Study of Prophylactic Action of Linseed Oil against Acetaminophen Induced Oxidative Stress and Liver Damage in Albino Rat

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Abstract: ***The protection conferred by linseed oil against acetaminophen (APAP) induced oxidative stress and liver damage has been evaluated in adult albino rat. Hepatotoxicity has been evaluated with the indication of elevated levels of enzymatic activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and bilirubin level in acetaminophen administered values as compared with control values. Co-treatment with linseed oil significantly restored the elevated levels of hepatic serum markers ALT, AST and ALP. This may result from cellular leakage due to peroxidative damage of the membrane. Significant reduction in antioxidant activities of SOD, GSH, CAT and GPx were obtained as compared with control values. At normal conditions, the body defense mechanism against oxidative stress, marked by endogenous antioxidant enzymes, such as SOD, GSH, CAT and GPx prevent cells damages induced by free radicals. The protective effects of linseed oil are dose and tissue-dependent, due to differences in the inherent susceptibility of each tissue in rat. Remarkable elevation in bilirubin level and reduced level of cholesterol in comparison to control group of rats were found in the study. While lowered the bilirubin level and higher the cholesterol level when compared with APAP treated group is attributed to hepato-cellular damage. Linseed supplementation in group III has returned the levels of bilirubin and total cholesterol to almost the same levels as the control while cholesterol decreased slightly below the control. The elevated bilirubin level in liver implies the overwhelming influence of oxidative stress generated by the APAP and likely due to the failure of antioxidant defense mechanisms. Importantly, linseed oil lowered the changes in total cholesterol level in the combination of acetaminophen. Acetaminophen-induced liver injury has also been marked by lipid oxidation. Thus, the augmented antioxidant enzymes levels in liver tissues of rat treated with linseed oil implies and justifies the antioxidant property of the oil.***

Keywords: Acetaminophen, oxidative stress, liver damage, Linseed oil

1. Introduction

Plant-derived products have attracted a lot of attention of many researchers today who find their medicinal potential for the treatment of various diseases. Now a days, most of the people believe solely on plant products as medicine to cure the diseases. Linseed oil is an antioxidant substance which derived from the dried rip seeds of flax plant (*Linum usitatissimum*, Linaceae). It contains 52 to 63% alpha Linolenic acid. The linolenic acid has beneficial effects in reducing inflammation leading to atherosclerosis (Thompson and Cunnane, 2003). Linseed oil may reduce cardiovascular risk through platelet function and inflammation (Mozaffarian, 2005). It has positive effect on femur bone mineral content, bone mineral density and lumbar vertebrae (Cohen *et al*., 2005). Linseed oil supplementation found to achieve a greater reduction in lung cancer and metastases (Chen *et al*., 2005). G. N. Rao (2000) found that high dose of linseed oil can also delay in the mammary cancer.

Acetaminophen, (N-acetyl-p-aminophenol, paracetamol, APAP) is an important antipyretic, analgesic and effective therapeutic medicine (Prescott, 2003). High dose of acetaminophen induces liver toxicity, which is usually characterized by chest pain, vomiting, diarrhoea, and sometimes shock in mammals. Oxidative stress is attributed to hepatotoxicity in mammals. Liver injury by the overdose of acetaminophen is caused by increased production of reactive oxygen species (ROS), antioxidant (glutathione) depletion and cytokine up-regulation (Dahlin *et al*., 1984; Ferret *et al*., 2001 and James *et al*., 2003). Hepatic failure, myocardial and renal dysfunction have also been reported in excessive ingestion of acetaminophen. APAP-induced hepatotoxicity is as a result of the formation of a reactive metabolite N-acetyl-P-amino-benzoquinone imine (NAPQI), which then deplete glutathione (GSH) level (Kumar *et al*., 2015 and Cristani *et al*., 2016). Inhibition of nitric oxide synthetase (NOS) in mitochondria, significant increase in manganese superoxide dismutase (MnSOD) nitration and Alanine aminotransferase (ALT) release during hepatotoxicity were found by Rakhi Agarwal *et al*., 2012. Acetaminophen is metabolized by cytochrome P-450 enzyme of the liver and detoxified by glucuronidation as well as sulfation. Depleted glutathione level permits the binding of free NAPQI to other thiol-containing compounds and a variety of cellular proteins, provoking oxidative stress and oxidative damage leading to cellular necrosis (Moshaie *et al*., 2018). Due to dose-dependent toxicity caused by APAP, APAP-induced hepatic damage can be studied in animal models and most mechanisms can be correlated to human being (Woolbright and Jaeschke, 2017). Several lines of evidence have implicated N-acetylcysteine (NAC) as the best therapeutic option to combating liver failure due to APAP toxicity. However, Clinical studies reveal untoward side effects of acetaminophen (Smilkstein *et al*., 1988 and Lee *et al*., 2015).

Liver plays an important role in the regulation of various physiological processes in the body such as carbohydrate metabolism and storage, fat metabolism, bile juice synthesis, and so forth besides being the most important organ involved in the detoxification of various drugs as well as xenobiotics in our body (Sharma *et al*., 2011). It is highly susceptible to damage by xenobiotics owing to its continuous exposure to these toxicants via the portal blood circulation (Pineiro and Pineiro, 2004). The liver is an important target for the toxicity of drugs in terms of oxidative stress. Various chemicals, like carbon tetrachloride (CCl_4), tert-butyl hydroperoxide (t-BHP), alcohol, paracetamol, galactosamine-N (Gal-N), and others can cause potential damage to the liver cells leading to progressive dysfunction. Most of the hepatotoxic chemicals cause damage to the hepatocytes by inducing lipid peroxidation. One of the most important liver toxicity mechanisms might be a consequence of cell damage by reactive oxygen species (ROS) and Reactive nitrogen species (RNS). Kupffer cells release ROS, cytokines, and chemokines, which induce neutrophil extravasations and activation. Also the liver expresses many cytochrome P450 isoforms, including ethanol-induced CYP2E1. CYP2E1 generates ROS, activates many toxicologically important substrates, and may be the central pathway by which some substances (ethanol, carbon tetrachloride, etc.) cause oxidative stress (Subramoniam and pushpangadan, 1999). Experimental liver injuries are induced by specific toxic compounds, because the formation of ROS is stimulated by a number of xenobiotics. Glutathione (GSH) plays an important role in protecting cells from electrophilic compounds and free radicals such as reactive oxygen species generated during cellular metabolism. Reduced glutathione can act as a reductant, reducing hydrogen peroxide and lipid hydroperoxides directly to H_2O, a reaction catalyzed by GSH-Px (Amar and Schiff, 2007). Depletion of intracellular GSH, under conditions of continuous intracellular oxidative stress, leads to oxidation and damage of lipids, proteins, and DNA by the reactive oxygen species (Aleksunes *et al*., 2008; Ruepp *et al*., 2002). Oxidative stress is an imbalance between the production and scavenging of reactive oxygen and nitrogen species (ROS and RNS) and free radicals that can induce lipid peroxidation, DNA fragmentation, and protein oxidation (Nencini *et al*., 2007). These damages result in the loss of membrane integrity, structural and functional changes in proteins, and gene mutations. Thus, the disorders associated with liver are numerous and varied. A number of models of hepatic disorders support the notion that ROS have a causal role in liver injuries. Therefore, this study was designed to determine the possible protective effects of linseed oil on acetaminophen-induced liver injury and regeneration, evaluating its ability to reduce oxidative stress after acetaminophen administration in rats.

2. Materials and Methods

2.1 Animal selection

A colony of 50 Adult (6-8 week old) Swiss albino rat (*Rattus norvegicus*) of body weight 160–200 gm have been selected from Animal Care Centre, Department of Zoology, University of Rajasthan, Jaipur. The colony has been taken care in compliance with the 'Principles of Laboratory Animal Care' formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals, prepared by the Academy of Sciences, 1996. Rats were kept in a 12-hour light/dark cycle, at a temperature of 22°C ± 2°C with relative humidity 50% ± 20%, and ventilated with filtered non-recycled air. They were fed on standard chow pellets which were purchased from Satya narayan Shriram kirana store, Jatia bajar, Sikar and tap water for the entire test period. The experimental procedure was approved by the local ethics committee, of Pandit Deendayal Upadhyay Shekhawati University, Sikar (Rajasthan) and all the experiments were designed and performed in the Department of Zoology, S. K. Govt. Girls College, Sikar.

2.2 Chemicals and reagents

Linseed oil was purchased from Khandelwal general store, dadi gate, Sikar. Acetominophen (APAP) and other chemicals used in this study were of high analytical grade and Kits for cholesterol, triglyceride and HDL-cholesterol, Kits for high sensitivity TNF-α kits, Quantikine Immunoassay kits, and PON-1 kit were purchased from Suthar chemicals, Sikar.

2.3 Experiment

Linseed oil (LSO) has been given orally to rat. Different concentrations of linseed oil were given to the rat for the selection of optimum dose in terms of ml/kg body weight. After supplementation of 15 days, sublethal concentration (0.5 mg/L) of pro-oxidant acetaminophen (APAP) has been injected to the animal. The survivability and body weight were checked up to 15 days. The dose of acetaminophen which produced 50% mortality within 30 days was estimated. The group which showed maximum survivability and healthier condition were selected for the study.

Swiss albino mice have been divided in to 3 groups.

a) Control group, which were not received any other treatment.
b) Second group were administered with single dose of acetaminophen or APAP.
c) Third group of were co-exposure of (APAP+LSO).

Mice were sacrificed at various intervals ranging between 1-15 days. Immediately after exsanguinations, the livers were removed, ringed, cleaned and weighed. Small portions of the livers were kept frozen at −80°C in order to analyze other antioxidant enzyme activity and estimation of bilirubin and cholesterol level.

2.4 Observation

Mice liver were studied time to time for qualitative and quantitative parameters color, weight of liver, histopathological and biochemical analysis by standard methods in Rajiv pathological laboratory and Rathi hospital and diagnostic center, Sikar. All serum samples were processed for the determination of the enzymatic activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), using a spectrophotometric method. Antioxidant level of SOD, GSH, CAT, GPx, were determined in serum samples with oxidative stress ELISA kit Art. No. K7960; Immundiagnostik AG, Bensheim, Germany). To estimate the Bilirubin and cholesterol level, a random-access chemistry analyser (RA-1000) was used. At least three random visual fields from each animal were scored in a blinded manner by two expert pathologists.

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Statistical analysis

The results are expressed as means ± SEM. All values were obtained from at least five animals. The obtained data were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran (1980). Least significant differences ($p<0.05$) were used to compare between means of treatment according to Walter and Duncan (1969) at probability 5%.

3. Results and Discussion

Oxidative stress is an important mechanism that has been implicated in acetaminophen toxicity. Exposure of rats to acetaminophen caused a significant elevation of hepatic serum markers ALT, AST and ALP (121.07%, 113.82% and 124.15% respectively) in comparison with the control group (Table-1 and figure-1& 2). This may result from cellular leakage of these enzymes into cytoplasmic circulation and peroxidative damage of the membrane produced evidence of hepatotoxicity. Acetaminophen or APAP toxicity causes the corresponding destruction of hepatocytes and thus resulting in the elevation of serum enzymes (Alkiyumi *et al*., 2012). Co-treatment with linseed oil significantly restored the elevated levels of hepatic serum markers ALT, AST and ALP (i.e. 103.34%, 101.40% and 100.53% respectively as in table-1). These levels affect and improved the basal antioxidant status of the cell.). Thus compounds that ameliorate oxidative stress can cause an improvement on oxidative damage to the liver (Figure- 3A, B and C). These biochemical restorations may be due to the inhibitory effects of the linseed oil on cytochrome P450 or/and promotion of APAP glucuronidation (Cavin *et al*., 2001). In the present study 250 mg/kg acetaminophen caused acute liver injury which was characterized by increased serum activity.

Significant reduction in antioxidant activities of SOD, GSH, CAT and GPx (43.83%, 45.20%, 70.28% and 81.67%) were obtained as compared with control values (Table-1 and figure-1&2). At normal conditions, the body defense mechanism against oxidative stress, marked by endogenous antioxidant enzymes, such as SOD, GSH, CAT and GPx prevent cells damages induced by free radicals (Prakash *et al*., 2001 and Gini & Muraleedhara, 2010). The protective effects of linseed oil are dose and tissue-dependent, probably because of differences in the inherent susceptibility of each tissue in rat. Remarkable elevation in bilirubin level (137.16%) and downward level of cholesterol (77.95%) in comparison to control group of rats were found (Table-1 and figure-1& 2) in the study. While lowered the bilirubin level (103.54%) and higher the cholesterol level (95.07%) when compared with APAP treated group is attributed to hepato-cellular damage (Figure-3B) . Linseed supplementation in group III has returned the levels of bilirubin and total cholesterol to almost the same levels as the control while cholesterol decreased slightly below the control. The elevated bilirubin level in liver implies the overwhelming influence of oxidative stress generated by the APAP and likely due to the failure of antioxidant defense mechanisms. Importantly, linseed oil lowered the changes in total cholesterol level in the combination of acetaminophen. Bilirubin is a conventional indicator of liver diseases (Achliya, 2004). Mounting evidence from previous studies Das *et al.*, (2007) and Biswas *et al*., (2000) supports what we found in the present study that acetaminophen-induced toxicity invoked elevated bilirubin level, depleted GSH level. After administration of linseed oil the deleterious effect of acetaminophen were significantly ameliorated in comparison to the acetaminophen group. Overall, these ameliorative potentials of linseed oil confirm the hepatoprotective effect of the oil (Figure-3C).

Therefore, measuring the levels of serum hepatic markers such as ALT, AST, ALP, total bilirubin and total cholesterol is vital for the identification of liver damage (Green *et al*., 2010 and Freitag *et al.*, 2015). Thus, increased formation of superoxide would result in hydrogen peroxide generation and peroxidation reactions (James *et al.*, 2003). Acetaminophen-induced liver injury has also been marked by lipid oxidation. Thus, the augmented antioxidant enzymes levels in liver tissues of animals treated with linseed oil implies and justifies the antioxidant property of the oil. As far as dose is concerned, author used the dose of linseed oil that was effective in acetaminophen-induced injury, but it proved inadequate for the acetaminophen intoxication model. Because author did not measure plasma concentrations, he is not able to conclude whether the sublethal dose reached effective plasma levels. Therefore, further studies examining the effect of different linseed oil doses on acetaminophen-induced liver injury are needed in order to investigate whether this oil can protect liver in this situation.

4. Conclusion

This study highlights the hepatoprotective effect of linseed oil improving defense mechanism of liver against oxidative stress believed to be caused by acetaminophen. The hepatoprotective effect of linseed oil may be associated with a reduction of oxidative stress and significant impact on liver inflammation.

Conflicts of interest: The authors declare that there is no conflict of interests regarding the publication of this research paper.

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Tables and Figures

Table 1: Antioxidant enzymes ALT, AST, ALP, SOD, GSH, CAT, GPx activities and bilirubin and cholesterol in control and experimental groups

Parameters (mg/dl)	Control Group [Group-I]	Single dose of APAP [Group-II]	Difference with control (In %)	APAP+Linseed oil Combined treated [Group-III]	Difference of Group-III with control
ALT activity	34.11±3.37	41.30±2.21	121.07%	35.25±2.54	103.34%
AST activity	159.20±13.12	181.20±1.34	113.82%	161.43±3.33	101.40%
ALP activity	133.30±6.23	165.50±1.20	124.15%	134.01±3.62	100.53%
SOD activity	7.30±0.47	3.20±0.41	43.83%	6.88±1.11	94.24%
GSH activity	21.16±0.27	9.52±0.21	45.20%	18.18±2.10	85.91%
CAT activity	4.24±0.19	2.98±0.18	70.28%	3.86±0.19	91.03%
GPx activity	34.55±1.21	28.22±1.20	81.67%	34.48±2.0	99.79%
Bilirubin level	41.20±4.08	56.51±3.11	137.16%	42.66±4.45	103.54%
Cholesterol level	65.60±3.26	51.14±4.84	77.95%	62.37±4.02	95.07%

Where ALT: Alanine amino transferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, SOD: Superoxide dismutase, GSH: Glutathione, CAT: Catalase, GPx: Glutathione peroxidase

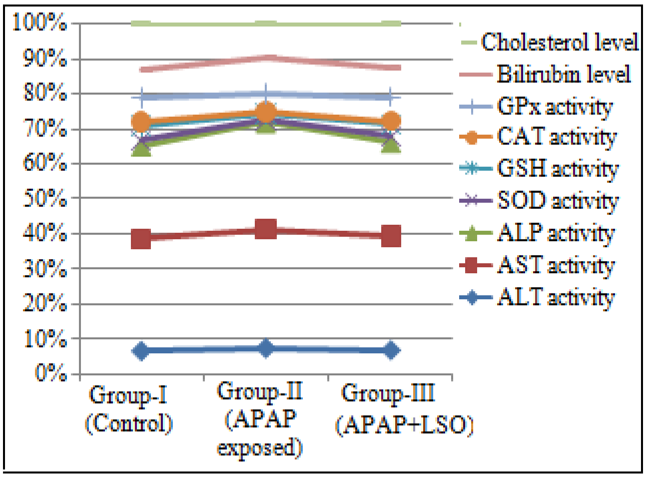
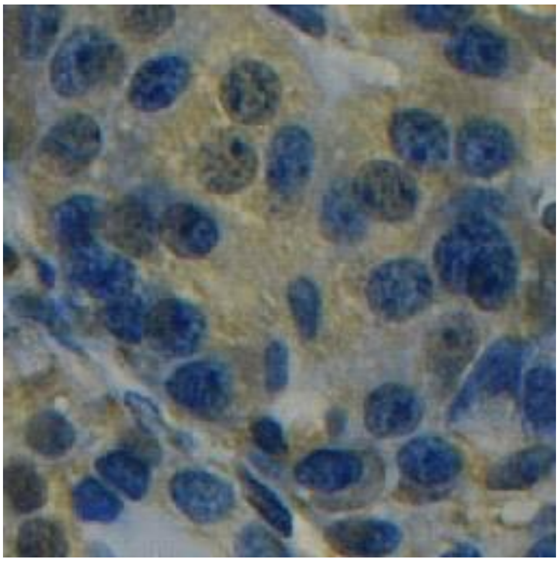


Figure 1: Hundred percent stacked line chart showing comparative study of ALT, AST, ALP, SOD, GSH, CAT, GPx activities and bilirubin and cholesterol levels in control and exposure groups.



A. Normal hepatocytes of control group

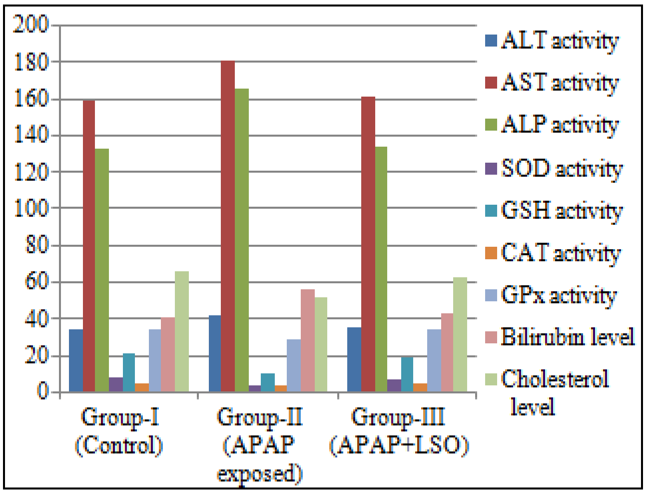
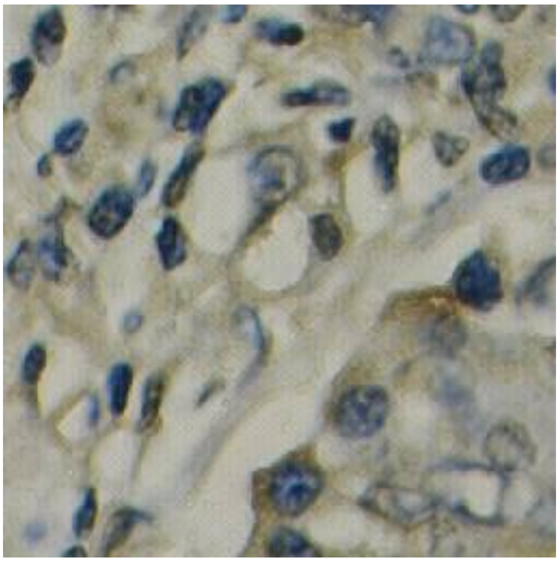
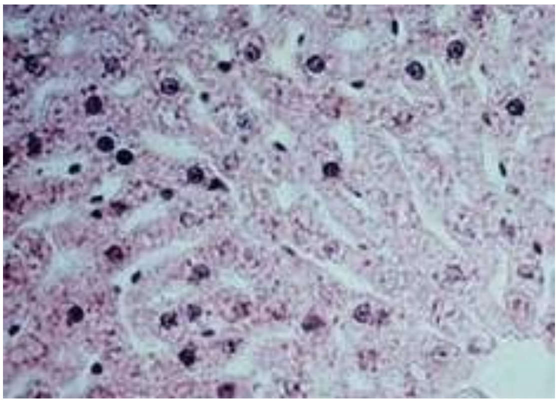


Figure 2: Column chart showing the values of ALT, AST, ALP, SOD, GSH, CAT, GPx activities and bilirubin and cholesterol levels in control and exposure groups.



B. Acetaminophen-induced liver injury



C. Hepatocytes co-treated with linseed oil

Figure 3: Section of liver showing normal, damaged and autorecovered hepatocytes (A,B and C)

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