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Impact of α-Tocopherol and Ascorbic Acid Pre-Treatment on Paracetamol Induced Liver Injury in Wistar Albino Male Rats

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Abstract **Original Research Article**

Background: Liver injury may occur by toxic dose of paracetamol administration where liver function markers are elevated and centrilobular hepatic necrosis is visible under a microscopic observation in hepatic lobule configuration. α-tocopherol and ascorbic acid are known antioxidant and they may have hepatoprotective effect against paracetamol induced liver damage. ***Aim of the study:*** The aim of this study was to evaluate the effects of α-tocopherol and ascorbic acid on paracetamol induced liver damage in Wistar albino male Rats. ***Methods:*** This was an animal model experimental study which was conducted in the Department of Physiology, Sir Salimullah Medical College, Dhaka, Bangladesh from 1st January to 31st December, 2017. Although primarily healthy Wister albino male rats, 90-120 days old, weighing from 160 to 200g were selected for this study, finally data were analyzed on 30 rats. Finalized 30 samples were divided into three groups Group I (baseline control group, n=10), Group II (paracetamol treated control group, n=10) and Group III (combined α-tocopherol with ascorbic acid pretreated & paracetamol treated group n=10). The dose of α-tocopherol 100 mg/kg Body weight and ascorbic acid 200 mg/kg Body weight per day orally were selected as pre-exposure prophylaxis. The statistical analyses were done by one-way ANOVA and Bonferroni test, paired 't' test and Fisher's Exact test as applicable. ***Results:*** The mean serum total bilirubin, ALT and AST were significantly increased in group II and III in comparison to that of group I except the serum levels of total bilirubin and ALT of group III which were increased than that of group I but it was not statistically significant. Again, the mean MDA concentration in liver was significantly increased in group II in comparison to that of group I whereas though this level was increased in group III than that of group I but it was statistically not significant. Furthermore, the mean serum levels of total bilirubin, ALT, AST and MDA concentration in liver tissue significantly decreased in group III in comparison to that of group II. Again, 100% normal histological findings of rat liver were observed in group I and abnormal histological findings were observed 100% in group II and 20% in group III. Statistically significant differences in histological findings of rat liver were observed between group I vs II (P<0.001) and group II vs III (P<0.01). ***Conclusion:*** The combined treatment of α-tocopherol and ascorbic acid have better hepatoprotective effects on paracetamol induced liver damage in Wistar albino male rats.

Keywords: α-Tocopherol, Ascorbic acid, Paracetamol, Liver damage, Male rats.

INTRODUCTION

Liver is the key metabolic organ in human body. It is essential for survival of life because it conducts a vast line-up of biochemical functions [1]. Liver diseases is a major cause of illness and death globally [2]. Exposure to certain elements, such as viruses, lipids, alcohol, and bio-transformed metabolites can cause hepatic damage to various degrees [3]. Most common causes of fulminant hepatic failure

include drug or toxin induced hepatic injury or viral hepatitis [4]. Hepatotoxicity caused by drugs, in particular idiosyncratic reactions and it is a major challenge to the pharmaceutical industry and physicians to solve the problem [5]. Drug-induced liver injury is a common adverse event seen in clinical practice., since a vast number of compounds, including phytomedicine and alternative medications are existing [6]. Paracetamol is an antipyretic and analgesic drug which is widely used to relieving fever, mild to moderate pain as an analgesic and it is also readily available as over the counter medicine [7]. Although paracetamol generally considered a safe and secure medication. But may cause of death and harms through overdose, idiopathic reaction or synergism with alcoholic liver disease, nephrotoxicity and hepatic failure [8]. The main role of vitamin E as an antioxidant which has its capacity of either reducing the harmful effect of free radical by preventing the oxidation of polyunsaturated fatty acid in the cell membrane [9]. It also can restore the normal lipid profile and can able to minimize the oxidative modification of LDL that has been distributed by paracetamol [10]. α-tocopherol or vitamin E is a group of fat-soluble compounds discovered in 1922 by Evans and Bishop and these compounds have unique antioxidant activities which is essential for health [11]. There are about eight tocopherols and tocotrienols with vitamin E activity found in nature. Among these, α-tocopherol is considered to be the most important tocopherol in animal tissues and having the highest biological activity in bioassay systems [12]. α-tocopherol may be found in a wide variety of foods and oils like wheat germ oil, peanut oil, maize oil, sun flower oil, cotton seed oil and large levels may also be found in green leafy vegetables, almonds, sunflower seeds, beet greens, pumpkin, meat, milk, butter and eggs [13]. In developing countries, populations may be at greater risk for nutritional deficiency due to limited intake of vitamin and higher prevalence of oxidative stressors. Deficiency of vitamin E is characterized by recurrent abortion, degenerative changes in spinal cord, peripheral neuropathy, ataxia, and haemolytic anemia [14]. The increased sensitivity to oxidative stress was largely reversed when glutathione-depleted cells were preloaded with ascorbic acid by exposure to dehydroascorbic acid. Vitamin C is an important independent antioxidant in protecting cells against death from oxidative damage due to redox imbalance [15]. Besides some study supports that, the hepatoprotection activity of Vitamin E and C occurred by modulating the antioxidant pathway. Therefore, Vitamin E and C have preventive action both on paracetamol induced hepatotoxicity in albino rats. It is due to the potential anti-oxidant mechanism of hepatoprotective action [16]. α-tocopherol and ascorbic acid are known antioxidant and they may have hepatoprotective effect against paracetamol induced liver damage. Therefore, Vitamin E and C have preventive action both on paracetamol induced hepatotoxicity in albino rats.

METHODOLOGY

This was an animal model experimental study which was conducted in the Department of Physiology, Sir Salimullah Medical College, Dhaka, Bangladesh. Although primarily healthy Wistar albino male rats, 90-120 days old, weighing from 160 to 200g were selected for this study, finally data were analyzed from 30 rats. Finalized 30 samples were divided into three groups; Group I (baseline control group, n=10), Group II (paracetamol treated control group, n=10) and Group III (combined α-tocopherol with ascorbic acid pretreated & paracetamol treated group, n=10). The study was approved by the ethical committee of the mentioned medical college hospital. The whole intervention was conducted in accordance with the principles of animal research specified in Institutional Animal Care & Use Committee *(IACUC)* [17]. As per the inclusion criteria of this study, rats with apparently healthy Wistar albino male, age 90 to 120 days and weight 160 to 200g were included. On the other hand, according to the exclusion criteria of this study, rats with disease were excluded. Group I received basal diet for 30 days and propylene glycol (2 ml/Kg bw, orally) on day 28, 29 and 30. In addition to basal diet, Group II received basal diet for 30 days and paracetamol (1500mg/ kg bw, orally) on day 28, 29 and 30. Group III received both α-tocopherol 100 mg/kg bw and ascorbic acid 200 mg/kg bw orally for 30 days and paracetamol (1500mg/ kg bw, orally) on day 28, 29 and 30. A pilot study was conducted to determine the toxic dose of paracetamol for liver damage in nine (9) Wistar albino male rats (3 rats for each dose) with 120 to 150 days old and 200 to 240 g weighted. After acclimatization of 10 days' blood samples were collected from tail vein. Then the rats were administered paracetamol solution orally with different doses like 1, 1.5, or 2 g per kg body weight once daily in the morning for 3 days sacrificed after 24 hours of last dose of paracetamol administration. Serum levels of alanine aminotransferase (ALT) was measured two times, before (as control) and after paracetamol administration. Histological findings of liver were also done to assess the optimum toxic dose of liver damage. Finally, the dose of paracetamol as 1500 mg/kg body weight once daily for 3 consecutive days was selected for induction of liver toxicity.

Study Procedure

All the animals were acclimatized for 10 days prior to intervention at 28 ±2°C room temperature under 12 hours' light/12 hours' dark cycle. During this period, the animals had free access to standard food pellets and allowed drinking water as desired. After 10 days of acclimatization, the total study period was 30 consecutive days. At the beginning of the study period (day-1), initial body weight of all the rats were measured and at the end of the study period after measuring the final body weight, all the rats were sacrificed on day 31. Blood samples were collected on day-1 from the tail vein of all rats to assess the liver function. The serum level of alanine aminotransferase was measured and rats with

normal level of ALT (10-40 U/L) were included in this experiment. Hepatotoxicity was induced by administration of single daily morning dose of paracetamol (1500 mg/kg bw orally) by intragastric gavage on day 28, 29 and 30 after overnight fasting in all groups of rats except group I. α-tocopherol (100 mg/kg bw) and ascorbic acid (200 mg/kg bw) were given in the experimental groups orally in the morning between 9:00 to 10:00 am. The experimental groups were received single daily dose of α-tocopherol and ascorbic acid as recommended for respective group for consecutive 30 days. The blood samples were collected from heart and the liver was removed and weighted from each rat after sacrifice. Serum levels of total bilirubin, alanine aminotransferase and aspartate aminotransferase were measured for assessment of liver function. Serum levels of total bilirubin, ALT and AST were measured in the department of Biochemistry, Bangabandhu Sheikh Mujib Medical University, Dhaka in Wroblewski and LaDue principle and Backman Culter AU 680 system. Assessment of malondialdehyde (MDA) content of liver tissue homogenate was done by using standard laboratory kits in the laboratory of department of Biochemistry and Molecular Biology, Jahangirnagar University, Dhaka. To find out the histopathological changes of liver tissue, histological slides were prepared with hematoxylin-eosin stained to be observed under the microscope and photomicrographs were taken by using standard laboratory procedure in the department of Pathology, Sir Salimullah Medical College, Dhaka, Bangladesh. After 24 hours of last dose of paracetamol administration, all the rats were anesthetized with the help of chloroform (30%) and then sacrificed. Then blood sample (5ml) were collected from the heart by using disposable syringe and were taken in separate clean and dry test tubes with proper identification numbers. The test tube was kept in standing position till formation of clot. Then blood was centrifuged at a rate of 3000 rpm for 10 minutes. After that, supernatant serum was collected in labeled Eppendorf tube and preserved in the refrigerator for estimation of all the biochemical parameters.

Statistical Analysis

Numerical data were presented as mean±SD. Statistical analysis was done by one-way ANOVA test. Post hoc Bonferroni test was performed to compare between groups and Fisher's exact test was done for histological analysis. Paired 't' test was also done. P value ≤0.05 was considered statistically significant. Statistical analyses were done by using Statistical Package of Social Science (SPSS) version 22.0.

RESULTS

In this study, the mean initial body weights of the rats (day-1) were 181.60 ± 6.08, 183.20 ± 7.04 and 184.40 ± 7.89 gm whereas final body weight (day-2) was 222.90±17.22, 220.40 ± 16.02, 224.50 ± 18.72 gm in group I, II and III respectively. Initial body weights of group I, II and III were almost similar and showed no statistically significant differences of these values among the groups. Again, final body weights of group I, II and III were also almost similar and no statistically significant differences of these values were observed among the groups. The mean % change of body weight from final to Initial were 22.62 ± 6.25, 20.21 ± 5.34 and 21.60 ± 6.06 % in group I, II and III respectively. Moreover, final body weights of all rats were significantly increased from initial body weight in each group. The mean liver weights of rats were 3.88 ± 0.53, 6.13 ± 1.28 and 4.37 ± 0.83 gm in group I, II and III respectively. In this study, the liver weight was increased in group II and group III in comparison to that of group I but the differences were not statistically significant. Again, the liver weight was significantly decreased in group I ($P<0.001$), and group III ($P<0.01$) than that of group II. The mean serum total bilirubin levels of the rats were 0.68 ± 0.30, 1.46 ± 0.31 and 1.79 ± 0.21 mg/dl in group I, II and III respectively. In this study, the serum total bilirubin level of rats was significantly increased in group II ($P<0.001$) and also in group III ($P<0.001$) in comparison to that of group I. The mean serum total bilirubin levels of the rats were 0.68±0.30, 2.41±0.59, and 1.10 ± 0.27mg/dl in group I, II and III respectively. In this study, the serum total bilirubin level of rats was significantly increased in group II ($P<0.001$) in comparison to that of group I. This change was significantly decreased in group III ($P<0.001$) when compared to that of group II. Moreover, no statistically significant differences were observed between group I vs III. The mean serum ALT levels of the rats were 35.90±9.10, 94.10±16.93 and 51.20±7.84 U/L in group I, II, III respectively. In this study, the serum ALT level was significantly increased in group II ($P<0.001$) than that of group I. This level was significantly decreased in group III($P<0.001$) in comparison to that of group II. The serum ALT level was increased in group III than that of group I but no statistically significant differences were found between these groups. The mean serum AST levels of the rats were 58.10±13.67, 138.30 ± 28.28, and 91.10 ± 17.73U/L in group I, II and III respectively. In this study, the serum AST level of rats were significantly increased in group II ($P<0.001$), and in group III ($P\leq0.05$) in comparison to that of group I. Again, serum AST level was significantly decreased in group III ($P<0.001$) than that of group II. The mean MDA concentration in liver of the rats were 3.93±2.31, 10.23±1.53 and 4.90±1.15 nmol/mg protein in group I, II, and III respectively. In this study, the MDA concentration in liver was significantly increased in group II ($P<0.001$) in comparison to that of group I. But the statistically significant differences were not observed between group I vs III although the MDA concentration in liver in group III was increased than that of group I. The mean MDA concentration in liver of the rats were significantly decreased in group III ($P<0.001$) than that of group II. In this study, histological examination of liver revealed normal findings in 100% of rats in group I, whereas abnormal histological findings were observed in 100% of rats in group II and 80% of rats in group III

showed normal findings. In this study, statistically significant differences in histological findings of rat liver were observed between group I vs II (P<0.001) and group II vs III (P<0.01). Again no statistically significant changes were observed in group I vs III.

Table 1: Body weight and percent change of body weight in different groups of rats, (N=30)

Groups	**Body weight (gm)**		**p value**	**Change (%)**
	Initial (I)	**Final (F)**		
	(Day-1)	**(Day-30)**		**[(F-I)/Ix100]**
I (n=10)	181.60±6.08	222.90 ± 17.22	<0.001	22.62 ± 6.25
II (n=10)	183.20 ± 7.04	220.40 ± 16.02	<0.001	20.21 ± 5.34
III (n=10)	184.40 ± 7.89	224.50 ± 18.72	<0.001	21.60 ± 6.06
Statistical analysis				
Groups	**P-value**			
	Initial (I)	**Final (F)**	**Change (%)**	
I vs II vs III	0.436	0.596	0.194	
I vs II	1.000	1.000	1.000	
I vs III	1.000	1.000	1.000	
II vs III	1.000	1.000	1.000	

Table 2: Liver weight of rat in different groups, (N=30)

Groups	**Liver weight**
	(gm)
I (n=10)	3.88±0.53
	(3.10-5.05)
II (n=10)	6.13 ± 1.28
	(4.72 - 8.12)
III (n=10)	4.37 ± 0.83
	(3.12-5.88)
Statistical analysis	
Groups	**P-value**
I vs II vs III	<0.001
I vs II	<0.001
I vs III	1.000
II vs III	0.001

Table 3: Serum total bilirubin level in different groups of rats, (N=30)

Groups	**Serum total bilirubin**
	(mg/dl)
I (n=10)	0.68 ± 0.30
	(0.29-1.05)
II (n=10)	2.41 ± 0.59
	(1.11-3.02)
III (n=10)	1.10 ± 0.27
	(0.81-1.60)
Statistical analysis	
Groups	**P-value**
	Serum total bilirubin
I vs II vs III	<0.001
I vs II	<0.001
I vs III	0.114
II vs III	<0.001

Table 4: Serum alanine aminotransferase (ALT) level in different groups of rats, (N=30)

Groups	**Serum ALT**
	(U/L)
I (n=10)	35.90 ± 9.10
	(26.00-58.00)
II (n=10)	94.10 ± 16.93

Groups	**Serum ALT**
	(U/L)
	(72.00-129.00)
III (n=10)	51.20 ± 7.84
	(41.00-65.00)
Statistical analysis	
Groups	**P-value**
I vs II vs III	<0.001
I vs II	<0.001
I vs III	0.076
II vs III	<0.001

Table 5: Serum aspartate aminotransferase (AST) level in different groups of rats, (N=30)

Groups	**Serum AST**
	(U/L)
I (n=10)	58.10 ± 13.67
	(42.0-90.0)
II (n=10)	138.30 ± 28.28
	(100.0-190.0)
III (n=10)	91.10 ± 17.73
	(69.0-119.0)
Statistical analysis	
Groups	**P-value**
I vs II vs III	<0.001
I vs II	<0.001
I vs III	0.023
II vs III	<0.001

Table 6: Malondialdehyde (MDA) concentration in liver in different groups of rats, (N=30)

Groups	**MDA**
	(nmol/mg protein)
I (n=10)	3.93 ± 2.31
	(0.09-7.16)
II (n=10)	10.23±1.53
	(8.08-12.39)
III (n=10)	4.90 ± 1.15
	(3.28-7.01)
Statistical analysis	
Groups	**P-value**
	MDA
I vs II vs III	<0.001
I vs II	<0.001
I vs III	1.000
II vs III	<0.001

Table 7: Distribution of rats by histological changes in liver, (N=30)

Groups	**Histological findings**	
	Normal	**Abnormal**
	n(%)	**n(%)**
I (n=10)	10(100.0)	0(0)
II (n=10)	0(0)	10(100.0)
III (n=10)	8(80)	2(20)

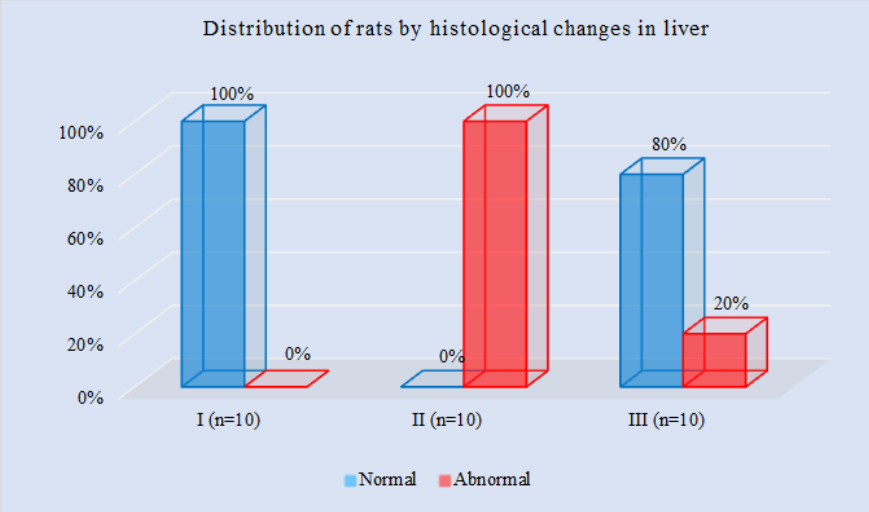
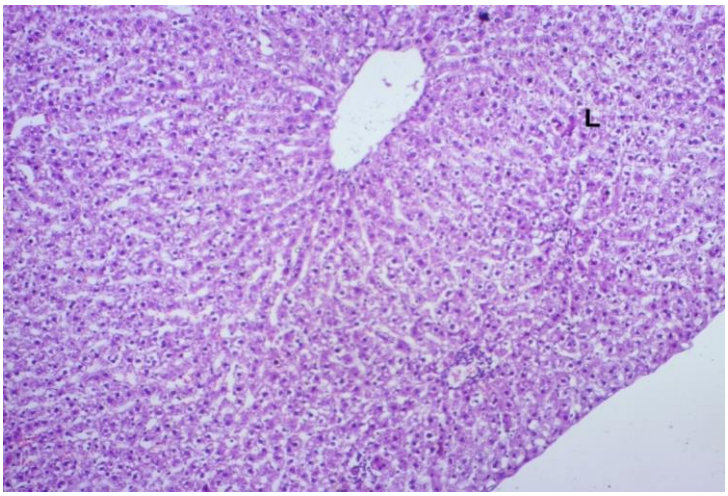
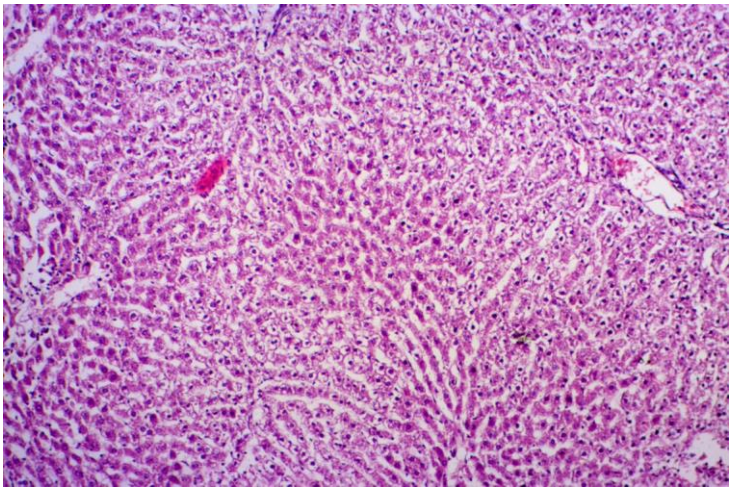


Figure I: Column chart showed histological changes in liver of the rats, (N=30)



Photomicrograph 1: Liver of paracetamol treated control rats (here 'L' represents lymphocyte infiltration in X 100)



Photomicrograph 2: Improvement of necrosis and other changes of liver in combined α-tocopherol with ascorbic acid pretreated and paracetamol treated rats (X 100)

DISCUSSION

The aim of this study was to evaluate the effects of α-tocopherol and ascorbic acid on paracetamol induced liver damage in Wistar albino male Rats. In this study, the mean serum total bilirubin, ALT and AST were significantly increased in group II and group III in

comparison to that of group I. Again, the mean MDA concentration in liver was significantly increased in group II (P<0.001) in comparison to that of group I whereas though this level was increased in group III than that of group I but it was statistically not significant. In this study, the MDA concentration in liver was significantly increased in group II (P<0.001) in comparison to that of group I. But the statistically significant differences were not observed between group I vs III although the MDA concentration in liver in group III was increased than that of group I. The mean MDA concentration in liver of the rats were significantly decreased in group III (P<0.001) than that of group II. Values of the study parameters such as serum levels of total bilirubin, ALT, AST, MDA concentration in liver of all the animals of baseline control group were nearly physiological limit and the histological findings of liver revealed normal histological architecture. These findings were almost similar to those, reported by various investigators of different countries [14, 18]. In this study, the final body weight of all the rats were increased than that of initial body weight and the difference was statistically significant in each group of rats. Almost similar finding was also observed by other researchers on hepatotoxicity [17, 19]. In this study, the mean serum levels of total bilirubin, ALT, AST and MDA concentration in liver tissue significantly increased in group II, Again, 100% normal histological findings of rat liver were observed in group I and abnormal histological findings were observed 100% in group II and 20% in group III. In this study, statistically significant differences in histological findings of rat liver were observed between group I vs II (P<0.001) and group II vs III (P<0.01) but no statistically significant changes were observed in group I vs III. The liver weight of rats was significantly increased in paracetamol treated control group than that of baseline control group. Almost similar finding was also found by other researchers [20]. Whereas, the liver weight was increased but statistically not significant in combined α-tocopherol with ascorbic acid pretreated & paracetamol treated group than that of baseline control group. In this study, the serum total bilirubin level of the rats was significantly increased in combined α-tocopherol with ascorbic acid pretreated and paracetamol treated control group than that of baseline control group. Similar findings were also observed by some other researchers [21]. Whereas, Bhavsar *et al*., found non-significant increase of bilirubin rats treated with ethanol extract of Citrus lemon [22]. In the present study, serum levels of ALT were significantly increased in paracetamol treated control group than that of baseline control group. This finding was equivocal with that of some other investigators [23, 24]. In the study, serum AST level of all the experimental rats were significantly increased in comparison to that of baseline control group. Almost similar finding was observed by some other researchers [25, 26]. In this study the MDA concentration in the liver tissue homogenate was significantly increased in paracetamol treated control group than that of baseline control group. Similar observation was made by some other researchers [27, 28]. Whereas, the MDA concentration was increased in combined α-tocopherol with ascorbic acid pretreated and paracetamol treated group than that of baseline control group but the difference was not statistically significant. In the present study, abnormal histological changes like centrilobular necrosis, disorganization of hepatic sinusoids, infiltration of lymphocytes and Kupffer cells, fatty changes and ballooning degeneration were observed in 100% of rats in paracetamol treated control group. Significant elevation of serum total bilirubin, ALT, AST and MDA concentration in liver tissue were observed in paracetamol treated rats that of combined vitamin pretreated rats. In addition, moderate histological changes of liver were also observed by microscopic examination [29]. Paracetamol is inactivated in the liver, mainly by conjugation as glucuronide and sulphate. Minor metabolites are also formed, one of them is N-acetyl-p-benzoquinone imine (NAPQI) which is chemically highly reactive. It is normally harmless by conjugation with glutathione. Again, paracetamol that undergo bioactivation to toxic intermediates cause necrosis of the cells surrounding the central veins (centri-lobular), because the components of the cytochrome P-450 system are found in those cells in abundance.

LIMITATION OF THE STUDY

Different doses of α-tocopherol and ascorbic acid were not used to find out the best effective dose. Anti-oxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) levels were not studied. Serum enzyme levels of α-tocopherol and ascorbic acid were not done. All of those are some limitations of this current study. Moreover, this was a single centered study with small sized samples.

CONCLUSION & RECOMMENDATION

In α-tocopherol with ascorbic acid pretreated & paracetamol treated Wister albino rats, the mean total bilirubin, ALT, AST and, MDA concentration in liver is found as significantly decreased than those in paracetamol treated Wistar albino rats', Histological findings also suggests better hepatic protection of combined α-tocopherol with ascorbic pretreatment I paracetamol induced liver injury. For getting more specific results we would like to recommend for conducting similar more studies in several places with larger sized samples.

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