Antioxidant effects of vitamin E on diclofenac-induced hepatotoxicity in male rats

Siva T¹, Girija Sivakumar², Sankaran PK³, Yuvaraj Maria Francis⁴, Gayathri T⁵, Kumaresan M⁶, Karunakaran Balaji⁷

¹Bharath University, Chennai, Tamil Nadu, India,
²Department of Anatomy, Karpaga Vinayaga Medical College, Mathuranthagam,
³Department of Anatomy, AllMS, Mangalagiri-
⁴Department of Anatomy, Saveetha Medical College Hospital Thandalam Chennai India
⁵Sri Ramachandra Institute of Higher Education and Research
⁶Department of Anatomy, Saveetha Medical College Hospital
⁷Department of Anatomy, Sri Ramachandra Institute of Higher education and research

Article History:
Received on: 03.03.2019
Revised on: 04.06.2019
Accepted on: 08.06.2019

Keywords:
Diclofenac, malondialdehyde, vitamin E and liver

ABSTRACT
Drug-induced liver injury possesses a major clinical problem and has become a leading cause of acute liver failure and transplantation. Overstressed liver compromises its detoxification role, which may expose it to a variety of diseases and disorders. The present study was to determine whether pre-administration of various doses of vitamin E would have a protective effect against diclofenac-induced hepatotoxicity in Wistar male rats. Twenty-four albino male rats weighing 180-200g were divided equally into four groups. In control group rats were administrated with physiological saline 2ml/kg b.wt /intramuscularly. Another group with 50mg/kg b.wt/ intramuscularly/seven days diclofenac was used for inducing toxicity. In experimental groups, rats were administrated with different doses of vitamin E along with diclofenac sodium [200 and 400 IU orally and 50mg/kg b.wt/ intramuscularly/seven days]. Showed that there was a rapid increase in the levels of liver function test in diclofenac-treated group, which was significantly decreased after pre-treatment with high dose than low dose of vitamin E. Although the exact mechanism by which diclofenac injuries liver is not understood, some studies explain the toxicity by affecting cytochrome P 450, leading to the production of active metabolites. Administration of different dose of diclofenac sodium induces severe adverse effects in the liver and kidney.

INTRODUCTION
The liver is a vital organ which plays a key role in detoxifying various drugs and xenobiotic compounds (Shati, 2014a). The liver is commonly damaged by many toxic and chemical substances, one among them is NSAIDS (Hamilton et al., 2016). NSAIDS are commonly used for the treatment of many acute and chronic inflammatory conditions, pain management, rheumatic disorder and ankylosing spondylitis (Vuppalanchi et al., 2007) (Skoutakis et al., 1988). Diclofenac sodium is a non-steroidal anti-inflammatory agent belongs to aryalkanoic group of phenylacetic acid (Menassè et al,
work as scavengers for this free radical (enzymatic antioxidants. Antioxidants like vitamins such as SOD, catalase, glutathione peroxidase and non-radicals leads to decrease enzymatic antioxidants (1668 ©PharmascopePublications

mine whether pre-administration of various doses Therefore, the aim of the present study was to determine whether pre-administration of various doses of vitamin E would have a protective effect against diclofenac-induced hepatotoxicity in wistar male rats.

MATERIALS AND METHODS

Experimental animals

The study was carried in 24 male albino Wistar male rats weighing 200-250g. The animals were housed individually in cages and maintained under standard laboratory conditions (temperature 25±2°C) 12 hrs light and 12 hrs dark cycle with free access to a standard commercial diet & water ad libitum throughout the experimental period. The rats were acclimatized to laboratory conditions for 7 days before commencement of the experiment. All the animals were reviewed and approved by the institutional animal ethical committee (IAEC), Saveetha Medical College and Hospital.

Experimental method

Rats were divided into four groups

1. Group 1: (n=6) control rats were treated with normal saline of 2ml/kg b.wt i.m for seven days
2. Group 2: (n=6) rats treated with diclofenac at dose of 50 mg/kg i.m for seven days
3. Group 3: (n=6) rats treated with vitamin E at dose of 200IU/kg orally followed by diclofenac at 50mg/kg i.m 2 hours later for seven days.
4. Group 4: (n=6) rats treated with vitamin E at dose of 400IU/kg orally followed by diclofenac at 50mg/kg i.m 2 hours later for seven days.

The animals were given over anesthesia with intramuscular injection of ketamine hydrochloride 50 mg/kg and sacrificed by cervical dislocation. The blood samples were taken by retroorbital vein puncture for analyzing the biochemical parameters like SGOT, SGPT, ALP, bilirubin, total protein. The liver tissue was dissected and fixed in 10% formalin solution for 24 hours and processed through paraffin embedding technique. Paraffin blocks were cut by microtome in to 5 microns, thin sections were stained by hematoxylin and eosin (H and E) for histology preparation. The dissected liver washed with ice cold saline and a 10% homogenate prepared in phosphate buffer (Ph 7.0). The portion of liver homogenates was centrifuged at 3000 rpm for 15 min at 4 °C and the supernatant was used for the estimation of TBARS and estimation of SOD (Misra and Fridovich, 1972).

Estimation of liver enzymes

1978). NSAIDS commonly inhibit the action of cytochrome P450, which cause inflammation in liver tissue. Diclofenac sodium has antipyretic, analgesic, and anti-inflammatory effects, which generally suppress the production of prostaglandin by inhibiting the activity of cyclooxygenase (Bessone, 2010).

Diclofenac sodium is metabolised in the liver by the action of cytochrome P450 enzymes which converts diclofenac into diclofenac 2,5 quinone imine and cytochrome P450 2C9 and uridine diphosphate glucuronate 2B7 enzymes converts diclofenac into 4 OH diclofenac acyl glucuronide this because diclofenac mediated hepatic injury.

The metabolites such as 4hydroxy 3diclofenac, 5hydroxy 4diclofenac and 5hydroxy 6 diclofenac are reported to cause diclofenac-induced hepatotoxicity (Tang et al., 1999). During diclofenac metabolism, the number of reactive oxygen species (ROS) can be increased and cause oxidative stress.

These ROS are highly unstable and rapidly react with proton and electron, and most of these ROS are converted to water molecule before they damage cell (Weltman et al., 1998). Increase in free radicals leads to decrease enzymatic antioxidants such as SOD, catalase, glutathione peroxide and non-enzymatic antioxidants. Antioxidants like vitamins work as scavengers for this free radical (Bolukbas et al., 2005) like superoxide radicals, catalase and glutathione peroxidase catalyse the removal of hydrogen peroxidase (Jadhav et al., 2010).

GSH is the one of the most active antioxidant present in the liver involved in the removal of free radicals such as hydrogen peroxide, superoxide anions and alkyl radicals (Meister, 1984). ROS cause lipid peroxidation of polyunsaturated fatty acid leading to destructive in cell membrane structure and function (Hickman and Macdonald, 2007). Vitamin E is a natural fat-soluble antioxidant which has been reported to have a hepatoprotective property which is primarily due to its ability to attenuate the oxidative stress in various tissues by retaining the antioxidant levels of GSH, SOD and CAT recover the affected liver cells.

More number of literatures have reported that vitamin E ameliorates drug mediate oxidative stress in the liver (Shati, 2014b). Researchers have reported that vitamin E has antioxidant effect against various components which induce hepatotoxicity such as CCL4, mercury, copper and cadmium (Al-Attar, 2011) (Chinoy et al., 2004) (Gaurav et al., 2010).

Therefore, the aim of the present study was to determine whether pre-administration of various doses...
Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) were determined calorimetrically according to the method (Reitman and Frankel, 1957) alkaline phosphate level was determined colourimetrically according to the method Kind and King (1954) total protein levels determined calorimetrically according to the method.

Statistical analysis

Results were statistically analysed and Calculations are done using computerized SPSS software version 16. Normality of the data were analyzed using Shapiro-Wilk test. Normally distributed data were analysed with parametric test one-way analysis of variance (ANOVA) and the data are represented as mean and standard deviation. Kruskal-Wallis test were performed for non-parametric test and the data are represented as median (interquartile range). A meaningful change was accepted at p<0.05.

RESULTS AND DISCUSSION

The results of present study are shown in Table 1 and Table 2. From these results it was observed that there was a significant increase (p<0.001) in serum liver enzymes AST, ALT, ALP and total bilirubin in diclofenac administrated group of rats when compared to control with the value of (33.33(2.16), 45.33(2.16), 72.17(4.792) and 0.8(0.7-0.8)) respectively whereas there was a significant decrease (p<0.001) in total protein levels when compared to control with value of (8.18(0.19)). In Table 1 group 3 after the pre-administration of vitamin E 200 IU followed by diclofenac 50mg/kg for seven days showed a significant (p<0.001) reduction in the values of serum AST, ALT, ALP and total bilirubin (44.67(2.65), 54.67(3.77), 84.83(2.63) and 0.8(0.7-0.9)) respectively when compared to diclofenac treated groups which is almost nearer to the value of control group and also there was an significant increase (p<0.001) in total protein level (7.64(0.23)) Table 1. Similar effects were seen in group 4 with pre-administration of vitamin E400IU followed by diclofenac 50mg/kg for seven days showed a significant decrease (p<0.001) in serum liver enzymes in AST, ALT, ALP and total bilirubin (52(49.50-55.50), 59.50(57.25-60.50) 85(83-88.75) and 0.8(0.8-0.9)) which is similar to the values of control (Table 1) and also significant (p<0.001) raise in total protein levels with the value of 37(7.88-8.88) when compared to diclofenac-treated group.

Table 2 shows changes in the antioxidant parameters after the diclofenac administration there was a significant reduction (p<0.001) in the SOD, GSH, GPX and CAT when compared to the control group with the values of (1.66(1.50-1.72), 16.09(0.40), 18.12(17.77-18.33) and 3.08 (3.01-3.1)) respectively however there was an significant (p<0.001) increased TBARS values (6.06(5.95-6.27)) seen in diclofenac group Table 2. After the pre-administration of vitamin E 200 IU reversed these values by resorting the antioxidant levels by showing a significant (p<0.001) increase on SOD, GSH, GPX and CAT levels (1.05(1.0-1.2),15.42(0.47),16.97(16.74-17.34) and 3.01(2.98-3.14)) respectively when compared to diclofenac-treated group and a reduced TBARS level (3.78(3.60-3.92) seen Table 2. The effects seen in pre-administration with vitamin E 400IU shows a more remarkable (p<0.001) increase in SOD, GSH, GPX and CAT (1.30(1.20-1.52),15.42(0.47),17.87(17.49-18.22) &3.02(3.01-3.09) respectively when compared to diclofenac and low dose group treated group and also there was an significant (p<0.001) decrease in TBARS value of (3.43(3.35-3.52)) noted Table 2.

Histopathological Changes:

The histopathology of liver sections was visualized using light microscopy and it was photographed. Changes observed in the slides were narrated in the order; Figure 1 control group shows the normal central vein, hepatocytes, and hepatic sinusoids. Figure 2 Diclofenac (50 mg/kg im) treated rats showing distorted central veins, oedematous enlargement of cytoplasm, nuclear degeneration, and centrilobular necrosis of hepatocytes Figure 3 Vitamin E low dose pre-treated 200 IU orally showing the normal arrangement of the central vein, hepatocyte, and hepatic sinusoids with minimal degenerated hepatocytes. Vitamin E pre-treated 400 IU orally showing the normal arrangement of the central vein, hepatocytes, and hepatic sinusoids which is seen like the control group Figure 4.

Liver is a versatile organ in the body mainly concerned with metabolism and detoxification of chemical substance. Drugs and chemicals substance ingested by humans and animals are produce adverse effects. The most common adverse effects are hepatotoxicity mainly produced by drugs such as NSAIDS. Therefore, damage to the liver induced by hepatotoxic agents is of grave consequences. It has been reported that administration of diclofenac sodium dose of 50 mg is known to cause acute liver damage within 24 hrs it causes increase in the liver biomarkers level within six hours (Cantoni et al., 2003)

In the present study administration of diclofenac intramuscular for 7 days showed a significant increase (p<0.001) in the liver enzymes such as AST,
Table 1: Changes in serum liver enzymes in rats treated with Diclofenac and Vitamin E groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Parameters</th>
<th>Group 3 (Vitamin E+ diclo)</th>
<th>Group 3 (Vitamin E+ diclo)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (IU/L)</td>
<td>33.33(2.16)</td>
<td>89.67(2.06)</td>
<td>44.67(2.65)</td>
<td></td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>45.33(2.16)</td>
<td>106.50(4.03)</td>
<td>54.67(3.77)</td>
<td></td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>72.17(4.79)</td>
<td>35.17(131-140.75)</td>
<td>84.83(2.63)</td>
<td></td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>8.18(0.19)</td>
<td>4.62(0.40)</td>
<td>7.64(0.23)</td>
<td></td>
</tr>
<tr>
<td>Total bilirubin (μmol/L)</td>
<td>0.8(0.7-0.8)</td>
<td>1.7(1.4-1.9)</td>
<td>0.8(0.7-0.9)</td>
<td>0.8(0.8-0.9)</td>
</tr>
</tbody>
</table>

All the values are expressed in Mean (SD) (ANOVA test). Total bilirubin value are expressed in median (interquartile range) (Kruskal Wallis test). P<0.05 significant difference with control.

Table 2: changes seen in antioxidant enzyme level in rats treated with diclofenac and Vitamin E groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (Control)</th>
<th>Group 2 (Diclofenac)</th>
<th>Group 3 (vitamin E + Diclofenac)</th>
<th>Group 4 (vitamin E + Diclofenac)</th>
<th>Pvalues</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol/MDA/g)</td>
<td>3.35(3.20-3.52)</td>
<td>6.06(5.95-6.27)</td>
<td>3.78(3.60-3.92)</td>
<td>3.43(3.35-3.52)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>1.66(1.50-1.72)</td>
<td>12.58(0.66)</td>
<td>14.06(13.54-14.22)</td>
<td>15.42(0.47)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GSH (μmol/g)</td>
<td>18.12(17.77-18.33)</td>
<td>15.42(0.47)</td>
<td>16.97(16.74-17.34)</td>
<td>17.87(17.49-18.22)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GPX (U/mg protein)</td>
<td>3.08(3.01-3.1)</td>
<td>3.04(2.03-2.07)</td>
<td>3.01(2.98-3.14)</td>
<td>3.02(3.01-3.09)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CAT (U/mg protein)</td>
<td>3.43(3.35-3.52)</td>
<td>1.30(1.20-1.52)</td>
<td>17.87(17.49-18.22)</td>
<td>3.02(3.01-3.09)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The values are expressed in Mean (SD) for parameter of GSH (ANOVA test). All other parameters values are expressed in median (interquartile range) (Kruskal Wallis test).

Figure 1: Control rats showing normal central vein (Red arrow) and hepatocytes (Green arrow) and hepatic sinusoids (Blue arrow)

Figure 2: Diclofenac treated rats shows coagulativenecrosis, Damaged Porta hepatis and distorted central vein
ALP, ALT and total bilirubin levels when compared to the control group. In addition, to biochemical we also confirmed by histological findings showed some severe necrosis in liver tissue with infiltration of cells and centrilobular vein congestion (Kretzrommel and Boelsterli, 1993) (Hargus et al., 1994). Previous studies show that administration of diclofenac sodium cause increase in the liver enzyme markers and reduction in body weight, which indicates severe liver damage (Spivak et al., 2018).

The measuring serum levels of specific liver enzymes such as AST, ALT, ALP and total bilirubin are the most commonly used biomarkers in hepatotoxic studies and their values are important for identification of liver damage (Freitag and Cardia, 2015) (Hu et al., 2014).

In our study the results showed that pre-administration of vitamin E with various doses showed a reduced level of serum AST, ALT, ALP and total bilirubin and improving from liver damage when compared to the diclofenac-treated group. However, administration of vitamin E at 400 IU orally showed higher response than vitamin E at 200 IU.

The liver histopathological analysis of both the vitamin E treated groups showed some mild lesion areas, no evidence of necrosis and infiltrations of liver cells and hepatocyte preserved. In group 4, vitamin E with 400 IU administration shows a normal morphology of liver parenchyma like the control group.

Similar effects have been reported by Khalifa et al. 2009 (Khalifa et al., 2009) demonstrated the ability of vitamin E to reverse chemical agents induced hepatotoxicity, results showed 0.2g/kg/day of vitamin E normalised AST, ALT and ALP levels elevated by ccl4 in rats.

Our results were also correlated with Awodele et al. 2010 (Awodele et al., 2010) reported that pre-treated with vitamin E before rifampicin administration showed a significant decrease in levels of AST, ALT and ALP when compared with rifampicin treated rats. Comparable results were seen in pre-administration of high dose of vitamin E (100 mg/kg) intraperitoneal for 2 months prevented both biochemical as well as histopathological changes on hepatic damage induced by isoniazid and rifampicin.

Few literatures reported that formation of reactive metabolites produced during the metabolism of diclofenac in the liver are the main cause for toxicity (z Yan, 2005) (Daly et al., 2007). These reactive species are likely to damage antioxidant levels in the cells and thereby increase the production of lipid peroxidation which damages the cell membrane and
cause leakage on cellular components (Linden et al., 2008)

Our results showed a significant increase in the serum AST, ALT, ALP and total bilirubin in diclofenac-treated group whereas there was a significant decrease in total protein level was observed when compared to the control group, which is an indicative of liver damage these comparable results were also observed by Basvaraj et al. (Thanagari et al., 2012), Afyaa et al. and Zeyeb et al. (El-Maddawy and El-Ashmawy, 2013).

Vitamin E are considered as the most important antioxidant in extra cellular fluids and are mainly concerned with effective scavenging the free radicals such as superoxide, hydrogen peroxide, hydroxyl and singlet oxygen radicals (Darbar et al., 2009).

The results of our study showed there was a significant decrease in the antioxidant parameters such as SOD, CAT, GSH and GPX on the administration of diclofenac-treated rats whereas there was an elevated TBARS levels. These observations indicated that reactive metabolism produced in liver which enhances the formation of lipid peroxidation.

However, on pre-administration of vitamin E reversed or restored the antioxidant levels and showed a significant increase in SOD, CAT, GSH and GPX when compared to diclofenac group. In addition, administration of high dose of vitamin E shows almost a normal value which is more significant when compared to low dose of vitamin E This has been evidenced by normal texture of hepatic cells.

Kamil et al. 2010 (Ahmed and Mohamed, 2010) who reported that vitamin E showed significant hepatoprotective effect by preventing the leakage of intracellular enzymes of protein and decreasing apoptosis. Similar hepatoprotective effects of vitamin E were reported by few authors on the administration of drug induced hepatotoxic effects administration in rats Zaki et al. Reported co administrate of amiodarone and vitamin E. Baran et al. 2004 observed vitamin E decreased valproic acid-induced hepatotoxicity. Vitamin E has been reported as a lipid-soluble antioxidant by preventing ROS damage in polyunsaturated fatty acids and act as membrane stabilizing agent (Bradford et al., 2003). Vitamin E prevents ROS produced cell membrane damage mainly by breaking the antioxidant chain (Brigelius-Flohé and Traber, 1999). Factor et al. (Factor et al., 2000) reported that vitamin E reduced ROS production directly by interfering in the union between membrane and NADPH oxidase complex.

The study has clearly concluded that pre-administration of high dose of vitamin E ameliorates the toxic effects of diclofenac-induced hepatotoxicity.

CONFLICTS OF INTEREST: NIL

REFERENCES


tioxidant effect of vitamin C and E against some common non-steroidal anti-inflammatory drugs induced hepatic damage in rats. Asian Journal of Chemistry.


