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Vitamins Protects Rat liver from acetaminophen-Induced Oxidative Stress and In- flammation

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ABSTRACT

The current study aims to investigate the rat hepatotoxicity caused by acetaminophen (APAP) which is the main factor for drug-induced liver failure. Although the precise mode with which APAP damages the liver is unknown, recent research has demonstrated that the enzyme cytochrome P450 is affected, resulting in increased metabolite production resulting in liver failure. In this study, rats were administered with 50mg/kg/b.w to induce hepatic damage and in contrast to damage, the hepatoprotective effects was ana- lyzed with various vitamins A, E, and C. The effects of vitamins A, E, and C were examined using general biochemical parameters, liver marker enzymes, hepatic antioxidants, inflammatory biomarkers, gene ex- pression, and histopathology assays. The results of this study indicate that through lowering ALT, AST, and ALP levels, all vitamins had hepatoprotective benefits against APAP-induced hepatoxicity. Vitamin E had the greatest impact when compared to vitamins A and C. Hence, we recommend that patients receiv- ing APAP treatment may take vitamin E as a supplement to minimize liver damage.

Keywords: Acetaminophen, Hepatotoxicity, Vitamins

Introduction

According to the World Health Organization (WHO), acetaminophen (paracetamol) or N-acetyl-p-aminophenol (APAP) is a widely and most frequently used popular drug. It is one of the leading causes of drug-induced acute liver injury in developing and developed countries. [1, 2] APAP is a highly reactivemetabolite, cheap, safe, and effective at recommended doses for treating pyrexia and pain-related disor- ders. [3] Nowadays, people take the easier route of self-medication with over-the-counter medicines with- out proper knowledge of the nature of clinical prognosis and take drugs in greater quantities than required, resulting in several side effects, including drug-related complications. The excess intake of APAP will re- sult in the synthesis of NAPQI (N-Acetyl-P-benzoquinone imine) and inhibits the synthesis of antioxidant enzymes such as GSH, GPX, SOD, etc. Although the exact mechanisms underlying highly complex intra- cellular and extracellular changes are unknown, APAP-induced liver toxic effects include pathophysiolog- ical process, metabolism, fragmentation, necrotic cell death, mitochondrial oxidative stress, ER stress, au- tophagy, dysfunction and liver regeneration. [2-5] The phosphatidylinositol 3 kinase/protein kinase B (PI3K/AKT) is essential to maintain the normal functions of hepatocytes. APAP alters the PI3K/AKT sig- naling pathway by synthesis and inhibiting the oxidative stress and antioxidant markers, respectively, which results in structural and functional changes in hepatocytes. Since so many factors are involved, early diagnosis of rapid deterioration effectively prevents morbidity and mortality. Based on the literature sur- vey, all vitamins have pharmacologically protective effects, including various cell regulatory roles. [6-10] Usually, liver injury can be caused by toxins, drugs, alcohol and medicinal herbs. Non-Steroidal Anti- inflammatory Drugs (NSAIDs) are being the core of pharmacotherapy used as antipyretics and analgesics in large numbers for most. Drug-induced hepatotoxicity is the most common cause of liver injury. [11-12] Long-term use of APAP can result in liver damage in humans and animals. “APAP” from the preferential COX-2 inhibitors of NSAIDs are used to manage pain in auto-immune and degenerative disorders. [13-16] APAP is known to be the most toxic associate of NSAIDs, which cause hepatic, gastrointestinal and renal damage. APAP hepatotoxicity resulted from covalent alteration of proteins, oxidative stress, idiosyncratic drug reaction and deployment of mitochondrial injury by ROS. [2,4,17] Several vitamins are being consid- ered as antioxidants that have shown hepatoprotective activities in contrast to the hepatotoxicity resulting from an overdose of APAP. The fat-soluble vitamin A in mice has been shown to augment a noticea- ble hepatoprotective action against CCl_4-induced liver damage. Vitamin E is a lipophilic antioxidant that stimulates the de-toxic effect of liver cells. [18] Vitamins E & C inhibit free radicals brought down by oxi- dative damage to lipoproteins and lipids in several cells and tissues. [19-24] Previous studies have ana- lyzed drug-induced toxicity by different vitamins separately. Hence, in the present study, we analyze the comparative potential hepatoprotective effects of vitamins A, E and C to select the best supplement to manage APAP-induced hepatotoxicity effectively.

Materials and Methods

Chemicals

Bovine Serum Albumin (BSA), Glutathione (GSH), Ethylene diamine tetra acetic acid (EDTA), and vitamins A, C and E were obtained from Sigma Chemical Company, St. Louis, MO, U.S.A. Folin's-Ciocalteau reagent and APAP were procured from Sisco Research Laboratories, Mumbai - India. EDTA, ANSA, DNPH, and Tetramethylethylenediamine (TEMED) were obtained from British Drug House Pvt. Ltd. All the chemicals and solvents were procured from analytical grade obtained from Ranbaxy laboratories. Thermo Fischer Scientific Company purchased the monoclonal antibody for P13K and AKT-308 proteins. The MDA, GSH, GPx, CAT and SOD standards were purchased from Elab Science Biotechnology Company.

Experimental animals

In the present investigation, male albino Wistar rats having 180g-200g body weight were used. They were acquired and protected with standard laboratory conditions separately in cages (25±2°C). Throughout the study, standard commercial diet and water *ad libitum.* Rats were adapted to the novel laboratory circumstances for 7 days before the experiment's initiation. The IAEC of Saveetha Institute of Medical and Technical Sciences, Chennai (SU/CLAR/RD/004/2016) approved the experiments ethically.

Experimental protocol

The Wistar rats weighing between 180-200g were selected and categorized into 5 groups consisting of 6 animals each.

Group 1: Normal rats were orally given 2 ml/kg body weight/day of physiological saline for 7 days.
Group 2: Rats treated with APAP sodium (50 mg/kg b.wt) i.m for 7 days.
Group 3: Vitamin A (400 IU/kg) treated orally, followed by APAP for 7 days.
Group 4: Vitamin C (200 IU/kg) treated orally, followed by APAP for 7 days.
Group 5: Vitamin E (200 IU/kg) treated orally, followed by APAP for 7 days.

Blood and tissue samples

Ketamine hydrochloride (50mg/kg) intramuscular injection was given for anesthetizing the animals. The blood samples were taken using a retro-orbital puncture to analyze the biochemical parameters. The dissected hepatic section was rinsed with ice-cold saline, and 10 percent homogenates were prepared in pH 7 phosphate buffers. A small portion of hepatic homogenate was run at 3000 rpm for 15min at 4°C, and the supernatant was taken for estimating antioxidants and other parameters.

Serum biochemical markers of liver functions

The serum was used to determine the activity of liver enzyme levels through the activity of AST, and ALT, ALP. [25]

Analysis of Oxidants and Antioxidants

The liver tissue was used to determine the activity of oxidative and antioxidant levels. MDA [25], GSH [26], GPx [27], CAT [28], SOD [29]

Tissue Preparation for Inflammation

After cutting hepatic tissue, weight was analyzed and PBS (pH 7.2–7.4) was added. Tissue was homogenized by tissue lyser and centrifuged for 20 min at the speed of 1200× g to remove supernatant. Supernatant was used for the analysis of inflammation and apoptosis.

Assay of Inflammation

Cytokine production in the hepatic tissue was evaluated by enzyme-linked immunosorbent assay (ELISA) using commercial kits according to manufacturer's instructions. Liver tumour necrosis factor-alpha (TNF-α) (sensitivity: 5.127 ng/L, assay range: 8–1000 ng/L) and Interleukin-6 (IL-6) level was measured using a rat ELISA kit (sensitivity: 20.118 pg/L; the minimum detectable dose of IL-6 is typically less than 3.0 pg/mL) from Uscn Life Science Inc. (Houston, TX, USA). The plates were read at 450 nm using the ELISA microplate reader (Bio-Tek, Winooski, VT, USA).

Histopathological assessment

Liver tissue was isolated from rats and fixed in 10% formalin. Later tissue was dehydrated gradually using different grades of alcohol, cleared in xylene, then embedded in paraffin wax. Sections were taken with 5 µm thickness using a rotatory microtome (INCO MRM-1120). The slides were stained with hematoxylin and eosin and observed under 40X magnification with a light microscope.

Statistical analysis

All statistical analyses were calculated using SPSS Statistical Package (version 22.0). Results were expressed as Mean ± SD (n=6) and the outcomes were subjected to ANOVA followed by Student's t-test and post hoc test for statistical analysis. Results with $P<0.05$ level was considered statistically significant.

Results

Effect of vitamins on APAP induced hepatotoxicity via serum Biomarkers

The findings revealed that liver enzymes such as AST, ALT and ALP were significantly elevated in the APAP-induced rats than the control group. However, pre-treatment with the different vitamins showed decline in the liver enzymes in comparison with the APAP group. Moreover, the Vitamin E with a dosage of 200 IU/kg showed protection near normal to control group than Vitamin A and C Figure 1.

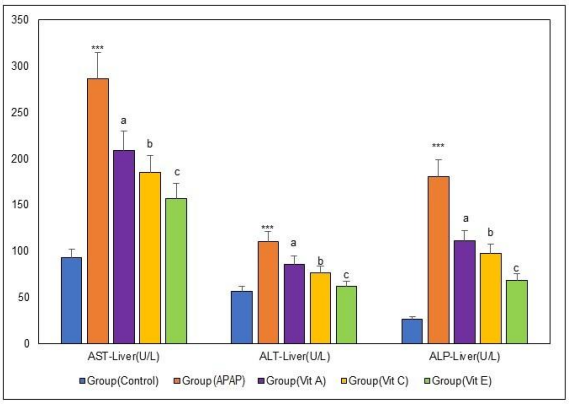

Figure 1 Effect of vitamins on liver marker enzymes in APAP induced hepatotoxicity rats.

Effect of vitamins on APAP induced hepatotoxicity via oxidative markers

APAP induced group showed a significant up surge in the liver enzymes (MDA) compared to controls ($p < 0.001$). The (APAP + Vitamins) treated group showed a noticeable down surge of MDA levels as compared to APAP-exposed group ($p < 0.01$). We reported that reduced liver enzymes were noted in (APAP + VIT A), (APAP + VIT C), (APAP + VIT E), treated group in comparison to APAP exposed group. There was significant decline in the enzymatic marker were observed in (APAP + VIT E) group than Vitamin A and C Figure 2.

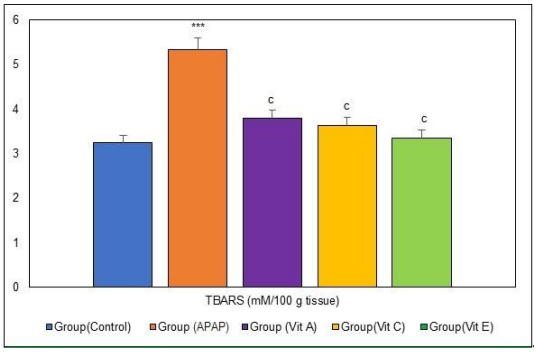

Figure 2 Effect of vitamins on TBARS levels in APAP-induced hepatotoxicity rats.

Effect of vitamins on APAP induced hepatotoxicity via Antioxidant enzymes

The influences of APAP induced toxicity and administration of vitamins on antioxidant enzymes of hepatic tissues were shown in (Figure. 3A and 3B). SOD, CAT, GPX and GSH enzymatic concentrations in hepatic tissues were significantly declined in APAP intoxicated group (G2) in comparison with the normal control (G1). Moreover, a noticeable increase in hepatic SOD, CAT, GPX and GSH was observed in G3,

G4, and G5 compared with APAP intoxicated group (G2). Meanwhile, a significant increase in enzymatic antioxidant activity was noticed in hepatic tissue administered with Vitamin C group compared to the control and Vitamin A and E.

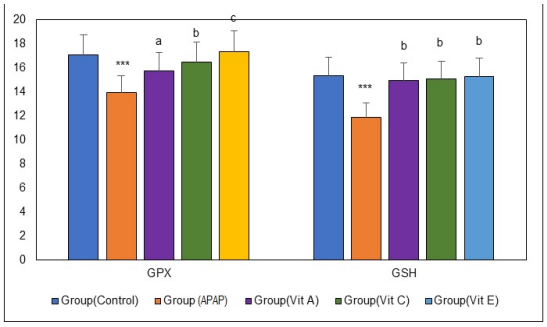

Figure 3A Effect of vitamins on levels of GPX and GSH in APAP induced hepatotoxicity rats.

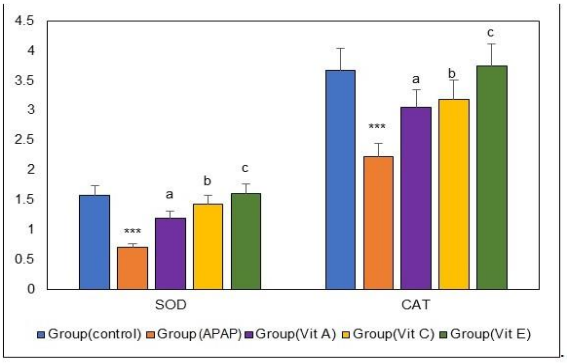

Figure 3B Effect of vitamins on levels of GPX and GSH in APAP induced hepatotoxicity rats.

Effect of vitamins on APAP induced hepatotoxicity via inflammatory markers

TNF-α, and IL-6 are the pro-inflammatory cytokines involved in the progression of APAP-induced hepatic injury. As shown in 4a and 4b, APAP induced group showed tremendous elevation of TNF-α, and IL-6 levels than control group ($p < 0.01$), Vitamins suppressed the upregulated cytokines induced by the APAP in a dose-dependent manner of various vitamins. TNF-α and IL-6 levels were significantly different between vitamin A, vitamin C and Vitamin E and the APAP group ($p < 0.05$ or $p < 0.01$).

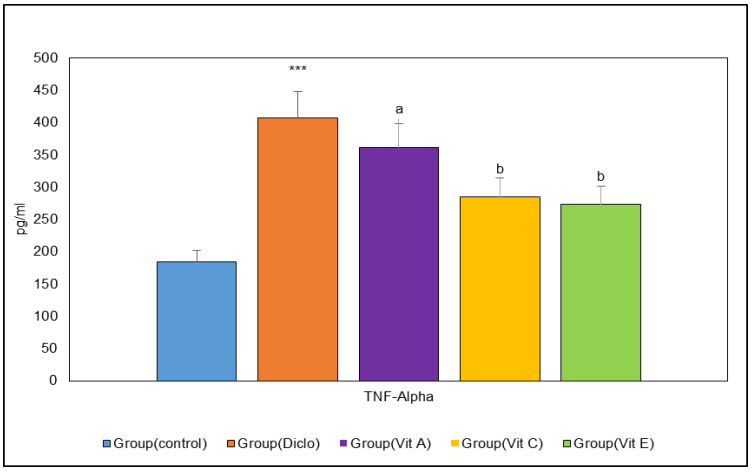

Figure 4a Effect of vitamins on TNF-α levels of in APAP induced hepatotoxicity rats

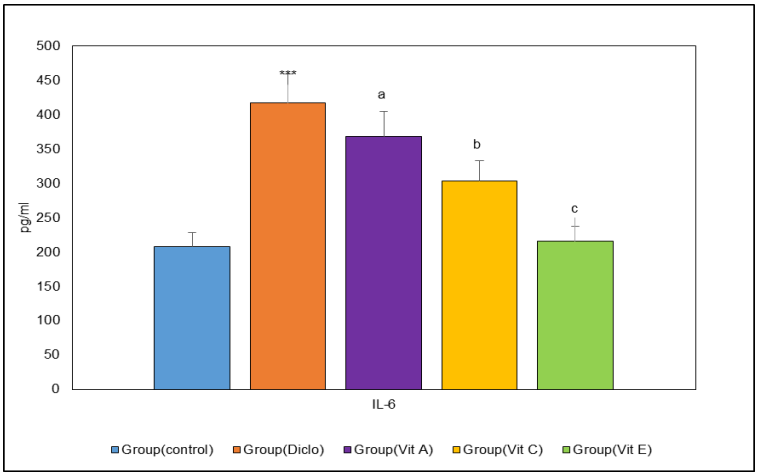

Figure 4b Effect of vitamins on IL-6 levels of in APAP induced hepatotoxicity rats

Effect of vitamins on APAP induced hepatotoxicity via histopathology

The control rats showed a normal cytoarchitecture in the hepatocytes. However, APAP induced rats exhibited hepatotoxicity (Figure 5) characterized by notable markings such as degeneration of vacuoles, infiltration of cells, and marked elevation in sinusoidal space, karyopyknosis and nuclear alterations. In the rats treated with Vitamin A and C at the dose of 200 IU/kg b.w. and mild APAP, focal degeneration and damaged hepatocytes of liver tissue. However, there were no markable histological changes observed in the vitamin E at the dose of 200IU/kg b.w. + APAP group compared to the normal rats. Overall, Vitamins provided hepatoprotective role against APAP induced hepatotoxicity.

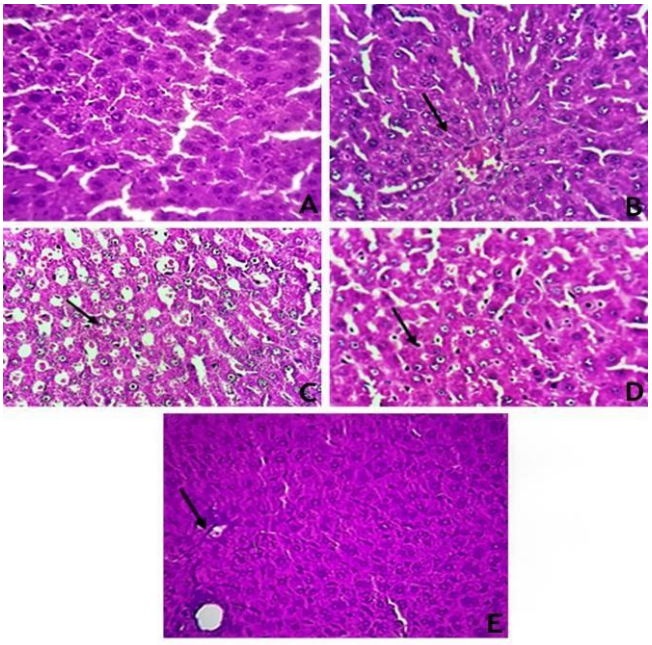

Figure 5 Histology study of liver tissue using hematoxylin and eosin staining (40x). Image A– Normal control; Image B–APAP induced; Image C–APAP + vitamin A; Image D–APAP + vitamin C; Image E-APAP + vitamin E. (Arrow mark denotes the hepatic infiltration, hepatic cell injury).

Discussion

The hepatocytes and their associated cells in the liver is chiefly involved in the detoxification and elimination of circulating compounds, which include environmental toxins, chemicals and drugs, as a mechanism in the preservation of individuals health. Toxic chemicals and drugs are metabolized by the hepatic tissue with subsequent release of liver tissue-damaging reactive oxygen species (ROS) or reactive nitrogen species (RNS) resulting in the leakage of liver enzymes into systemic and pulmonary circulation.

Drug induced hepatic damage is the primary cause of liver diseases in hospitals and the incidence of drug-induced hepatic damage seems to be on the rise along with the upsurge in the new drugs. Among the drug-induced hepatic injury, APAP-induced hepatic damage has taken the lead. APAP is safe antipyretic and analgesics in therapeutic doses but is a major exposed factor in acute hepatic injury once exceeds. [1] The overdose of APAP leads to destruction of hepatocytes resulting in the upsurge of serum biomarkers. The evaluation of serum biomarkers like ALT, AST, and ALP, are commonly used markers in hepatotoxic studies. In this study, administration of 50 mg/kg of APAP caused hepatic injury in rats, characterized by an upsurge in serum activity of transaminase and phosphatases (AST, ALT and ALP). Similarly, many studies showed elevation in the serum biomarkers in APAP induced hepatotoxicity. [2, 3] In the present study, serum biomarker levels in the groups treated with APAP+ vitamin A, E and C were significantly decreased compared with the APAP treated only group. Vitamins pre-treatment attenuated hepatic damage by reducing serum toxicity markers such as AST, ALT and ALP against the APAP-induced hepatotoxicity. MDA is a secondary product of lipid peroxidation and it is utilized as a tissue marker for vital organ damage resulting from reactions [4]. Many research studies have explored that hepatic oxidative stress is induced by elevation in LPO. The previous studies shows that APAP-induced rats showed noticeable lipidperoxides in hepatic tissue [5]. Similarly, in this study rats exposed to APAP showed elevation of MDA compared to control. In contrast, the MDA levels in the APAP+Vit A, APAP+Vit C and APAP+Vit E groups were considerably lowered in comparison to the APAP-treated group. Our findings revealed that vitamins reduced MDA levels in the hepatic tissue. However, significant were reduction observed in Vitamin E than A and C.

SOD, GSH, GPX and CAT are enzymatic and non-enzymatic antioxidants that protects the various tissues from lipid peroxidation. Tissue damage results in synthesis of free radicals, resulting in decrease of above mentioned enzymes [6]. In this study rats treated with APAP, shows decreased levels of antioxidant enzymes like SOD, GSH, GPX and CAT than control group. However, rats pre-treated with various vitamins upregulated the antioxidant enzymes than APAP induced group. Moreover, Rats treated with the Vitamin E showed full protection in par with the control group group, while vitamin A and Vitamin E showed partial protection. Similarly, Ram et al., 2021showed protective effects by vitamins against Non-Alcoholic Fatty Liver Disease. [7] In this study, we evaluated the APAP induced inflammatory reaction by increasing the TNF-α levels than control group. However, Vitamin supplementation showed a protective role against hepatotoxicity induced by APAP. These findings suggest that Vitamins has anti-inflammatory effects by reducing pro-inflammatory cytokine expression. IL-6 is a cytokine and excreted throughout the entire body in organ damage. In this study, IL-6 levels were considerably upregulated in the APAP group in comparison with the control rats. Alternatively, Vitamins declined the levels of IL-6. These findings showed that Vitamins exerts anti-inflammatory effects by reducing the levels of various pro-inflammatory cytokines. Moreover, the Vitamin E has more promising beneficial effects than A and C. The findings were corroborated with Kandemir et al., 2017 by chrysin against APAP induced hepatotoxicity. [8]

The liver histopathological examination in groups treated with APAP showed morphological alterations such as including inflammatory infiltrate, necrosis of hepatocytes, congestion of sinusoids, and nuclear alterations. Similar findings were observed by Chariyakornkul et al., 2022 against APAP induced hepato-

toxicity. In contrast, the rats pretreated with various vitamins showed remarkable restoration of hepatocytes, infiltrated cells equivalent to control, mild lesion, and the histomorphology of the liver parenchyma were similar to the control group, suggesting a possible protective effect of Vitamin E. The findings were consistent with the Okiljevic et al., 2024 and Menon et al., 2023, in which Silymarin and Annona muricata treatment restored the morphology hepatic tissue against by APAP induced hepatotoxicity respectively.

Conclusion

The present work showed a predominantly protective role of vitamin E over vitamins A and C in APAP-induced hepatotoxicity in Wistar rats through their antioxidant properties. However, Vitamin E has promising effects by restoring the SOD, CAT, GPx & GSH levels. In addition, Vitamin E showed hepatoprotection more than Vitamins A and C through the restoration of liver morphology by APAP-induced liver toxicity.

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