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| RESEARCH ARTICLE

The Protective Role of Vitamin E on the Liver, Kidney, and Male Reproductive Functions of Paracetamol Overdose in Male Rabbits

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| ABSTRACT
Pharmaceutical harm may result from both planned effects and pharmaceutical errors. Although paracetamol is often used as an antipyretic and painkiller, an excessive amount of it may be toxic to the liver and create free radicals that are harmful to human health. Thirty adult male rabbits were divided into three groups. Group I was orally administered normal saline (control). Group II (Paracetamol toxic dose) was orally administered paracetamol (1500mg /kg b.w) dissolved in normal saline. Group III (Paracetamol & vitE) (1500;400)mg/kg b.w), respectively. All group doses were given for three weeks daily. The findings revealed that a toxic dose of paracetamol increased oxidative stress (MDA), liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AS.T), alkaline phosphatase (ALP), the levels of serum creatinine (Cr), urea, and blood urea nitrogen (BUN), and decreased testosterone hormone. Additionally, the findings revealed a notable improvement in the liver and kidney functions. This study demonstrates that paracetamol in overdose elevated oxidative stress and hepatotoxicity, and nephrotoxicity reduced testosterone hor, but on the other hand, vitamin E had a protective effect of eliminating this disruptor.

| KEYWORDS

Paracetamol, vitamin E, oxidative stress, Liver enzymes, and kidney functions.

| ARTICLE INFORMATION

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1. Introduction

The research proved that a toxic dose of paracetamol increased oxidative stress (MDA), liver enzyme levels of alanine aminotransferase (ALT), and aspartate aminot. Transferase (AST), alkaline phosphatase (AL.P), serum levels of creatinine (Cr), urea, blood urea nitrogen (BUN), and testosterone hormone levels. Additionally, the results showed that the liver and renal functions had significantly improved. (Schilling, 2010) One of the most frequent global causes of poisoning is the use of paracetamol. Its poisoning may result from ingesting too many dosages, too often, or repeatedly. Subtherapeutic intake usually occurs as a severe clinical issue. However, paracetamol excess or prolonged usage has well-known adverse effects, such as hepatotoxicity, nephrotoxicity, loss of reproductive ability, modification of testicular structure, and induction of oxidative stress. (Baniasadi, 2010, Potter, 1987) Paracetamol (Para), (acetaminophen[a] or para-hydroxy acetanilide) (acetaminophen) belongs to para-aminophenol, a class of NSA.Ids. (Ranganathan, 2006) The availability and affordability of paracetamol, which is offered both with and without a prescription, contribute significantly to its popularity. The cytochrome P450 enzyme produces the reactive intermediate N-acetyl-p-benzoquiinone imine (NAPQI), the first stage of its toxicity. At therapeutic dosages, NAPQI is eliminated by conjugating with glutathione (GSH). The cellular GSH is depleted by high paracetamol dosages, which makes it possible for NAPQI to bind to cells.lar proteins and start lip. In peroxidation, which may be hazardous. (Farag, 2015, Saritas, 2014) By reducing an oxyg.en molecule, the liver creates reactive oxygen species (ROS) in the form of free radicals during the metabolism of paracetamol. These free radicals harm the mitochondria in cells and change how the hepatocytes in the liver operate. Overdosing or using paracetamol often may have well-known negative consequences, such as diminished reproductive potential, altered uterine and ultrastructure, and reduced sperm quality. 2,3. In both humans and laboratory animals, acute paracetamol (N-acetyl-amino. phenol; APAP) overdose

can lead to testicular toxicity. (Cekmen, 2009). Renal-hepatic necrosis and apoptosis outcomes when Para ingestion exceeds 150 mg/kg body weight in adults. (Kett, 2011) When it accumulated in renal tissues, it was considered to start a series of biochemical processes that led to acute or chronic nephropathies. (Afshar, 2008) Paracetamol overdose was used to study glutathione depletion. Glutathione loss caused paracetamol metabolites to accumulate intracellularly and form covalent bonds with cellular proteins rather than being eliminated from the body. Lipid peroxidation starts in the renal tubular cells, disrupting the body's natural antioxidant system and causing renal tubular cells to die. (Niki, 2012) The reactive oxygen species (ROS) or free radicals are essential mediators of paracetamol-induced nephrotoxicity. (Bessems, 2001). Vitamin E is the collective term for a group of fat-soluble compounds (Asad, 2013). Vitamin E is fat-soluble; it is present in foods that contain fat and are stored in the mammal's fatty tissues. (Al-Attar, 2011) It is also present in foods like seeds, nuts, and vegetable oils, which have high levels of -tocopherol. Green leafy vegetables and fortified cereals also have significant amounts of vitamin E. Reactive oxygen species (ROS) molecules cannot be produced when fat is exposed to oxidation because vitamin E, a potent antioxidant that breaks chains, blocks the propagation of the free radical reaction. (Aziz, 2012). Its most substantial protective effect may be seen in the membranes of cells and organelles, where it was first discovered. Numerous studies have shown that oxidative damage that occurs after paracetamol intoxication causes oxidant parameters to rise and antioxidant parameters to fall. In this research, adult rabbits' liver, kidneys, and male reproductive systems were examined for the toxic effects of paracetamol as a result of prescription mistakes. Vitamin E is an antioxidant that may remove this toxicity.

1.1 Aim

The present study was undertaken to evaluate the protective efficacy of vitamin E regarding altered oxidative parameters, hepatotoxicity, nephrotoxicity, and testosterone activities subsequent to paracetamol overdose in male rabbits.

2. Material and methods

Thirty mature male rabbits weighing between 2000 and 2500 grams each were employed. They were kept in dedicated cages under regular laboratory settings and fed at will with access to tap water. 1000 mg of paracetamol pills

2.1 Experimental design

The adult male rabbits were randomly distributed into three experimental groups, each containing (10) rabbits. Normal saline (control) was given orally to Group I. Group II (Paracetamol Toxic Dose) received 1500 mg/kg b.w. of paracetamol dissolved in normal saline orally. Group III received dosages of paracetamol and vitamin E (1500 and 400 mg/kg b.w., respectively) for three weeks daily.

2.2 Blood Samples

Fasting blood samples (about 5ml) were collected at 7:30 a.m. from the hearts of animals for each group at the end of the experimental period (three weeks). Samples were let until clotting, then centrifuged at 300 rpm for ten min. to be used as serum samples immediately for detection in this study. The rest of the serum was stored at -20ºC.

2.3 Serum assays

According to the technique (Nursah, 2022), malondialdehyde, one of the primary end products of lipid peroxidation, will be measured in serum. Thiobarbituric acid reactive substances (TBARs) are created when thiobarbituric acid (TBA) and MD.A interact. The resulting pink product's absorbance was measured at 535nm. The total testosterone was calculated using a procedure (Ijaz, 2016). The alkaline phosphatase (ALP) average was calculated using the technique of reference (Asif, 2019); the aspartate aminotransferase (A.ST) activity was measured by method (Tenenbein, 2004) using the ELISA approach, whereas the level of alanine aminotransferase (ALT) was analyzed using the technique given by (Richardson, 2000). Using a specialized chemical kit (Agape Hills, Dist, Ernakulam Kerala.India_683 562, blood urea and creatinine were determined enzymatically (Tenenbein, 2004).

2.4 The Statistical Analysis

ANOVA was used to evaluate the mean values of the serum indices. The results showed mean differences and standard deviations (mean ± SD) between the treatment and control groups; P-values of less than 0.05 are considered statistically significant. (Niki, 2012)

3. Results

The results indicate a significant increase in the MDA at ($P < 0.05$) when the animals were treated with paracetamol compared to the control group but a significant decrease in MDA in the treatment group Vit. E, Table 1, And the same table found a significant reduction ($P < 0.05$) in the testosterone level in the group that received paracetamol at a toxic dose compared to standard control, whereas results showed administration of Vit. E plus paracetamol pronounced elevation in the testosterone level compared to the group received alone. The results presented in Table 2 showed a significant increase in AST, ALT, and ALP levels in the rabbits treated with a toxic dose of paracetamol. According to Table 3 findings, which were in line with other research (Baniasadi, 2010),

there was a substantial rise in kidney function tests (urea, creatinine, and blood urea nitrogen concentrations) in the group treated with a hazardous dosage of paracetamol.

Table 1. Serum MDA and Test esostoren levels in the control group and treated groups with a toxic dose of paracetamol plus Vit. E in male rabbits. Data are expressed as mean ± SD (n=10). The letters refer to significant differences (p≤ 0.05) para: paracetamol; vit E: Vitamine E.

Parameters / Groups	MDA µ mol / L	Testosterone ng/ml
Control	0.54±0.039 c	7.35 ±0.48 a
Paracetamol (1500mg /kg/ day)	2.10 ±0.102 a	3.25 ±0.38 b
Para (1500mg /kg/ day) vit. E (400/kg)	1.13 ±1.861 b	6.62 ±0.38 a
LSD	0.1303	1.5932

Table 2. Serum AST, ALT, and ALP were used in the control group, and the treated groups were treated with a toxic dose of paracetamol plus vitamin C. E in male rabbits. Data are expressed as mean ± SD (n=10). The different letters refer to significant differences (p≤ 0.05) para: paracetamol vit E: Vitamin E

Parameters / Groups	AST unit/L	ALT unit/L	ALP unit/L
Control	42.87±0.29 b	62.68 ±0.68 c	116.27 ±0.69 b
Paracetamol (1500mg /kg/ day)	86.70 ±0.59 a	142.36 ±0.61 a	234.30 ±0.37 a
Para+Vit.E. (1500mg/kg/ day)+ (400mg/kg)	48.48 ±0.73 b	89.53 ±1.36 b	125.80 ±0.88 b
LSD	9.73 ±0.25	6.80 ± 0.89	12.29±3.96

Table 3. Serum Creatinine, Urea, Blood urea nitrogen, Uric acid, and Total Protein Concentrations in the control group and treated groups with a toxic dose of paracetamol plus Vit. E in male rabbits. Data are expressed as mean ± SD (n=10). The letters refer to significant differences (p≤ 0.05) para: paracetamol vit E: Vitamine E.

Parameters / Groups	Creatinine mg/dl	Urea mg/dl	BUN
Control	0.54 ±0.02c	23.53 ± 2.23 c	10.92±1.0c
Paracetamol (1500mg /kg/ day)	2.11 ±0.17 a	44.08 ±2.56 a	21.38 ±1.19 a
Para+Vit.E. (1500+400)mg/kg	0.57 ±0.01c	27.76±1.80 b	12.88 ±0.83c
LCD	1.6± 0.2	2.81 ±0.67	3.01 ±0.17

4. Discussion

A significant increase in the MDA at (P < 0.05) resulted from administering paracetamol at a toxic dose associated with oxidative stress and lipid peroxidation. This finding seems to be in concordance with the findings of other authors who showed that paracetamol-induced oxidative stress and change in the structure and/ or function of cell membranes (Wafaa, 1999; Factor, 2000).

Uboh FE. et al. (2012) reported that at the appropriate MDA level, polyunsaturated fatty acid peroxidation results in the formation of conjugated dienes (CD), which are then broken down into reactive aldehydes such as 4-hydroxy-2,3-transnmoveal, malondrialdehyde, and 4-hydroxy-2,3-transhexual, also known as thiobabitunc acid-reacting substances (TBARs). Lipid peroxidation's byproduct causes reactive species to damage DNA and cellular membranes more severely. A paracetamol overdose causes lipid peroxidation, as seen by a high amount of MDA and increased oxidative stress in tissues, according to prior research that has been conclusively shown by Alderman et al.(2002) Also, the results that vitamin E inhibits oxidative damage to membrane polyunsaturated fatty acid, the role of vitamin E on Paracetamol-induced oxidative damage in male rabbits has endogenous antioxidant that scavenges free radical directly and inhibits biomolecule oxidation, these results were discussed by Melnyk et al. (2010) investigated the vitamin E suppressed generation and accumulation of intracellular ROS which converts cellular redox balance toward oxidative stress under inflammatory condition, the considerable blood testosterone level decline in male rabbits during the course of the drug's continuous use. Because the oxidative damage in the testes and sperms largely relied on their oxidative defense level, paracetamol was hypothesized to cause oxidative stress in different organs, including the testes. (Poma, 2012) The reduction in estradiol levels is related to a decline in circulating testosterone levels. this may be attributed to the fact that a toxic dose of paracetamol acted directly on the tests and affected the androgen biosynthesis in interstitial cells. Abnormal interstitial cells decreased the steroid. Genic potential of the testis. This was agreed with researchers Wolf (2005), who reported that injury caused by paracetamol (paracetamol) is apparent at presentation and related to dose magnitude and duration. However, the precise mechanism by which paracetamol administration of vitamin E improves the levels of FSH, LHof, and testosterone hormones in animals indicates the ability of vitamin to ameliorate paracetamol toxic-induced testicular damage (Larson, 2005). These results may be explained by the androgenic activities of vitamin E, which are reflected by the increase of testis weight serum hormones level. Jaeschke et al. concluded that vitamin vit. E supplementation causes efficiency spermatogenesis by serum testosterone, which increases sperm quality, motility, and density and consequently increases fertility in male rats; these results revealed that vitamin E. acts as an antioxidant, preventing lipid peroxidation in biological membranes. (Jaeschke, 2003) The results present in Table 2 showed that there was a significant increase in the levels of AS.T, ALT, and AL, P levels in the rabbits treated with toxic do.se of paracetamol; this elevation due to liver damage and leakage of cytosolic enzymes from hepatocytes Similar finding was reported by, Atkuri et al. (2007) who found Mechanisms of acetaminophen toxicity due to excessive formation of a highly reactive intermediate metabolite, N-acetyl-para-benzoquinone-imine (NA.PQI), occurs when large doses of the drug are ingested. It has been claimed that taking too much paracetamol results in an increase in the generation of free radicals (Saritas, 2014). These reactive oxygen species bring on hepatocyte mortality, mitochondrial malfunction, the development of fatty livers, lipid peroxidation, and eventually liver cell destruction. However, co-administration of vitamin E and paracetamol led to a restoration of the enzyme levels in the rabbits' serum, indicating vitamin E's protective impact against liver damage brought on by a toxic dosage of paracetamol. (Li, 2013) Giving vitamin E to rabbits supplied with paracetamol had a noticeable ameliorative impact on the hepatotoxicity of acetaminophen exposure. (Mercan, 2004). The vitamins increased the antioxidant capacity of various endogenous antioxidant components and counteracted the hepatotoxic impact linked to paracetamol-generated free radicals. According to reports, vitamin E performs two crucial roles in membranes: protecting polyunsaturated fatty acids from ROS damage as a liposoluble antioxidant and preventing damage to phospholipids as a membrane-stabilizing agent. This is in line with the assertion made by Ratnasooriya et al. (2000) that vitamins C and E have a beneficial impact on hepatotoxicity. The adverse effects of paracetamol on the kidney, which result in nephrotoxicity, were discovered to be the source of this rise in these parameters through one or more common pathogenic processes. These include crystal nephropathy, inflammation, and altered tubular cell toxicity. Three possible factors might explain the current study's findings of an increase in serum urea: 1) reduced protein metabolism, 2) impaired hepatic function, and 3) decreased renal urea filtration rate. (Shah, 2011) When functional nephrons are damaged, there is an increase in blood creatinine levels. (Trujillo, 2013; Tamaddonfard, 2014) At toxic doses, paracetamol has been linked to lipid peroxidation, glutathione depletion, and intracellular accumulation of its reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI), which results in cell death and deterioration. These events are linked to electrolyte imbalance, elevated creatinine levels, and unstable blood urea nitrogen levels. (Baniasadi, 2010) A paracetamol overdose has been demonstrated to target renal tissues, which raises blood levels of Cr and BUN. (Poma, 2012; Du, 2016) These findings from the current study corroborated those from earlier research in which rats were given gentamicin and acetaminophen to cause nephrotoxicity. (Trujillo, 2013) Urea, creatinine, and blood urea nitrogen concentrations decreased ($P \leq 0.05$) when combined with paracetamol and vitamin E. Plasma creatinine urea levels had been depletion by vit.E. These results are in agreement with other research (Mohamed, 2013, Omidi, 2014) which reported nephroprotective activity of vit. E was due to possessed strong antioxidant activity. Similarly, Harumngana madmagascariensi, Pimpinellas tirupatigensis, Cardiospermoum halicacabum, Cana. rium schweinfurthi, Tapinantphus globuliferous, and Spathodea campanulate also reduced the Cr and BU.N levels in paracetamol induced nephrotoxic experimental animals. (Piper, 1998)

4.1 Study limitations

The study's limitations are those characteristics of design or methodology that impacted or influenced the interpretation of the findings from your research. This section is not mandatory. (Niki, 2012)

5. Conclusion

This study demonstrates that paracetamol in overdose elevated oxidative stress and hepatotoxicity; nephron-toxicity reduced testosterone hormone, but on the other hand, it. E. had a protective effect on the elimination of these disruptors

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Declarations

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Conflicts of interest: No conflict of interest.

Data availability: No datasets were generated or analyzed during the current study.

Ethics approval: The Institutional Scientific Committee at the University of Thi-Qar approved this study according to the Declaration of Helsinki for human studies 1975, and all were followed for the care and use (Project identification 1877).

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