

PROTECTIVE EFFECTS OF GINGER ON ALCOHOL INDUCED STEATOTIC LIVER OF RATS IN LAHORE PAKISTAN

Muhammad Rashid, Sameer Anjum, Ghulam Rasool, Nadia Naseem, Abdul Hannan Nagi

Department of Morbid Anatomy Histopathology University of Health Sciences Lahore Pakistan

ABSTRACT

Objective: To observe the histological and biochemical alterations that may occur in the liver tissue of rat by ethanol intoxication and to investigate the role of Ginger supplementation against these alterations.

Study design: Experimental study.

Place & duration of study: Department of morbid anatomy and Histopathology, University of health sciences Lahore from January to December 2016.

Materials and methods: Thirty rats were divided into three Groups 1, Group 2, and Group 3. Group 1 which was a negative control group, was kept on normal routine diet. Group 2, positive control was fed with 5% w/w Alcohol (Ethanol). Group 3 which was experimental group was fed with Ginger 100mg/kg body weight thrice a week in addition to Alcohol. Experiment continued for four weeks. Blood samples were taken at day 0 and 29 at the end of experiment. Serum parameters and liver histopathology was done before and after the end of experiment to see Hepatic lobular architecture, Hepatocyte morphology, Inflammation, Stromal reaction, Steatosis, Central vein and Fibrosis.

Results: Positive control group 2 animals showed significant alteration in the liver histology due to Alcohol intoxication as compared to negative control group 1 animals. Experimental group 3 showed abrogation of toxic effects induced by Alcohol intoxication. Similarly, positive control group 2 revealed significant elevation in the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and triglycerides (TG). Serum levels of low density lipoprotein (LDL) Cholesterol was significantly reduced in G 2 animals. Experimental group G3 animals showed significant reduction in serum levels of ALT, AST and TG. There was no significant difference in serum LDL cholesterol level of Group 3 as compared to positive control group 2.

Conclusion: Alcohol (Ethanol) induces liver parenchymal injury in the form of inflammation, distortion of hepatic lobular architecture and fatty change in the rat liver. Herbal supplement has hepatoprotective effect against hepatotoxicity induced by Alcohol.

Key words: Alcohol, Alcoholic hepatitis, Steatosis, Luteolin

This article can be cited as: Rashid M, Anjum S, Rasool G, Naseem N, Nagi AH. Protective effects of ginger on alcohol induced steatotic liver of rats in Lahore Pakistan. Pak J Pathol. 2017; 28(2): 77-84.

INTRODUCTION

Alcohol was previously consumed in developed countries on a large scale. It was considered a huge burden on budget to treat alcohol related diseases. From a few decades, in developing regions of the world alcoholism is becoming a rising trend and significant population is developing alcohol related diseases [1, 2]. Health is not the only thing affected by drinking but social aspects are also affected by drinking [3]. Alcohol has wide range of

effects on human body and all these effects are dose and time dependent. Alcohol has tendency to accumulate in different organs of the body like brain, gastrointestinal tract, heart and liver where it causes neurological, gastrointestinal, cardiac, metabolic and hepatic symptoms [4].

Alcoholic Liver Disease: Liver is one of the major organ damaged by alcohol drinking. Alcoholic liver disease is the 12th major cause of death in the United States and death rates are higher in France and Spain. World health organization's global data base exists and can be used to measure the world-

Correspondence: Dr. Muhammad Rashid, Department of Morbid Anatomy Histopathology University of Health Sciences, Lahore Pakistan

Email: malik_rashid111@yahoo.com

Received: 17 Mar 2017; Revised: 29 Apr 2017; Accepted: 27 May 2017

wide patterns of alcohol consumption and to look into morbid conditions and mortality related to drinking.

Alcoholic liver disease encompasses a wide range of spectrum in the form of symptoms and histology. There are basically three stages of alcoholic liver disease like steatosis, hepatitis and more advance fibrosis and cirrhosis [5]. On one hand, there is steatosis due to lipid accumulation which is a reversible phase with abstinence. Second phase of disease spectrum is alcoholic hepatitis which can also be reversed with abstinence. Last stage of disease is fibrosis and cirrhosis which is an irreversible stage and has poor prognosis [6].

Pathophysiology of Alcoholic Liver Disease: Alcohol affects almost every organ of the body either on subclinical level or it leads to frank organ failure. While considering the pathophysiology of liver disease, there are many pathways linked to the pathogenesis of alcoholic liver disease. Most significant of them are lipogenic pathway and free radical injury pathway.

Lipogenic pathway: Two main lipogenic pathways mainly triggered by alcohol ingestion in animal model of study are sterol regulatory element-binding protein (SREBP-1) activation and adenosine monophosphate (AMP) activated protein kinase (AMPK) inhibition. AMPK is inhibited but SREBP-1 is activated due to alcohol intoxication and influences the up-regulation of lipogenic genes and lipids accumulation in the liver parenchymal cells [7].

Reactive oxygen species pathway (ROS): Free radicals like reactive oxygen species produced in the body due to metabolic pathways are eliminated from the body through homeostasis. Liver cells have scavenger system like superoxide dismutase and

many other pathways to neutralize the ROS. Metabolic pathways that utilize alcohol to metabolize it, produce excessive amount of ROS. These excessive levels of ROS lead to oxidative stress that leads to cell injury [9]. ROS have different mechanisms to disrupt the normal physiological function and integrity of cell. ROS can modify the functions of signaling pathways regulating the metabolism of lipids, carbohydrates and proteins. In addition to this, ROS can directly modify the function of proteins and react with DNA. By neutralizing the oxidative stress produced due to excess ROS, hepatocellular injury can be hampered [8].

Role of mitochondria: One of the most important organelle in priming and sensitizing the effects of alcohol is mitochondria. Mitochondria not only provide energy to cells for its normal physiological functions but they also provide scavenger system to neutralize reactive oxygen species. In context to this view experimental work was done on rats. It showed that mitochondria isolated from alcohol fed rat cells produced more ROS [10]. Depletion of mitochondrial glutathione (GSH) in alcohol fed rat liver cells was found in mitochondria and cytosol [11].

MATERIALS & METHODS

Study design: This is an experimental model of study conducted at the experimental research laboratory of university of health sciences Lahore.

Study setting: Animals were divided into three groups with a weight range of 150-200 grams. Each group was acclimatized to the laboratory environment and enough sun light exposure was given. All the research work related to animal feeding

was done in animal research laboratory of university of health sciences (UHS).

Preparation of ginger extract: Almost 2-kilogram fresh ginger roots were prepared for extraction. Ginger was cut into small pieces and dried in sun light. It was crushed into powder form and soaked in distilled water. Excess water was evaporated by Heidolph rotary evaporator and dry extract in the form of powder was prepared. All the setting was done in pharmacology department of UHS Lahore.

Sample size: A total of 30 male Wister rats were obtained from experimental research laboratory of university of health sciences Lahore. Their weight range was 150-200gm and they were divided into 3 groups each containing 10 animals.

Negative control group (G 1): Negative Control group containing 10 rats was separated and given a normal routine diet.

Positive control group (G 2): This group included 10 rats and was given 5% w/w 6g/kg body weight alcohol for 4 weeks daily through oral lavage.

Experimental group (G 3): This group included 10 rats and was given 5% alcohol 6g/kg body weight daily along with Ginger extract (100mg/kg) 3 times a week for 4 weeks through oral lavage.

Laboratory Processing

Experimental schedule: All rats were weighed before the start of experiment. All rats were given nutritionally standard diet and water. Rats were kept in separate cages. Each rat was marked for its identification by giving a number on its back. Before

the start and after the end of experiment blood was drawn through heart puncture for pre and post experiment serum parameters like ALT, AST, TG and LDL Cholesterol.

Method of determining serum levels of parameters: All the serum parameters ALT, AST, TG and LDL Cholesterol were measured by using commercially available kits (HUMAN, GERMANY) with the help of chemistry analyzer. LDL cholesterol was measured by indirect method with the help of Friedewald equation [12].

STATISTICAL ANALYSIS

Data was analyzed using SPSS 24 (Statistical package for Social Sciences). Mean \pm SD was calculated for quantitative variables like ALT, AST, TG & LDL Cholesterol. Qualitative variables like hepatic lobular architecture, hepatocyte morphology, inflammation, steatosis, central vein congestion, and fibrosis were given in frequencies and percentages.

One-way ANOVA test was applied to observe the mean differences between quantitative variables of groups. A p-value of <0.05 was considered statistically significant.

Fischer's exact test was applied to compare qualitative variables. P value of <0.05 was considered significant.

RESULTS

Histological Parameters

Negative control group (G 1): Histological examination of the negative control group animal livers had preserved Lobular architecture of parenchyma. No ballooning degeneration and fatty change was seen in hepatocytes. Portal triad was

containing branch of bile duct, portal vein and hepatic artery. Liver stroma was unremarkable. There was no evidence of inflammation in the portal, Periportal and

in liver parenchyma. Central veins were not congested and there was no fibrosis and cirrhosis.

Table-1: Histological parameters with their frequencies, percentages in groups and P values.

Histologic variables	Positive control group G 2 N(%)	Experimental group G 3 N(%)	P value
Preserved Hepatic lobular architecture	5(50%)	7(70%)	0.028*
Ballooning degeneration	10(100%)	10(100%)	0.025*
Apoptosis	8(80%)	4(40%)	0.075
Multi-binucleat cells	8(80%)	8(80%)	0.075
Inflammation	10(100%)	7(70%)	0.150
Steatosis	10(100%)	10(100%)	.166
Central vein congestion	6(60%)	4(40%)	0.383
Fibrosis	0(0.0%)	0(0.0%)	1.000

P value less than 0.05 was considered significant.

Table-2: Comparison of post experimental mean serum levels of parameters in G 2 and G 3.

GROUP	ALT (IU/L)	AST (IU/L)	TG (mg/dl)	LDL cholesterol (mg/dl)
Positive control group G 2	65.55 ± 8.39	231 ± 38.99	144 ± 28.89	3.13 ± 11.45
Experimental group G 3	40.62 ± 7.95	182.3 ± 15.82	107 ± 29.66	11.74 ± 9.33
P value	<0.00001*	0.001792*	0.010472*	0.091219

P value of less than 0.05 was considered statistically significant.

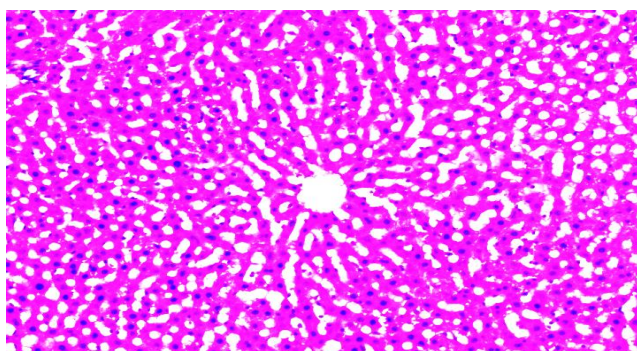


Figure-1: Photomicrograph of liver from negative control group (G 1) showing preserved hepatic architecture in which cells are radiating in the form of cords from central vein and there is no central vein congestion (H & E 40X).

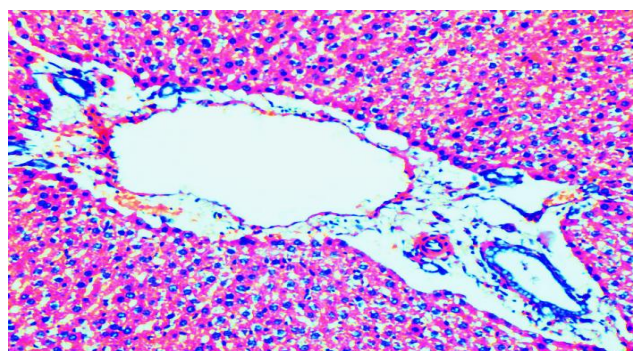


Figure-2: Photomicrograph of liver from negative control group (G 1) showing portal triade with no inflammatory cell infiltrate (H & E 40X).

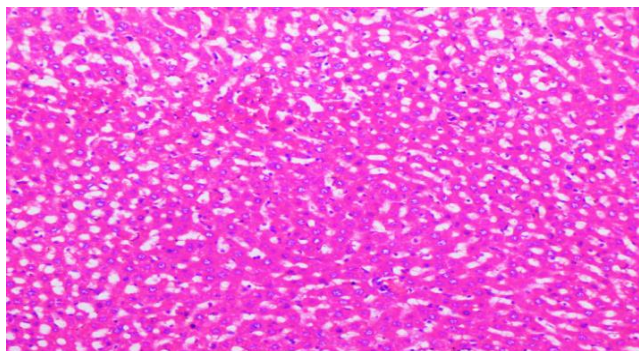


Figure-3: Photomicrograph of liver from negative control group G 1 showing normal hepatocytes without steatosis (H & E 40X).

Positive control group (G 2): Positive control group animals showed 70% lobular architecture distortion and 100% animal livers had Steatosis, 60% having focal and 40% diffuse type. Steatosis was both micro and macrovesicular type. Positive control group animal livers revealed chronic inflammatory cell infiltrate in portal triade in 100% cases, binucleat cells in 80% of cases. All animals showed ballooning degeneration in which cells swell 2-3 times their original size with central nucleus and cytoplasmic clearing. None of the animal liver showed stromal reaction in the form of fibrosis and septa formation. About 60% of animal livers showed central vein congestion.

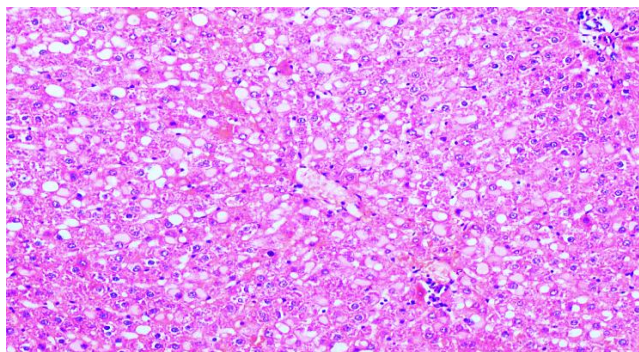


Figure-4: Photomicrograph of liver from positive control group (G 2) showing hepatic architecture distortion and fatty change in liver cells (H & E 40X).

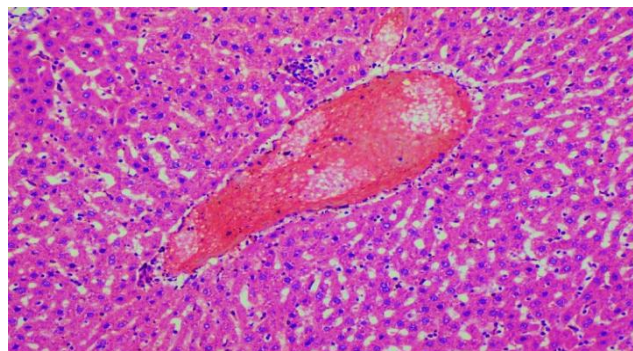


Figure-5: Photomicrograph of liver from positive control group (G 2) showing central vein congestion and mild inflammation (H & E 40X).

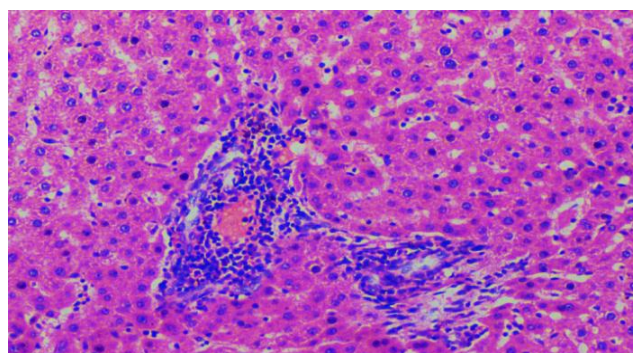


Figure-6: Photomicrograph of liver from positive control group (G 2) showing portal triade inflammatory cell infiltrate (H & E 40X).

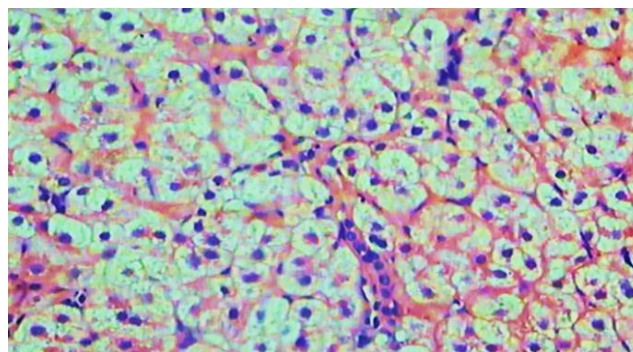


Figure-7: Photomicrograph of liver from positive control group (G 2) showing ballooning degeneration of liver hepatocytes (H & E 40X).

Experimental group (G 3): Experimental group animal livers showed protective effects of Ginger feed. Hepatic lobular architecture was preserved in Ginger feed group as compared to G2. Preserved architecture was observed in 70% cases

as mentioned in table 1. Central vein congestion was also decreased in this group as compared to G2. Steatosis was present in all groups but it was focal in 70% cases as compared to G2 in which steatosis was more in diffuse form. G3 animals showed reduction in inflammation to 70%.

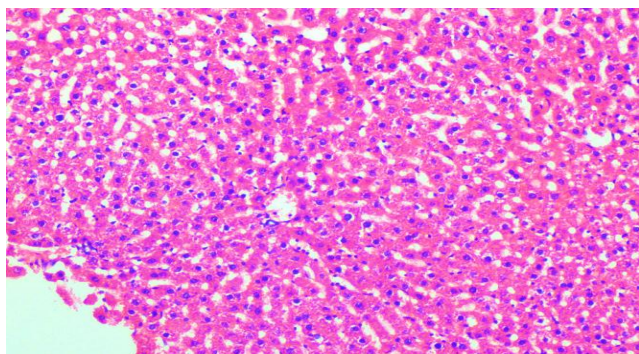


Figure-8: Photomicrograph from the liver of experimental group (G 3) showing relatively preserved hepatic lobular architecture (H & E 40X).

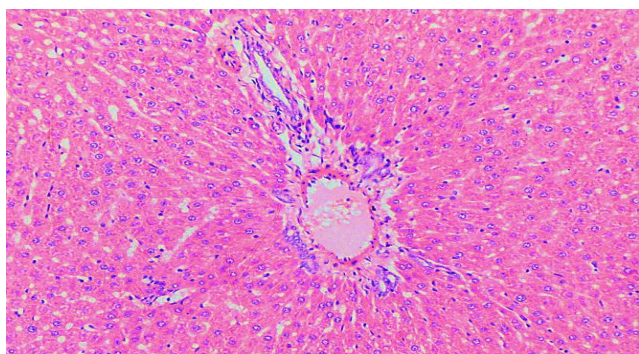


Figure-9: Photomicrograph of liver from experimental group (G 3) showing no inflammatory cell infiltrate in portal triade (H & E 40X).

BIOCHEMICAL PARAMETERS

Serum levels of ALT, AST, TG and LDL cholesterol were measured before and after the end of experiment. There was no significant difference in the serum levels of these parameters before the start of experiment but there was significant difference in serum levels of these parameters in Negative control

group G 1 and Positive control group G 2 after the completion of experiment. Similarly, experimental group G 3 which was fed with Ginger in addition to Alcohol showed significant reduction in the serum levels of ALT, AST and TG. There is no significant difference the serum levels of LDL cholesterol at the end of experiment.

DISCUSSION

The aim of present study was to see role of Ginger on alcohol induced steatotic liver of rats and altered serum biochemical profile. Only male rats weighing 150-200 gram were included in this study to avoid any gender related variability. Duration of experiment was 4 weeks. Positive control group G2 showed variable changes in liver histology due to Alcohol feeding. Early phase of Alcoholic liver disease was developed in which different histological parameters showed variable percentages and frequencies. G2 showed statistically significant variation in liver histology due to Alcohol intoxication with the P value of less than 0.05. About 70% of animals showed distorted lobular architecture, 100% of cases showed ballooning degeneration, 80% revealed apoptosis, and 80% revealed multi-binucleat cells. There was no stromal reaction in any case. Chronic Inflammation (Periportal) was seen in 100% of cases, which was mild in nature. Parenchymal inflammation was also seen in 50% of cases. Steatosis (fatty change) was seen in 100% of cases in which 60% was focal and 40% diffuse type table 1.

Experimental group G3 which was fed with Ginger in addition to Alcohol when compared with positive group animals significantly abrogated liver parenchymal injury, inflammation and steatosis Liver injury and lipid accumulation was reduced significantly [13]. Oxidative stress induced by alcohol ingestion, results in cell injury and resultant

inflammation, Ginger supplement reduced the severity of liver parenchymal injury by preservation of hepatic lobular architecture and reducing the serum levels of ALT [14]. Experimental group serum parameters were compared with G 2. There was a significant reduction the serum levels of ALT, AST, TG with the p value of <0.00001, <0.001792, 0.010472 respectively which is statistically significant. There was no major difference the serum LDL cholesterol levels of G3 when compared with G 2 with the P value of 0.091219 which is statistically insignificant as mentioned in table 2.

Our results are similar to the results of Bhandari *et al.*, 2003; Shati and Elsaid, 2009 [16, 17] in which they showed that Ginger extract not only have histological evidence of hepatoprotective effects but it also decreases the serum levels of liver enzymes. Lai *et al.*, in 2016 [13] also revealed similar results in their animal study that serum levels of TG were significantly reduced when Ginger oil was given to mice along with high fat diet.

CONCLUSION

Alcohol (Ethanol) induces liver parenchymal injury in the form of inflammation, distortion of hepatic lobular architecture and fatty change in the rat liver. Herbal supplement has hepatoprotective effect against hepatotoxicity induced by Alcohol. Ginger as a potent antioxidant and anti-inflammatory agent, not only corrects elevated serum biochemical parameters (ALT, AST and TG) due to Alcohol toxicity but it also ameliorates the toxic effects of Alcohol on liver histology. The lipolytic properties of this herbal supplement lead to lipid lowering effect in serum as well as abrogates the fatty change in liver parenchyma.

AUTHORS CONTRIBUTION

Muhammad Rashid: Concept, design, data collection, and manuscript writing.

Abdul Hannan Nagi: Concept, design, manuscript revision.

Nadia Naseem: Concept, design, manuscript revision.

Sameer Anjum: Histopathological lab work-up.

Ghulam Rasool: Histopathological lab work-up.

REFERENCES

1. Ezzati M, Lopez AD, Rodgers A, Vander Hoorn S, Murray CJ. Selected major risk factors and global and regional burden of disease. *The Lancet*. 2002; 360(9343), 1347-60.
2. World Health Organization, 2014. Global status report on alcohol and health. World Health Organization.
3. Brewer RD, Swahn MH. Binge drinking and violence. *Jama*. 2005; 294(5):616-8.
4. Vonghia L, Leggio L, Ferrulli A, Bertini M, Gasbarrini G, Addolorato G. Alcoholism treatment study group. Acute alcohol intoxication. *European J of Internal Medicine*. 2008; 31; 19(8): 561-7.
5. MacSween RNM, Burt AD. Histologic spectrum of alcoholic liver disease. In *Seminars in liver disease*. 1986; 6(03):221-32).
6. Aldahmash BA, El-Nagar DM. Histological study on the hazardous effects of ethanol on liver and spleen in Swiss albino mice. *J of Society for Development in New Net Environment in B & H*. 2013; 7(8): 2445-52.
7. You M, Crabb DW. Molecular mechanisms of alcoholic fatty liver: Role of sterol regulatory

- element-binding proteins. Alcohol. 2004; 34(1): 39-43.
8. Stoilova I, Krastanov A, Stoyanova A, Denev P, Gargova S. Antioxidant activity of a ginger extract (*Zingiber officinale*). Food chemistry. 2007; 102(3): 764-70.
9. Wu D, Cederbaum AI. Alcohol, oxidative stress, and free radical damage. Alcohol Research and Health. 2003; 27: 277-84.
10. Kukielka E, Dicker E, Cederbaum AI. Increased production of reactive oxygen species by rat liver mitochondria after chronic ethanol treatment. Archives of Biochemistry and Biophysics. 1994; 309(2): 377-86.
11. Fernandez-Checa JC, Ookhtens M, Kaplowitz N. Effects of chronic ethanol feeding on rat hepatocytic glutathione. Relationship of cytosolic glutathione to efflux and mitochondrial sequestration. J of Clinical Investigation. 1989; 83(4): 1247.
12. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry. 1972; 18(6): 499-502.
13. Lai YS, Lee WC, Lin YE, Ho CT, Lu KH, Lin SH, et al. Ginger essential oil ameliorates hepatic injury and lipid accumulation in high fat diet-induced nonalcoholic fatty liver disease. J of Agricultural and Food Chemistry. 2016; 64(10): 2062-71.
14. El-Ghonaimy NM. Role of ginger (*zingiber officinale*) against metalaxyl induced hepatotoxicity in male albino rats: a histological and immunohistochemical study. J of Histology & Histopath. 2015; 2: 1-10.
15. Gupta S, Figueredo VM. Alcohol and lipids. OA Alcohol. 2014; 2(1): 3-15.
16. Bhandari U, Shamsheer AA, Pillai KK, Khan MSY. Antihepatotoxic activity of ginger ethanol extract in rats. Pharmaceutical biology. 2003; 41(1): 68-71.
17. Shati AA, Elsaid FG. Effects of water extracts of thyme (*Thymus vulgaris*) and ginger (*Zingiber officinale* Roscoe) on alcohol abuse. Food and Chemical Toxicology. 2009; 47(8): 1945-9.