1467 -1476.
- Zahr NM, Luong R, Sullivan EV and Pfefferbaum (2010). Measurement of serum, liver, and brain cytokine induction, thiamine levels and hepatopathology in rats exposed to a 4-day alcohol binge protocol. *Alcohol Clinical Experimental Research*, 34(11): 1858—1870.
- Zakhari S and Li TK (2007). Determinants of alcohol use and abuse: impact of quantity and frequency patterns on liver disease. *Hepatology*, 46(6): 2032—2039.