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Full Length Research Paper

Histological and biochemical study of the effects of garlic oil and vitamin E in paracetamol induced hepatotoxicity

Adejoke Yetunde ONAOLAPO[1] and Olakunle James ONAOLAPO[2]

[1] Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University, Ogbomoso, Oyo State.

[2] Department of Pharmacology, Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University, Oshogbo , Osun State.

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The effects of over-the-counter garlic oil capsules and vitamin E on the liver following paracetamol (APAP) overdose was studied. This was with a view to comparing the possible hepatoprotective effects of vitamin E and garlic oil. Thirty adult Wistar rats weighing between 150 and 200 g were randomly divided into five groups of 6 rats each. Garlic oil (3 mg/kg) or vitamin E (200 mg/kg) was administered concurrently with a daily oral dose of 500 mg/kg of APAP. Serum levels of Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP) were measured on the 15th day following which animals were sacrificed; liver sections were processed for histological study. Statistical analysis was carried out using a one way analysis of variance (ANOVA) followed by a post-hoc test; results were expressed as mean ±S.E.M. The results showed significant weight reduction and elevation of liver enzymes and extensive liver cell injury in animals in the paracetamol control (group B) with significant improvement seen in the groups that received either garlic oil or vitamin E although there was no significant difference in the effects of vitamin E over garlic oil. The study concluded that both vitamin E and garlic oil are hepatoprotective in paracetamol induced liver injury with neither showing any advantage over the other.

Keywords: Paracetamol, Hepatotoxicity, Garlic oil, Vitamin E.

INTRODUCTION

Paracetamol (Acetaminophen, APAP) hepatotoxicity is a common cause of acute liver failure worldwide (Larson et al., 2005). Paracetamol overdose is the cause of more hospital admission than overdose from other pharmacological agents (Lee, 2004). APAP is marketed under different trade names all over the world and is a very effective and well tolerated over-the-counter analgesic (Jalan et al., 2006). Paracetamol over dose following acute or chronic use causes fulminant hepatic failure particularly if daily doses exceed 4g in adults (Ostapowicz, 2002; Nourjah et al., 2002). Recently, the safety profile of acetaminophen even at therapeutic doses has become a cause of concern (Jalan et al., 2006).

Acetaminophen toxicity has been recorded at other wise therapeutic doses thereby reopening the controversies on the safety of acetaminophen (Kwan et al., 1995: Yin et al., 2001; Bernal, 2003).

Acetaminophen-induced hepatic injury is sequel to increased lipid peroxidation in the liver cells, options for treatment and prophylaxis are limited; thus the search for newer prophylactic agents has become imperative (Schnellman et al., 2001: Bessems and Vermeulen, 2001; James et al., 2003).

Garlic is a perennial plant originally from central Asia but now cultivated worldwide (Amagase et al., 2001). It also has a wide range of uses topmost being antioxidant

*Corresponding author. E-mail: olakunleonaolapo@yahoo.co.uk. Tel: 2348062240434

properties (Borrelli, 2007; Berthold, 1998; Dorant, 1993).

Vitamin E is a fat-soluble vitamin that functions mainly as antioxidant and free radical scavengers in lipophilic media (Bell, 1987; Azzi, 2007); the most important function of vitamin E is in cell signaling and not necessarily in antioxidant metabolism (Azzi, 2004). However, it is still the most abundant and effective antioxidant in the human body (Frei et al., 1989).

In view of the antioxidant and possible hepatoprotective property of vitamin E and increasing evidence of the antioxidant properties of garlic oil, the present study was designed to investigate the comparative effects of garlic oil and vitamin E in acetaminophen overdose.

MATERIALS AND METHODS

Equipments and Apparatus

Electronic precision balance, metallic animal cages, sterile disposable syringes (1, 5, and 10 ml) needles, cotton wool and oral cannula. .

Chemicals and drugs

Vitamin E (Sigma Chemical Co. St. Louis, U.S.A.), Garlic oil capsules (Shanthou Pharmacy, China), Acetaminophen tablet (Juhel Pharmaceuticals, Lagos), and Normal saline. All other reagents used in this study were analytical grade.

Animals

Thirty healthy adult male Wistar rats with weights in the range of 150 to 200g purchased from the Empire animal farms in Osogbo, Osun State, Nigeria, were used. The animals were randomly assigned into five groups. The animals were housed in metallic cages measuring 64"x 36"x32" (6 rats in each cage). All animals had free access to food and water *ad libitum*. They were maintained under standard laboratory conditions. The experimental protocol was approved by the Animal Ethics Committee of the Ladoke Akintola University of Technology, Ogbomoso. All rules applying to animal safety and care were observed.

Experimental methods

The animals were assigned into five groups A, B, C, D and E of six rats each. Group A and group E served as normal control and were given normal saline and corn oil daily. Animals in group B received 500 mg/kg of paracetamol dissolved in normal saline daily, while animals in group C and D were given 500 mg/kg of paracetamol daily (Ratnasooriya and Jayakody, 2000) and then received either garlic oil (3 mg/kg) or vitamin E (200 mg/kg) daily dissolved in corn oil respectively.

Experimental period lasted 14 days. On day 15 after an overnight fast, the animals were sacrificed by cervical dislocation and samples for alkaline phosphate (ALP); alanine and aspartate transaminase (ALT, AST) levels were taken via intracardiac puncture and analysed immediately. Sections of the right lobe of the liver were obtained, processed, sectioned and stained with Hematoxylin & Eosin (H&E) for histological study. An Olympus BX50 digital light microscope was used to examine the slides and acquire photomicrographs. All the data for all biochemical parameters were analyzed by analysis of variance (ANOVA), and post-hoc tests (Student Newman Keuls) were used to determine the source of a significant effect. Results were expressed as mean ±S.E.M, $p<0.05$ was taken as accepted level of significant difference.

Determination of weight

Animals were weighed weekly using a Mettler weighing balance (Mettler Toledo Type BD6000, Greifensee, Switzerland)

Collection and analysis of blood samples for liver biochemistry

Blood was collected from each rat on the 15^{th} day by intracardiac puncture; rats were fasted for 8 hours before samples were collected. Samples were collected into lithium heparinised bottles, blood was allowed to clot and plasma separated by centrifugation at 3500 rpm for 10 minutes using a hematocrit centrifuge (JICA, Japan). The serum was assayed immediately; alanine (ALT) and aspartate (AST) aminotransferases and alkaline phosphatise (ALP) were assayed using Boehringer Mannheim diagnostic kits.

RESULTS

The effect of garlic oil and vitamin E on body weight

The mean body weights monitored weekly for a period of 14 days in all experimental groups are presented in Figure 1.

Animals in groups A C, D and E gained weight gradually throughout the experimental period. There was however a reduction in the weight of animals in the paracetamol control group (B) compared to normal control (A and E). Animals in group C (garlic oil) gained more weight than animals in group D (vitamin E); this weight difference was however not significant.

The effect of garlic oil and vitamin E on liver enzymes

Figure 2 shows the mean levels of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in animals following

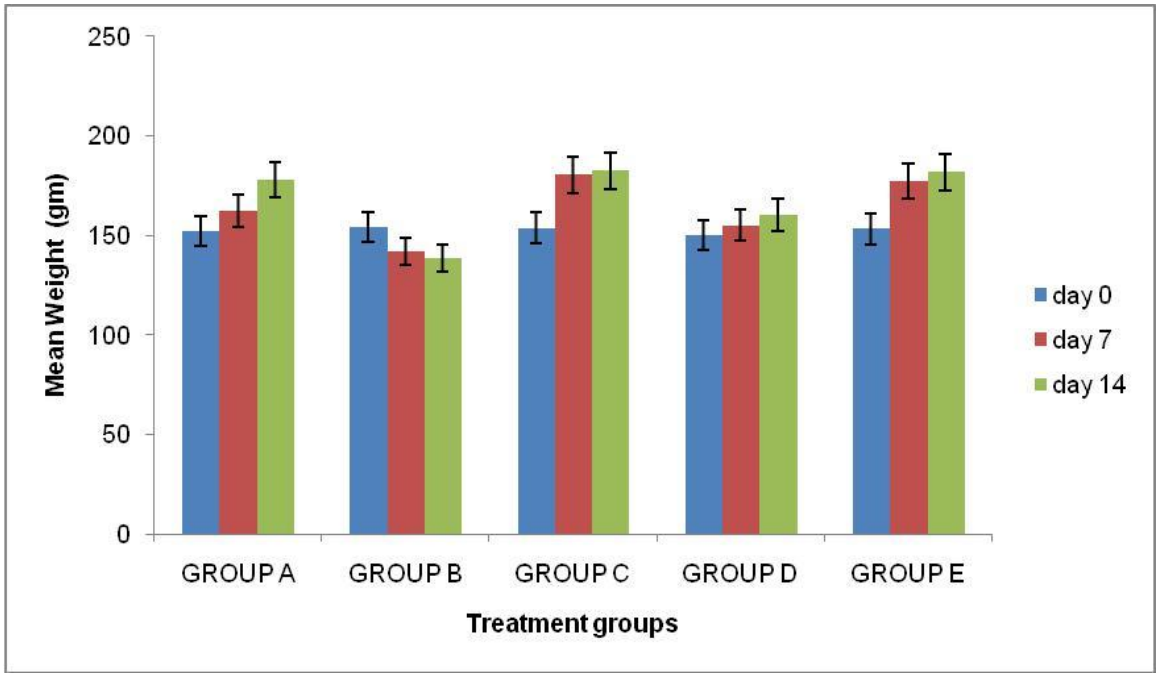

Figure 1. Effects of garlic oil or vitamin E on body weight. Each line represents mean ± S.E.M, *p<0.05 group by group comparison, n = 6. Groups A and E were controls, group B paracetamol control, groups C and D received garlic oil or vitamin E respectively.

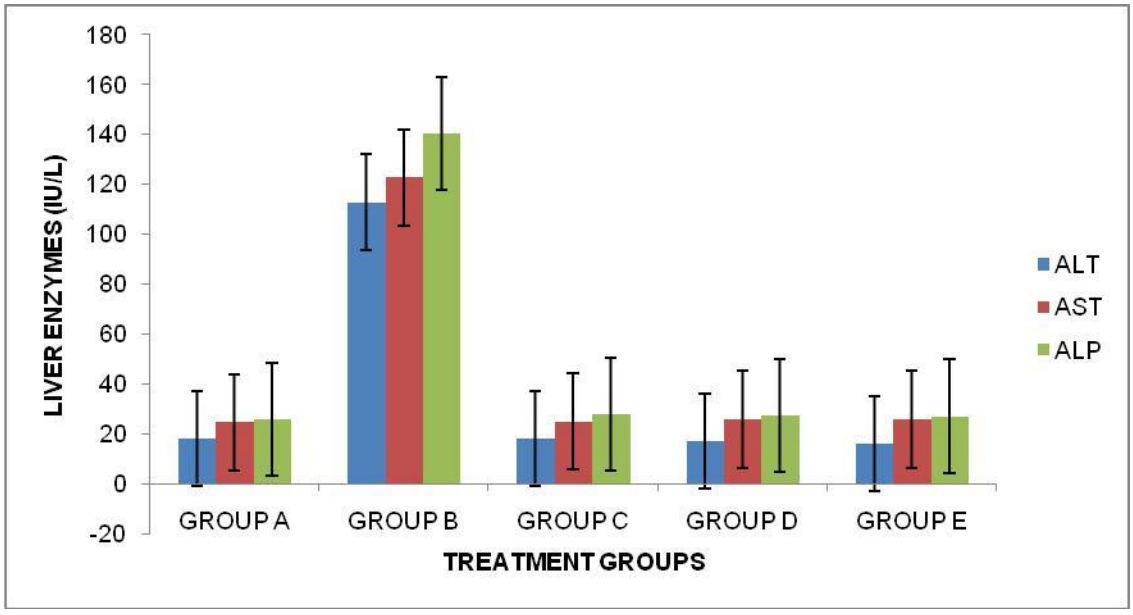

Figure 2. Effects of garlic oil and vitamin E on liver enzymes. Each line represents, mean ± S.E.M, *p<0.05 group by group comparison, n = 6. Groups A and E were controls, group B paracetamol control, groups C and D received garlic oil or vitamin E respectively.

paracetamol overdose. The levels of serum AST (f=173.09, p<0.05), ALT (f=436, p<0.05) and ALP (f=11063.24, p<0.05) increased significantly in animals in group B (paracetamol control) compared with the normal controls (A and E). Paracetamol overdose resulted in a 300% increase in the liver enzymes, while administration of either garlic oil or vitamin E caused a significant reduction in these enzymes. There was no significant difference in the effect seen with vitamin E over that seen following administration of garlic oil.

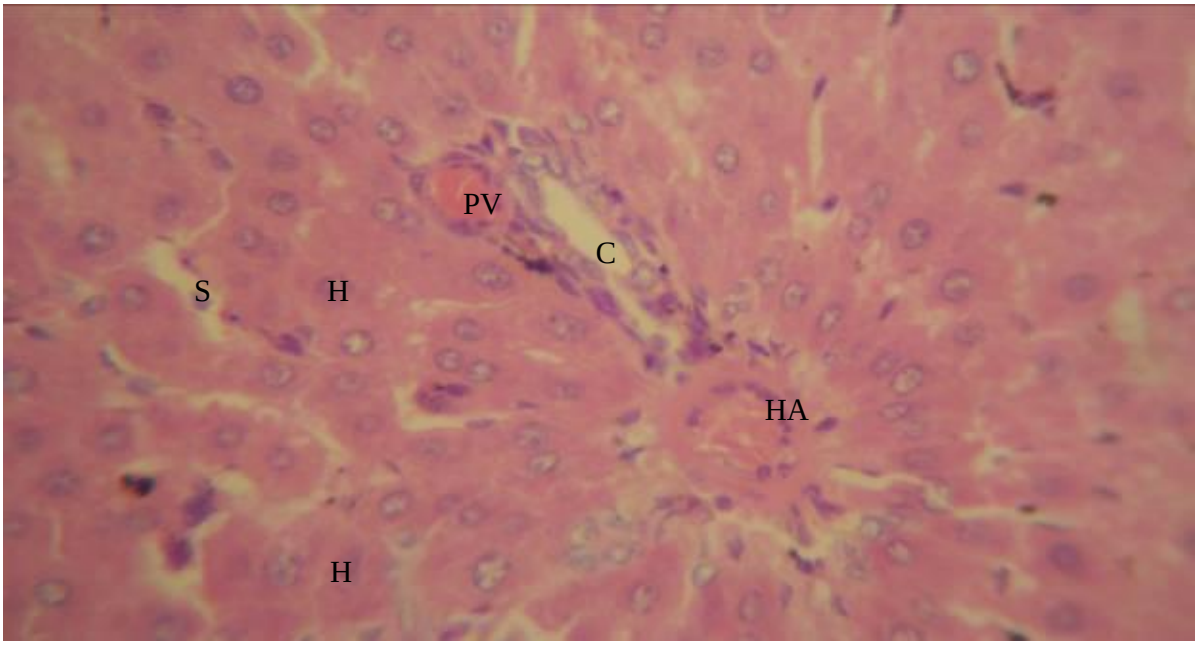

Plate 1. Group A showing normal liver architecture: hepatocyte with prominent nuclei (H), intervening sinusoids (S) central vein (C) hepatic artery (HA) and portal vein (PV). (H&E ×400).

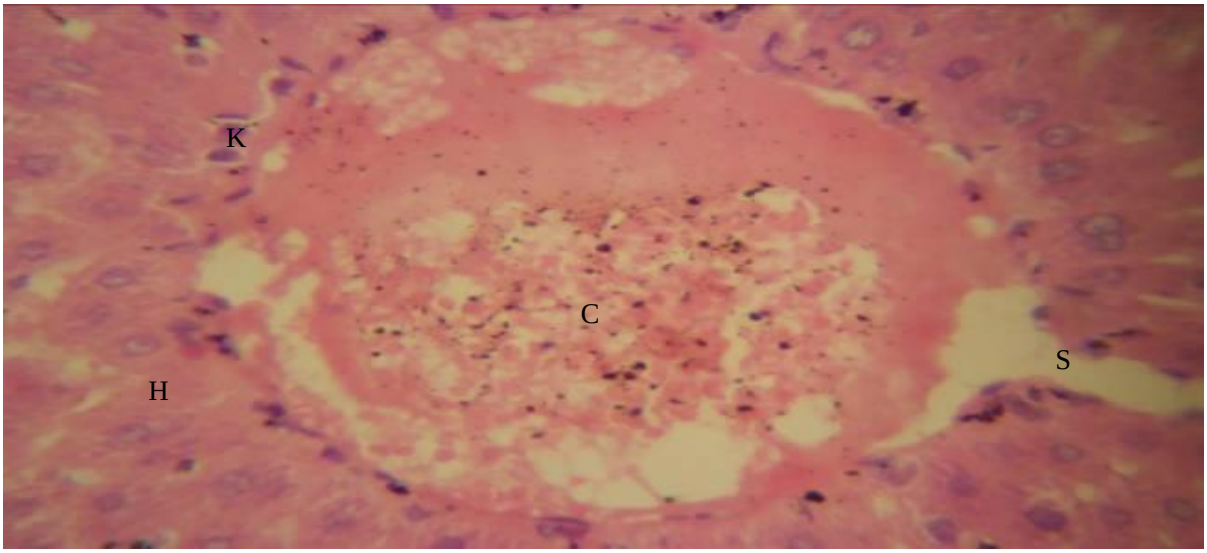

Plate 2. Group B animals showing loss of liver architecture with grossly dilated central vein (C) filled with blood and debris the central vein ruptures into one of the sinusoids (S). The hepatocytes are shrunken with pale nuclei (H) the central vein is also surrounded with inflammatory cells (K).

Result of liver histology

Examinations showed grossly normal livers in Groups A C, D and E. Livers taken from animal in Group B was slightly enlarged.

Slides from sections of the right lobe of the liver of Group A animals (Plate 1) revealed radially arranged cords of hepatocytes around terminal hepatic venule. There were intervening sinusoidal spaces between each cord and plate of hepatocytes; normal central veins were also seen. These are in keeping with normal hepatic histology. Liver slides from groups B (Plate 2) animals

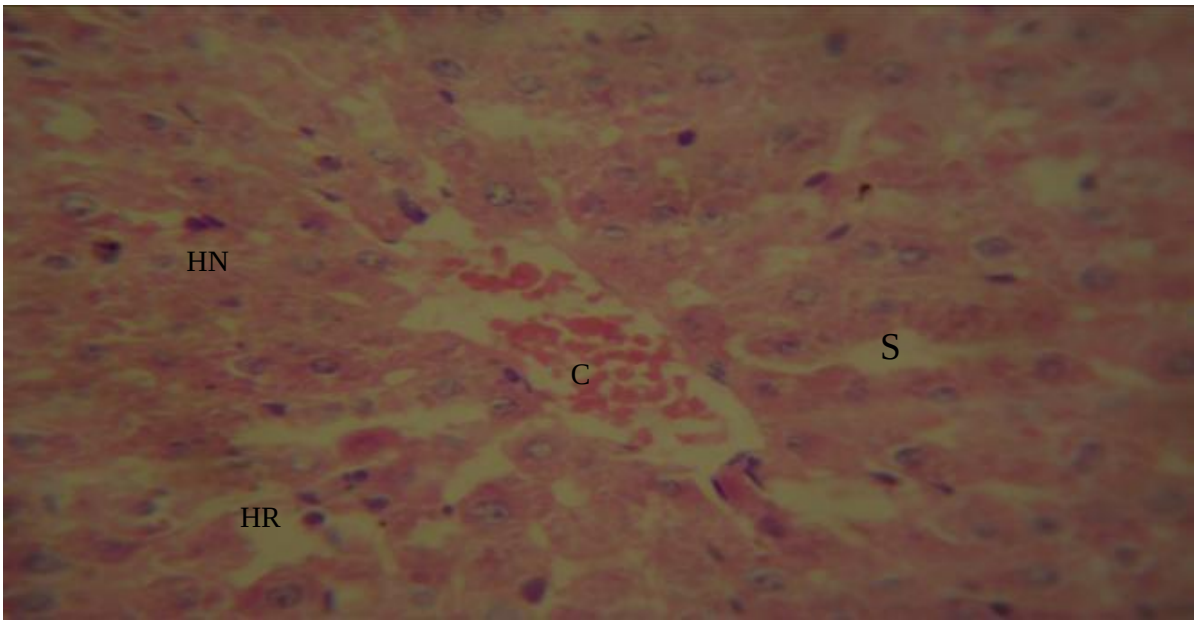

Plate 3. Slide from group C animals showing mild loss of liver architecture with normal sized central vien (C) and intervening sinusoids (S). Some of the hepatocytes are ruptured with pale staining nuclei (HR), others have deeply staining nuclei (HN).H&E ×400.

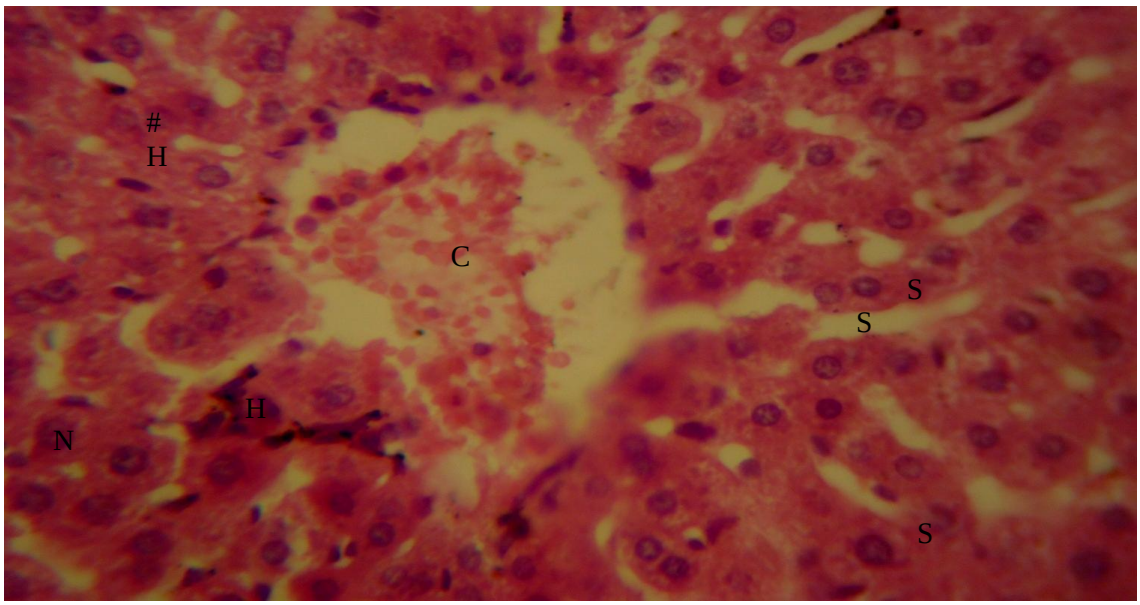

Plate 4. Group D animals showing mild loss of liver architecture with central vein (C) and intervening sinusoids (S). In some of the hepatocytes there is healing of the hepatocytes (H) and deeply staining nuclei (N).H&E ×400.

showed loss of normal liver architecture with rupturing of some of the hepatocytes. There were varying sizes and shapes of nuclei most of them pale staining. There was also central vein congestion and inflammatory cells seen surrounding the central vein; this features are in keeping with a possible drug induced hepatic injury. The examination of the liver slides of animals in Group C and D (Plates 3 and 4) showed varying degrees of recovery from liver injury as evidenced by mild loss of normal liver architecture with numerous radially arranged cords of

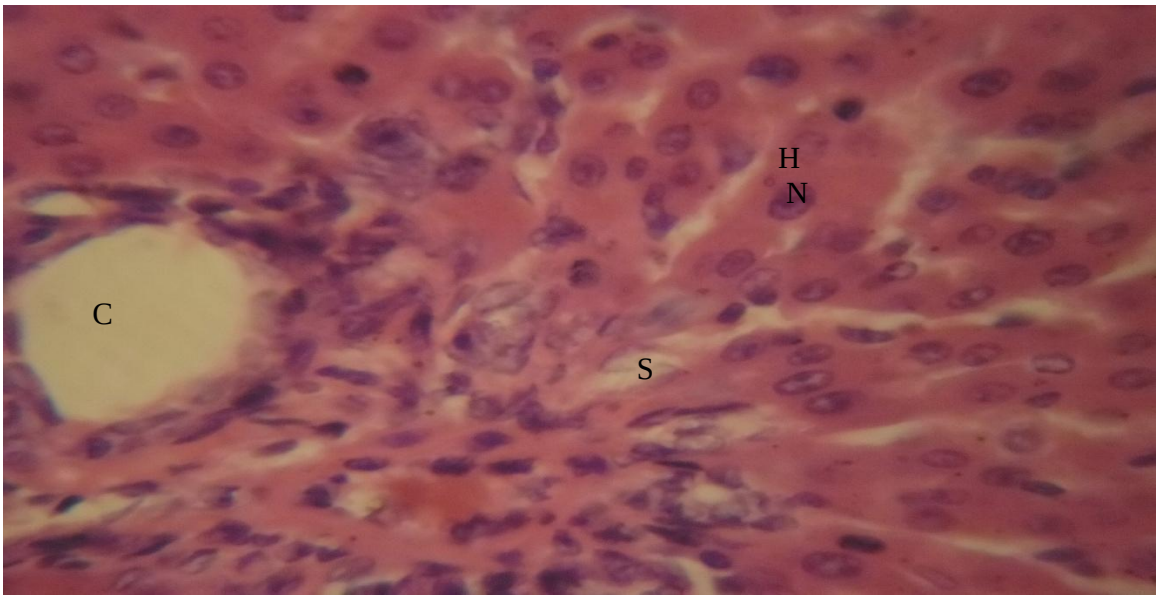

Plate 5. Group E showing normal sized central vein,(C), radially arranged hepatocyte, (H) with prominent nuclei (N) and intervening sinusoids (S) H&E ×400.

hepatocytes with normal nuclei, a few hepatocytes with either pale or deeply staining nuclei. There was also mild central vein congestion. These features are in keeping with probable recovery from paracetamol overdose. Slides from animals in Group E (Plate 5) that received corn oil were in keeping with normal.

DISCUSSION

Paracetamol is an over-the-counter analgesic whose affordability and accessibility makes it one of the commonest causes of acute fulminant hepatic failure from its overdose (Bonkovsky et al., 1984). In the present study, acetaminophen was administered daily at 500 mg/kg, for 14 days (Ratnasooriya and Jayakody, 2000) and protective effects of garlic oil and vitamin E studied and the difference in these effects compared.

The results showed significant weight reduction and elevation of liver enzymes and extensive liver cell injury in animals following administration of paracetamol with significant improvement seen in the groups that received either garlic oil or vitamin E although there was no significant difference in the effects of vitamin E over garlic oil. G Garlic oil and vitamin E both resulted in weight gain which was an improvement over the weight loss that resulted following paracetamol overdose; administration of garlic oil however resulted in more weight gain than vitamin E although this weight increase was only visual. In toxicological studies, body weight and relative organ weights are important criteria for evaluation of toxicity (Crissman et al., 2004). Animals in the paracetamol control group showed weight reduction compared to normal control; this weight loss may be attributed to decrease food intake (anorexia or food avoidance) or poor food palatability due to treatment related toxicity. Paracetamol overdose may also induce oxidative stress leading to generation of free radicals and alterations in antioxidant levels which cause metabolic disorder and weight loss; all these are possibly corrected by garlic oil and vitamin E supplementation.

The liver plays a central role in transforming and clearing chemicals and is consequently susceptible to the toxicity induced from these agents. Many enzymes are present in the liver; those routinely used in diagnosis include the transaminases (AST, ALT) and alkaline phosphatase (ALP) increased levels are markers of toxicity (Govindwar and Dalvi, 1990).An increase in the activities of these enzymes are the early indices of toxic hepatitis. Liver enzymes were significantly increased in the paracetamol control group. Paracetamol toxicity results following depletion of glutathione, N-acetyl-para-benzoquinone-imine (NAPQI) which ordinarily forms conjugates with glutathione now accumulates and stable acetaminophen adducts (Comporti et al., 1991). In addition highly reactive free radicals are generated by oxidative reaction of cytochrome P450; this free radicals cause lipid peroxidation and hepatic cell injury (Farrell, 1994). Administration of garlic oil or vitamin caused a significant drop in liver enzymes although neither showed any significant advantage over the other.

Garlic oil and vitamin E have well documented antioxidant properties (Idogun and Ajala, 2004; Saravanan and Prakash, 2004) hence the effect seen here may be due to their ability to mop up free radicals and so halt the chain reactions of acetaminophen-

generated free radicals or scavenge the reactive free radicals before reaching their hepatic targets. Results of the present study suggests vitamin E's ameliorating effects to be likely mediated via inhibition of free radicals generation and/or free radical scavenging activity. Garlic oil producing a comparable effect to that seen with vitamin E may suggest that it has as much antioxidant capacity as vitamin E. A number of studies have also supported the antioxidant as well as gluthatione restoring ability of garlic oil (Saravanan and Prakash, 2004). Pretreatment with garlic preparations restored glutathione levels and offered significant protection to the myocardium in isoproterenol induced myocardial infarction in rats (Saravanan and Prakash, 2004); garlic oil has also been shown to reduce oxidative stress in streptozotocin induced diabetes (Anwar and Meki, 2004).

The result of liver histopathology showed that paracetamol induced hepatic injury in the slides from animals in the paracetamol control compared to the normal control, while administration of garlic oil and vitamin E resulted in significant qualitative improvement in the liver architecture compared to paracetamol control; however, vitamin E administration resulted in a near normal recovery of the liver cells compared to changes seen as a result of garlic oil administration. This could mean that the protective effect of garlic oil against hepatic structural defects possibly lagged behind vitamin E, allowing more hepatic injury to occur here than seen with vitamin E. Another school of thought would be that protection against paracetamol induced hepatic injury requires higher doses of garlic oil than used here for it to show a comparable effect to that seen with vitamin E.

CONCLUSIONS

In conclusion, this study showed that both garlic oil and vitamin E have comparable hepatoprotective effects in paracetamol induced hepatic injury although further studies intend to compare them against recognized standard medications.

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