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Vitamin E and C Long-Term Treatment Effect on paraquat Poisoned Liver in Rat

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Abstract: Paraquat toxicant causes serious herbicide intoxication generally and typically because of intentional or unintentional ingestion. Vitamin C and E are awesome antioxidants that respond quickly to oxidative stress and modify lipid peroxidation. This study was aimed at assessing the impact of Vitamin E and C combined treatment on chronic toxicity of paraquat on liver organs in Wistar rats. Exactly 200 male rats were used for the study. The rodents were isolated into four groups of 50 rats in each group (A, B, C, D) and was further subgrouped into two (0 and VEC), having 25rats per subgroup. Each of the "0" subgroups (A0, B0, C0 and D0) aside from A0 were treated with paraquat in 0.02g, 0.04g and 0.06g respectively for 3 months every 2 weeks. All "VEC" subgroups (AVEC, BVEC, CVEC and DVEC) aside from AVEC were treated with paraquat also, after which they were treated with vitamin E (500mg) and C (2000mg/L) week after week for three months. AVEC was treated with vitamin E and C alone. Blood samples were obtained and tested for liver function (total, conjugated, unconjugated bilirubin, total protein, albumin and globulin). There was a significant difference (p-value<0.05) in the level of the liver parameters among the "Ao", "Bo", "Co" and "Do". The outcome additionally showed that there were significant difference (p-value<0.05) in the levels of the liver parameters within the groups (Bo vs BVEC, Co vs CVEC, and Do vs DVEC). However, was no significant difference in the levels of globulin among the subgroups. This study has affirmed that vitamin E and C combined therapy has a helpful impact in male Wistar rats on week after week treatment for 3 months.

Keywords: Vitamin E and C, paraquat, rat, antioxidant, liver markers.

1. Introduction

Paraquat causes serious herbicide intoxication generally and normally because of intentional ingestion. The primary driver of death in paraquat poisoning is respiratory failure because of an oxidative damage to the alveolar epithelium which thus leads to fibrosis [1]. There are as of now no pharmacological treatment against paraquat and no agent to chelate the toxic substance in blood or different tissues, this has made the administration of paraquat poisoning to remain a critical health concern with its high absorption in the gastrointestinal tract [1].

Paraquat has an extremely high death rate in spite of its helpful role treatment, regardless of how much is ingested. It ought to be noticed that the harmful impacts of

paraquat is actualized through oxidative stress induced toxicity [2], and future management ought to be pointed towards lessening the intense alveolitis and pneumonic fibrosis by the use of antioxidants [2] as has been displayed in a few past studies. Two investigations conducted on the assessment of the impact of melatonin as an antioxidant on the cell toxicity and result of paraquat-intoxicated rats revealed that melatonin increased survival time and gives valuable impacts against paraquat poisoning [3,4]. A similar impact was found in another review utilizing the antioxidant, N-acetylcysteine [5].

The liver plays a significant part in the digestion of xenobiotic compounds with biochemical changes that occur in a few poisonous circumstances [13]. Cytochrome P450 (CYP) and its structures particularly CYP1A1, CYP1A2 and CYP2E1 have been seen to work with development of Receptive Oxygen Species (ROS) during xenobiotic digestion therefore adding to oxidative stress incited harm [14]. Direct association of CYP-induced free radical has been seen in pesticides by a few authors [15]. Anyway the metabolism of paraquat is extremely poor making it be discharged practically unaltered in urine. The metabolism of paraquat has been displayed to happen through methylation (monomethyl dipyridone particle) or oxidation (Paraquat pyridine particle and Paraquat dipyridone particle) [16]. It ought to be noted additionally that CYP2E1 enhance production of superoxide radicals and hydrogen peroxide in vitro [16].

Vitamin C and E are remarkable antioxidants that respond quickly with free radicals and decrease lipid peroxidation. The fluid in lungs contains high level of L-ascorbic acid to safeguard against free radicals that are created by toxicants in air. It has been accepted in like manner that the two vitamins C and E are consumed during oxidative stress [6,7]. A study reported that L-ascorbic acid diminishes following 48 hours of intraperitoneal administration of paraquat in rats, this proposes that L-ascorbic acid straightforwardly showed the oxidative stress in the lungs [8]. Another review [9] detailed that when the antioxidant status is increased by high dose of L-ascorbic acid, it could be valuable as a free radical scavenger for paraquat-induced toxicity in patients. This is on the grounds that L-ascorbic acid can extinguish free radical produced by redox cycling of paraquat [9].

Vitamin E assumes a significant part in paraquat poisoning as has been displayed in a few examinations where lack of vitamin E achieved the improvement of intense paraquat harmfulness in creatures. Two unique examinations demonstrated that lack of vitamin E potentiated a lessening in endurance and achieved histological lung harm in rodents [10], it likewise diminished the LD50 in mice that are presented to paraquat [11]. Concentrates additionally show that the organization of L-ascorbic acid turned around the potentiation of intense paraquat harmfulness by lack of vitamin E [10].

Regardless of the number of studies conducted on the effect of L-ascorbic acid and E combined therapy against paraquat intoxication, there are limited research work done on the liver. Thus, it is accordingly essential to assess the ameliorative impact of Vitamin E and C combined effect on paraquat induced toxicity on liver.

2. Material and Techniques

2.1. Study Area/Population

This study was conducted in the Department of Medical Laboratory Science, River State University. A total of 200 healthy adult male wistar rats with a mean weight of 0.2±0.02kg were used for this review. The rats were acquired from Animal House, Department of Biology, Rivers State University. The rats were moved to the study site and allowed to adapt for fourteen day prior to commencement of the study.

Groupings and Treatment of Animal

Two hundred (200) male wistar rats were utilized for this study and were classified into 4 groups (A, B, C and D) with each group containing fifty (50) rats each. Group A was taken as the control group; they were not induced with paraquat. Group B was induced with 0.02g of paraquat per kg of rat every 2 weeks for 3 months. Group C was induced with 0.04g of paraquat per kg of rat every 2 weeks for 3 months. Group D was induced with 0.06g per kg of paraquat per kg of rat every 2 weeks for 3 months.

Every main group had subgroups. "A" group had "Ao" and "Avec" subgroups; "B" group had "Bo" and "Bvec" subgroups; "C" group had "Co" and "Cvec" subgroups; "D" group had "Do" and "Dvec". "Ao", "Bo", "Co" and "Do" subgroups were not treated with vitamin E and C. "Avec", "Bvec", "Cvec" and "Dvec" subgroups were treated orally with 500mg of vitamin E and 2000mg/dl of L-ascorbic acid every week for one month.

After one month of week by week treatment with Vit E and C, the rats were sacrificed and their blood sample collected for laboratory analysis of liver function.

Method for Paraquat Administration

Administration of paraquat was through oral gavage. The rats were held at the skin over the head and turned so the mouth was looked vertical and the body brought down towards the holder. The syringe was then positioned into the mouth of the rat a bit horizontally in a manner to keep away from the teeth. The content in the syringe was then discharged into the mouth of the rat slowly [17].

Sample Collection Method

Under 70% chloroform sedation cardiac puncture technique was employed for blood sample collection from the rat's heart into the lithium heparin bottle and used for investigation of liver parameters [17].

2.2. Laboratory analysis

Bilirubin Determination as described by Okolonkwo et al. (2022)) [17].

Procedure

Total Bilirubin: 1.5mls of reagent-1 (Sulphanilic acid, HCl and Dimethylsulphoxide) was added to two glass-tubes labeled 'Blank' and 'Test' respectively. 50μL of reagent-3 (Sodium nitrite) was added to the tube for test only and mixed; subsequently 100μL

of sample was added to the 'Blank' and 'Test' tubes, mixed and incubated for exactly 5 minutes at room temperature. After which the absorbance were read spectrophotometrically at 530 – 580nm and 15 – 25^{0}C, with the instrument adjusted to zero with distilled water.

Calculation: Readings of (Sample – Sample blank) X 19.1 = Result in (mg/dL). Conversion factor: mg/dL X 17.1 = Result (*μL*/L).

Direct Bilirubin: 1.5mls of reagent-2 (Sulphanilic acid and HCl) was added to two glass-tubes labeled 'Blank' and 'Test' respectively. 50*μL* of reagent-3 (Sodium nitrite) was added to the tube for test only and mixed; subsequently 100*μL* of sample was added to the 'Blank' and 'Test' tubes, mixed and incubated for exactly 5 minutes at room temperature. After which the absorbance were read spectrophotometrically at 530 – 580nm and 15 – 25^{0}C, with the instrument adjusted to zero with distilled water.

Calculation: Readings of (Sample – Sample blank) X 14 = Result in (mg/dL). Conversion factor: mg/dL X 17.1 = Result (μL/L).

Total protein (Biuret colorimetric method as described by Okolonkwo et al. (2022)) [17].

Principle: Proteins give an intensive violet-blue complex with copper salts in an alkaline medium. Iodide is included as an antioxidant. The intensity of the colour formed is proportional to the total protein concentration in the sample.

Procedure

1mL of Biuret reagent was each added to three glass tubes labeled 'Blank', 'Standard' and 'Test', followed by 25*μL* each of Standard (7g/dL) and Sample added to the 'Standard' and 'Test' tubes respectively. The contents were mixed and incubated for 10 minutes at room temperature, after which, the absorbance (A) of the 'Test' and 'Standard' were read against the 'Blank'. The colour produced is stable for at least 30 minutes at room temperature.

Calculation: [A(Test) ÷ A(Standard)] X 7(Standard concentration)
= Result in g/dL

Albumin (Bromocresol green method as described by Okolonkwo et al. (2022)) [17].

Principle: The measurement of serum albumin is based on its quantitative binding to the indicator 3,3^{l},5,5^{l}-tetrabromo-m-cresol sulphonephthalein (bromocresol green, BCG). The albumin-BCG-complex absorbs maximally at 578 nm, the absorbance being directly proportional to the concentration of albumin in the sample.

Procedure

3mls of Bromocresol green reagent was each added to three glass tubes labeled 'Blank', 'Standard' and 'Test', followed by 10*μL* each of Water, Standard (7g/dL) and Sample added to the 'Blank', 'Standard' and 'Test' tubes respectively. The contents were mixed and incubated for 10 minutes at 20 – 25^{0}C, after which, the absorbance (A) of the 'Test' and 'Standard' were read against the 'Blank'. The colour produced is stable for at least 30 minutes at room temperature.

Calculation: [A (Test) ÷ A (Standard)] X 7 (Standard concentration)

= Result in g/dL

Globulin calculation method as described by Okolonkwo et al. (2022) [17].

Globulin values are calculated as a difference when albumin values are subtracted from the value of the total protein gotten from the same sample.

Globulin (g/dl) = Total protein (g/dl) – Albumin (unit in g/dl).

2.3. Statistical Analysis

The data gathered from the result of this research work was analyzed using Statistical Package for Social Sciences (SPSS) version 25.0 for descriptive and inferential statistics (ANOVA) for inter-group comparison and T-test for intra-group (sub-group) comparison at test significance, P-value<0.05.

Results

The results presented in Table 1.0 shows changes in liver parameters after Vitamin EC treatment on paraquat poisoned rats. The results show that there was significant dose-dependent rise (p-value<0.05) in total bilirubin and conjugated bilirubin and significant decrease (p-value<0.05) in total protein and albumin levels in paraquat poisoned rats. On the other hand, after treatment with vitamin EC, there was significant decrease (p-value<0.05) in total bilirubin and significant increase (p-value<0.05) in total protein and albumin levels but there was no significant difference (p-value>0.05) in globulin levels in paraquat and vitamin EC treatment groups.

Table 1. Changes in some liver parameters after three months treatment period.

Sub-group	Tot. Bilirubin (µmol/L)	D.Bilirubin (µmol/L)	T. Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
A_0	4.35 ± 3.55	0.30 ± 0.02	7.83 ± 0.04	5.06 ± 0.04	2.78 ± 0.08
A_{VEC}	1.85 ± 0.95	0.35 ± 0.01	8.09 ± 0.03	5.13 ± 0.00	2.96 ± 0.00
B_0	7.75 ± 0.55^{a}	1.45 ± 0.04^{a}	4.85 ± 0.02^{a}	2.34 ± 0.01^{a}	2.51 ± 0.01
B_{VEC}	3.65 ± 1.35^{a,b}	0.20 ± 0.01^{a,b}	5.14 ± 0.07^{a,b}	2.87 ± 0.01^{a,b}	2.27 ± 0.02
C_0	9.75 ± 1.05^{a}	1.70 ± 0.08^{a}	4.36 ± 0.08^{a}	2.12 ± 0.01^{a}	2.24 ± 0.02
C_{VEC}	2.45 ± 0.75^{a,b}	1.60 ± 0.07^{a,b}	5.00 ± 0.01^{a,b}	2.59 ± 0.01^{a,b}	2.42 ± 0.01
D_0	19.70 ± 0.80^{a}	2.30 ± 0.03^{a}	4.03 ± 0.02^{a}	1.68 ± 0.02^{a}	2.35 ± 0.02
D_{VEC}	15.40 ± 0.90^{a,b}	1.40 ± 0.07^{a,b}	4.93 ± 0.04^{a,b}	2.12 ± 0.01^{a,b}	2.81 ± 0.01

Statistical significance: P <0.05.

“a” signifies significant difference among groups treated with paraquat alone and control (A0, B0, C0 and D0).

“b” signifies significant difference between groups of “paraquat treated group” and “paraquat and Vit EC treated groups”

3. Discussion

The course of this study was to supply research evidence of the role of vitamin E and C combination therapy in restoration of liver function brought about by paraquat inflicted liver damage in rats. So some rats were poisoned with paraquat alone while some other rats were poisoned with paraquat and later treated with vitamin E and C. A comparison was made to determine if there was ameliorative success since reports have shown that paraquat is an exceptionally poisonous when presented to cells and tissues [18, 20].

The findings obtained from this study with paraquat treatment alone at different doses, and with vitamin E and C treatment on paraquat inflicted toxicity in rats, affirmed the toxic effect of paraquat and furthermore the ameliorative benefit of vitamin E and C. Comparison on the effect of paraquat among the different subgroups was completed. Subgroups A0, B0, C0, and D0 were compared and the study revealed that there was dose-dependent effect on T. bilirubin, D. bilirubin, T. protein and albumin. This means that the degree of toxicity experienced in the liver was dependent on the dose of paraquat administered. There were significant rise in total bilirubin and conjugated bilirubin which is a clear indication that paraquat destroyed the hepatocytes that were responsible for metabolism and excretion of bilirubin. On the other hand, the significant drops in total protein and albumin across the groups were indications that paraquat alters the synthetic function of the liver in a manner that is dependent on the dose of the paraquat administered. This was revealed as the group with the highest dose shown marked drop in total protein and albumin levels. This might be because of the reactive oxygen species that are produced by the paraquat. This outcome is in concurrence with those saw in the concentrate by Rizvi et al. (2014) and Howard et al. (2011) [21, 22].

After paraquat poisoning and the inflicted liver damage, vitamin E and C were both administered to all subgroups previous treated with paraquat to evaluate the ameliorative potential of this vitamin as a therapeutic for cases paraquat poisoning. The result revealed that all paraquat poisoned-rats showed recovery from liver injury after vitamin E and C treatment. This was demonstrated following the laboratory results of liver parameters. Total bilirubin and conjugated bilirubin values that were previously due to paraquat inflicted toxicity became decreased in all groups irrespective of the paraquat dose administered. Also, there was a restoration of the synthetic function of the liver after vitamin E and C treatment because declining values of total protein and albumin began to rise again in all dose-dependent groups. This has shown that irrespective of the dose of paraquat administered, vitamin E and C combined treatment can restore liver function in rats. This study concurs with those completed by Rizvi et al. (2014) and Howard et al. (2011) [20, 21]. This may be because of the antioxidative capacity of vitamin E and C. Such combined or synergistic effects may have resulted in the remarkable hepatocellular recovery reported in this study.

4. Conclusion

The findings gotten from this study has demonstrated that Vitamin E and C combined treatment amelioratively affects the liver against paraquat irrespective of the dose administered.

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