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PROTECTIVE EFFECTS OF VITAMIN E ON NITAZOXANIDE ADVERSE EFFECTS

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ABSTRACT

This study to evaluate the protective effect of Vitamin E (18mg/kg, P.O. once daily) on Nitazoxanide adverse effects (18mg/kg, P.O. once daily) and its effect on liver, kidney, blood and antioxidant enzymes for three weeks. Then tissue and blood samples were collected on 1st, 7th, 14th and 21th days post-treatment to assess the protective effect of Vitamin E. Our results indicated that Vitamin E has hepato-nephro-protective and there was a decrease in the elevation of liver enzymes caused by Nitazoxanide like ALT, AST and ALP and kidney parameters like creatinine and urea, and there was a normalize in all blood parameters which decrease by Nitazoxanide and finally there was a significant increase in antioxidant enzymes like CAT, SOD, GPX and a significant decrease in MDA, beside showing decrease in fatty change of liver caused by Nitazoxanide administration, as demonstrated by hepatic histopathology. Also, showing decrease in aggregation of chronic inflammatory cell caused by Nitazoxanide administration, as demonstrated by kidney histopathology. Therefore, Vitamin E should be taken with Nitazoxanide to decrease its side effects.

KEYWORDS: Nitazoxanide, Parasitic diseases, Vitamin E.

INTRODUCTION

Nitazoxanide, 2-acetyloxy-N-(5-nitro-2-thiazolyl) benzamide, is a new nitrothiazole benzamide compound. Rossignol and Cavier, 1975.[1] Nitazoxanide is a broad-spectrum antiparasitic and broad-spectrum antiviral drug that is used in medicine for the treatment of various helminthic, protozoal, and viral infections. Sisson et al., 2002.[2] Nitazoxanide (NTZ) exhibits broad-spectrum activity against anaerobic bacteria and parasites and the ulcer-causing pathogen *Helicobacter pylori*. It have been shown that NTZ is a noncompetitive inhibitor of the pyruvate: ferredoxin / flavodoxin oxido reductases (PFORs) of Trichomonas vaginalis,

Entamoeba histolytica, Giardia intestinalis, Clostridium difficile, Clostridium perfringens, H. pylori, and Campylobacter jejuni and is weakly active against the pyruvate dehydrogenase of Escherichia coli. Hoffman et al., 2007.[3] Nitazoxanide is absorbed from the gastrointestinal tract, with approximately one-third of the oral dose excreted in urine and two-thirds excreted in feces. In blood, nitazoxanide is rapidly hydrolyzed by plasma esterases into its desacetyl derivative, tizoxanide (desacetyl-nitazoxanide). Broekhuysen et al., 2000.[4] Tizoxanide is the active metabolite in vivo and the only measurable species in plasma. Following oral administration of nitazoxanide, a maximum tizoxanide plasma concentration of 2 mg/L is observed within 1–4 h. Tizoxanide is extensively bound to plasma proteins (>99%), and its urinary elimination half-life is 7.3 h. Tizoxanide then undergoes glucuronidation to form tizoxanide glucuronide. The parent drug, nitazoxanide, is not detected in plasma, urine, bile, or feces. Tizoxanide is found in plasma, urine, bile, and feces, and tizoxanide glucuronide is found in plasma, urine, and bile. Stockis et al.,1996.[5] Tizoxanide does not significantly inhibit cytochrome P450 enzymes *in vitro* and, therefore, no significant interaction is expected when nitazoxanide is administered concurrently with agents that are metabolised or inhibited by cytochrome P450 enzymes. With the high plasma protein binding of tizoxanide, caution is warranted when other drugs with high plasma protein binding(>99.9%) and small therapeutic indices are used concurrently. However, nitazoxanide did not appear to affect the pharmacokinetic parameters of warfarin when the two were administered concomitantly. Romark, 2006.[6] Nitazoxanide side effects have included abdominal pain, diarrhea, vomiting, and headache, Dizziness, somnolence, insomnia, tremor, and hypesthesia, asthenia, fever, pain (pelvic/back), flu syndrome, ear ache, and chills, increased creatinine, SGPT, pruritus, rash, and sweat, pale yellow eye discoloration, epistaxis, rhinitis, lung disease, and pharyngitis, discolored urine, dysuria, amenorrhea, metrorrhagia, and edema labia, myalgia, leg cramps, and spontaneous bone fracture, tachycardia, syncope, and hypertension, anemia. Stockis et al., 2002.[7] Antioxidant have been defined as substances that prevent the formation of reactive oxygen species (ROS) or other oxidants, scavenge them or repair the damage they cause. Antioxidant defenses act as a balanced and coordinated system and each relies on the action of the other. Hallowell, 1995.[8] Antioxidant is a molecules. Oxidation is a chemical reaction that transfer electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as anthills,

ascorbic acid, or polyphenols, tocopherols and thiols. Zidenberg-Cherr and Keen, 1991.[9] Antioxidant defenses consist of low molecular mass antioxidant such as Vit. E and enzymes e.g. SOD, CAT, GPX. Evans and Halliwell, 2001.[10] Vitamin E (VE) is an important antioxidant in biological system that diminishes the peroxidation of un structural lipids by chain breaking free radical (FR), thus it contributes to the stability of cellular membranes Kosther et al., 1995.[11] Vit.E (α-Tocopherol) is the most important lipid phase antioxidant. Esterbauer et al., 1991.[12] Nitazoxanide was approved that it had hepatic and renal disorder in rats. This review covers key studies of hepato-nephro protective effects of Vitamin E (18mg/kg P.O. once daily) against Nitazoxanide (18mg/kg P.O. once daily) toxicity for three weeks when Vitamin E was co-administered with Nitazoxanide. Then tissue and blood samples were collected and the protective effect of Vitamin E on liver and kidney appreciated through measuring biochemical constituents in sera such as serum creatinine, urea levels, liver injury biomarkers as serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total proteins, albumin and total bilirubin and oxidative stress biomarkers such as catalase (CAT), superoxidase dismutase (SOD), glutathione peroxidase (GPX) and malondialdehyde (MDA) (marker of lipid peroxidation). The obtained results indicated that Vitamin E has hepato-nephro protection through prohibiting the raise in liver and kidney injury biomarkers, and also the current histo-pathological results enhanced this effect.

MATERIAL AND METHODS

Drugs and Chemicals

Nitazoxanide (ALINIA®) was supplied by Romark laboratories. Nitazoxanide is dissolved in normal saline. Vitamin E (Vitamin E capsule) It was supplied by PHARCO pharmaceutical CO., Alex., Egypt. Vitamin E dissolved in corn oil.

Animals

The present study was carried on 80 adult female albino rats weight 150-200 g, obtained from laboratory Animal Farm, Faculty of Veterinary Medicine, Zagazig university. All animals were kept under observation for two weeks for acclimatization to the laboratory environment before starting the experiments. The animals were kept under hygienic condition in metal cages and fed on barely and milk all over the experimental time and water was provided.

Experimental design

Rats were allocated into four groups and each group contains 20 rats. 1st group were left non treated, 2nd group were treated with Nitazoxanide (18mg/kg b.wt orally once daily) the dose

calculated according to Paget and Barnes,1964.[13] 3rd group were treated with Vitamin E (18mg/kg b.wt orally once daily) and the 4th group treated with the combination on Nitazoxanide (18mg/kg b.wt orally once daily) and Vitamin E (18mg/kg b.wt orally once daily).

Preparation of serum sample and tissue sampling

At the end of the experiment (24 hrs. after the last dose), rats were sacrificed and the following samples were collected: Blood was collected and allowed to clot for 30 minutes at 25°C. Thereafter, they were centrifuged at 3000 rpm for 10 min, and the top yellow layers of serum were pipette off without distributing the white buffy layer. Serum was stored at -20°C and thawed just before use for the determination of liver and the kidney enzymes. The liver and the kidney of each rat were isolated and kept in 10% phosphate-buffered formalin for histopathological evaluation.

Biochemical markers of liver and kidney injury

Determination of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and serum alkaline phosphatase (ALP) activities was established according to the principle described previously Tiez,N.W., 1976.[14] Evaluation of creatinine according to the method described previously Henry etal.,1974.[15] and urea has been done according to the method described previously Vassault etal.,1986.[16] Determination of these parameters was carried out through commercial kits from Spectrum Diagnostics (Cairo, Egypt).

Hepatic and nephro histopathological evaluation

Liver and kidney tissues were fixed in 10 % neutral buffered formalin solutions for 24 hrs. Then, tissue processing and paraffin blocks preparation were done. Masson's trichome and hematoxylin-eosin stains were used to evaluate fibrotic areas and necro inflammation activity Suvarna etal.,2013.[17]

Biochemical markers of antioxidant activity

Determination of catalase activity (CAT), superoxidase dismutase activity (SOD), glutathione peroxidase activity (GPX) and malondialdehyde activity (MDA) by method according to the previous principles Aebi, H., 1984[18], Nishikimi etal., 1972[19] and Paglia, D.E and Valentine, W.N., 1967.[20]

Statistical analysis

The data was analyzed by using computerized SPSS program. Statistical evaluations of the results, except the histopathological results, were done by using methods as one way and two-way analysis of variance (ANOVA).

RESULTS

Effect of combination between Nitazoxanide (18mg/kg, P.O. once daily) and Vit.E (18mg/kg, P.O. once daily) and their combination on biochemical markers of liver injury.

Effect of combination between Nitazoxanide and Vit. E on ALT: in the 1st day post treatment, resulted in decrease in serum ALT level (28.8± 7.66 U/L) compared with (54.5± 6.65U/L) for Nitazoxanide group. In the 7th day post treatment, resulted in decrease in ALT serum activity (15.1 ± 1.7U/L) compared with (35.5 ± 4.7U/L) for Nitazoxanide group. In the 14th day post treatment, resulted in decrease in serum ALT activity (15.6± 1.68) compared with (21.8 ± 1.9U/L) for Nitazoxanide group. In the 21th day post treatment, resulted in decrease in ALT serum activity (18.1± 1.8U/L) compared with (19.3± 0.9U/L) for Nitazoxanide group.

Effect of combination between Nitazoxanide and Vit. E on AST: in the 1st day post treatment, resulted in decrease in serum AST level (24.8± 4.08U/L) compared with (34.9± 3.50U/L) for Nitazoxanide group. In the 7th day post treatment, resulted in decrease in AST serum activity (19.5 ± 1.36U/L) compared with (29.1 ± 1.3U/L) for Nitazoxanide group. In the 14th day post treatment, resulted in decrease in serum AST activity (16.7± 1.51) compared with (20.5 ± 2.41U/L) for Nitazoxanide group. In the 21th day post treatment, resulted in decrease in AST serum activity (17.7±0.66U/L) compared with (19.6±1.09U/L) for Nitazoxanide group.

Effect of combination between Nitazoxanide and Vit. E on ALP: in the 1st day post treatment, resulted in decrease in serum ALP level (81.1± 4.5 U/L) compared with (102.9± 4.9U/L) for Nitazoxanide group. In the 7th day post treatment, resulted in decrease in ALP serum activity (68.3 ± 7.9U/L) compared with (87.2 ± 3.6U/L) for Nitazoxanide group. In the 14th day post treatment, resulted in decrease in serum ALP activity (71.8 ± 2.9U/L) compared with (68.1 ± 1.6U/L) for Nitazoxanide group. In the 21th day post treatment, resulted in decrease in ALP serum activity (71.9± 3.11 U/L) compared with (69.3± 0.85U/L) for Nitazoxanide group.

Effect of combination between Nitazoxanide and Vit. E on total protein: in the 1st day post treatment, resulted in increase in total protein level (6.30 ± 0.26 g/dl) compared with (5.07 ± 0.48 g/dl) for Nitazoxanide group. In the 7th day post treatment, resulted in increase in total

protein level (7.42 ± 0.48g/dl) compared with (6.15 ± 0.33g/dl) for Nitazoxanide group. In the 14th day post treatment, resulted in increase in total protein level (8.20 ± 0.22g/dl) compared with (7.52 ± 0.23g/dl) for Nitazoxanide group. In the 21th day post treatment, resulted in increase in total protein level (8.30± 0.18 g/dl) compared with (8.05±0.27g/dl) for Nitazoxanide group.

Effect of combination between Nitazoxanide and Vit. E on Albumin: in the 1st day post treatment, resulted in increase in albumin level (2.95 ± 0.17 g/dl) compared with (1.92 ± 0.18 g/dl) for Nitazoxanide group. In the 7th day post treatment, resulted in increase in albumin level (3.75 ± 0.15g/dl) compared with (3.17 ± 0.13g/dl) for Nitazoxanide group. In the 14th day post treatment, resulted in increase in albumin level (4.77 ± 0.40 g/dl) compared with (4.55 ± 0.21g/dl) for Nitazoxanide group. In the 21th day post treatment, resulted in increase in albumin level (5.20 ± 0.12 g/dl) compared with (4.92± 0.45g/dl) for Nitazoxanide group.

Table. (1): The effect of Vit.E (18mg/kg, P.O. once daily), Nitazoxanide (18mg/kg, P.O. once daily), and their combination on liver parameters of rats at 1st, 7th,14th and 21thday post treatment.

		ALT	AST	ALP	Total protein	Albumin	Total Bilirubin
1st day	Control	14.67± 0.6	10.93± 0.94	62.22± 1.8	7.60± 0.25	4.00± 0.18	2.45± 0.17
	Nitazoxanide	54.5± 6.65	34.9± 3.50	102.9± 4.9	5.07± 0.48	1.92± 0.18	2.42± 0.25
	Vit.E	15.3± 1.92	11.2± 2.0	62.62± 2.9	7.70± 0.28	4.05± 0.41	2.27± 0.39
	Nitazoxanide + Vit.E	28.8± 7.66	24.8± 4.08	81.1± 4.5	6.30± 0.26	2.95± 0.17	2.32± 0.35
7th day	Control	13.11± 0.9	16.8± 1.1	64.7± 4.08	8.22± 0.13	4.32± 0.27	2.17± 0.17
	Nitazoxanide	35.5 ± 4.7	29.1± 1.3	87.2± 3.6	6.15± 0.33	3.17± 0.13	2.00± 0.34
	Vit.E	12.6± 0.83	16.4± 1.07	68.6± 4.9	7.97± 0.49	4.40± 0.30	2.52± 0.11
	Nitazoxanide + Vit.E	15.1 ± 1.7	19.5± 1.36	68.3± 7.9	7.42± 0.48	3.75± 0.15	2.12± 0.39
14thday	Control	16.7± 0.69	16.6± 0.91	71.9± 3.1	8.22± 0.11	4.97± 0.13	2.10± 0.40
	Nitazoxanide	21.8 ± 1.9	20.5± 2.41^{B}	68.1± 1.6	7.52± 0.23	4.55± 0.21	1.92± 0.13
	Vit.E	16.9 ± 1.11	16.6± 0.44^{A}	71.5± 2.3	8.30± 0.16	4.92± 0.40	1.97± 0.25
	Nitazoxanide + Vit.E	15.6± 1.68	16.7± 1.51^{B}	71.8± 2.9	8.20± 0.22	4.77± 0.40	1.83± 0.45
21thday	Control	17.74± 0.6	17.2± 0.79	69.4± 1.17	8.45± 0.23	5.15± 0.19	1.85± 0.11
	Nitazoxanide	19.3± 0.9	19.6± 1.09	69.3± 0.85	8.05± 0.27	4.92± 0.45	1.97± 0.25
	Vit.E	17.79± 0.9	17.1± 0.71	71.8± 2.08	8.27± 0.29	5.25± 0.22	1.76± 0.29
	Nitazoxanide + Vit.E	18.1± 1.8	17.7± 0.66	71.9± 3.11	8.30± 0.18	5.20± 0.12	1.75± 0.34

Effect of combination between Nitazoxanide (18mg/kg, P.O. once daily) and Vit.E (18mg/kg, P.O. once daily) and their combination on biochemical markers of kidney injury: Effect of combination between Nitazoxanide and Vit. E on serum creatinine: in the 1st day post treatment, resulted in decrease in serum creatinine level (1.75± 0.23 mg/dl) compared with (3.40± 0.33mg/dl) for Nitazoxanide group. In the 7th day post treatment, resulted in decrease in serum creatinine activity (1.10 ± 0.14mg/dl) compared with (2.00 ± 0.13mg/dl) for Nitazoxanide group. In the 14th day post treatment, resulted in decrease in serum creatinine activity (0.93 ± 0.12mg/dl) compared with (1.14 ± 0.18mg/dl) for Nitazoxanide group. In the 21th day post treatment, resulted in decrease in serum creatinine activity (0.89± 0.15 mg/dl) compared with (1.08 ± 0.21mg/dl) for Nitazoxanide group.

Effect of combination between Nitazoxanide and Vit. E on serum urea: in the 1st day post treatment, resulted in decrease in serum urea level (39.4± 1.57 mg/dl) compared with (53.8± 2.45mg/dl) for Nitazoxanide group. In the 7th day post treatment, resulted in decrease in serum urea activity (21.8 ± 1.53mg/dl) compared with (32.1 ± 1.86 mg/dl) for Nitazoxanide group. In the 14th day post treatment, resulted in decrease in serum urea activity (28.5 ± 2.16mg/dl) compared with (29.4 ± 2.48mg/dl) for Nitazoxanide group. In the 21th day post treatment, resulted in decrease in serum urea activity (19.4±1.85 mg/dl) compared with (22.4 ±2.50mg/dl) for Nitazoxanide group.

Table. (2): The effect of Vit.E (18mg/kg, P.O. once daily), Nitazoxanide (18mg/kg, P.O. once daily), and their combination on kidney parameters of rats at 1st, 7th,14th and 21thday post treatment.

		Creatinine	Urea
1st day	Control	0.94± 0.02	25.12± 1.48
	Nitazoxanide	3.40± 0.33	53.8± 2.45
	Vit.E	0.83± 0.10	24.47± 1.3
	Nitazoxanide + Vit.E	1.75± 0.23	39.4± 1.57
7th day	Control	0.85± 0.18	20.9± 0.51
	Nitazoxanide	2.00± 0.13	32.1± 1.86
	Vit.E	0.91± 0.08	22.1± 2.80
	Nitazoxanide + Vit.E	1.10± 0.14	21.8± 1.53
14thday	Control	0.89± 0.13	25.6± 2.04
	Nitazoxanide	1.14± 0.18	29.4± 2.48
	Vit.E	0.88± 0.09	27.05± 2.5
	Nitazoxanide + Vit.E	0.93± 0.12	28.5± 2.16
21thday	Control	0.76± 0.08	19.6± 1.04
	Nitazoxanide	1.08± 0.21	22.4± 2.50
	Vit.E	0.75± 0.11	20.4± 1.05
	Nitazoxanide + Vit.E	0.89± 0.15	19.4± 1.85

Effect of combination between Nitazoxanide (18mg/kg, P.O. once daily) and Vit.E (18mg/kg, P.O. once daily) and their combination on biochemical markers of antioxidant enzymes.

Effect of combination between Nitazoxanide and Vit. E on Catalase: in the 1st day post treatment, resulted in increase in catalase activity (192.1± 9.4 U/L) compared with (153.7± 7.2 U/L) for Nitazoxanide group. In the 7th day post treatment, resulted in increase in catalase activity (251.3 ± 23.9U/L) compared with (207.5 ± 9.04 U/L) for Nitazoxanide group. In the 14th day post treatment, resulted in increase in catalase activity (282.7 ± 7.9U/L) compared with (253.4 ± 8.7U/L) for Nitazoxanide group. In the 21th day post treatment, resulted in increase in catalase activity (276.4±3.7 U/L) compared with (263.2±2.8U/L) for Nitazoxanide group.

Effect of combination between Nitazoxanide and Vit. E on Superoxide dismutase: in the 1st day post treatment, resulted in increase in superoxide dismutase activity (49.3± 3.28 U/L) compared with (30.9 ± 2.5 U/L) for Nitazoxanide group. In the 7th day post treatment, resulted in increase in superoxide dismutase activity (74.2 ± 9.5U/L) compared with (57.03 ± 3.2 U/L) for Nitazoxanide group. In the 14th day post treatment, resulted in increase in superoxide dismutase activity (73.5 ± 5.05U/L) compared with (68.5 ± 3.1 U/L) for Nitazoxanide group. In the 21th day post treatment, resulted in increase in superoxide dismutase activity (78.66 ± 2.38 U/L) compared with (76.6± 2.2U/L) for Nitazoxanide group.

Effect of combination between Nitazoxanide and Vit. E on Glutathione peroxidase: in the 1st day post treatment, resulted in increase in Glutathione peroxidase activity (2.12± 0.16 U/L) compared with (1.18 ± 0.20 U/L) for Nitazoxanide group. In the 7th day post treatment, resulted in increase in Glutathione peroxidase activity (4.93 ± 0.43U/L) compared with (2.62 ± 0.52 U/L) for Nitazoxanide group. In the 14th day post treatment, resulted in increase in Glutathione peroxidase activity (6.18 ± 0.18U/L) compared with (4.62 ± 0.56 U/L) for Nitazoxanide group. In the 21th day post treatment, resulted in increase in Glutathione peroxidase activity (6.75±0.10 U/L) compared with (5.93±0.64U/L) for Nitazoxanide group.

Effect of combination between Nitazoxanide and Vit. E on MDA: in the 1st day post treatment, resulted in decrease in MDA activity (28.8± 1.6 U/L) compared with (47.3 ± 2.00 U/L) for Nitazoxanide group. In the 7th day post treatment, resulted in decrease in MDA activity (20.9 ± 0.77U/L) compared with (35.2 ± 3.00 U/L) for Nitazoxanide group. In the 14th day post treatment, resulted in decrease in MDA activity (20.51 ± 3.6U/L) compared with (23.2 ± 3.58

U/L) for Nitazoxanide group. In the 21th day post treatment, resulted in decrease in MDA activity (20.04 ±2.43 U/L) compared with (24.1±2.9U/L) for Nitazoxanide group.

Table. (3): The effect of Vit.E (18mg/kg, P.O. once daily), Nitazoxanide (18mg/kg, P.O. once daily), and their combination on antioxidant enzymes activities of rats at 1st, 7th,14th and 21th day post treatment.

		CAT	SOD	GPX	MDA
1st day	Control	247.1± 3.36	67.7± 2.4	3.62± 0.56	19.5± 1.14
	Nitazoxanide	153.7± 7.2	30.9± 2.5	1.18± 0.20	47.3± 2.00
	Vit.E	242.4± 6.5	68.9± 4.14	3.31± 0.6	19.9± 1.6
	Nitazoxanide + Vit.E	192.1± 9.4	49.3± 3.28	2.12± 0.16	28.8± 1.6
7th day	Control	274.2± 5.1	81.6± 1.59	4.87± 0.99	20.7± 1.69
	Nitazoxanide	207.5± 9.04	57.03± 3.2	2.62± 0.52	35.2± 3.00
	Vit.E	276.7± 8.9	80.8± 4.46	5.31± 1.5	20.3± 1.04
	Nitazoxanide + Vit.E	251.3± 23.9	74.2± 9.5	4.93± 0.43	20.9± 0.77
14th day	Control	286.0± 2.4	82.1± 1.82	5.93± 0.31	19.3± 0.81
	Nitazoxanide	253.4± 8.7	68.5± 3.1	4.62± 0.56	23.2± 3.58
	Vit.E	283.5± 5.4	81.09± 4.6	5.93± 0.4	20.4± 1.00
	Nitazoxanide + Vit.E	282.7± 7.9	73.5± 5.05	6.18± 0.18	20.51± 3.6
21th day	Control	278.5± 3.7	80.5± 1.6	6.37± 0.16	19.1± 0.40
	Nitazoxanide	263.2± 2.8	76.6± 2.2	5.93± 0.64	24.1± 2.9
	Vit.E	278.4± 5.9	80.4± 3.3	6.37± 0.50	20.5± 0.72
	Nitazoxanide + Vit.E	276.4± 3.7	78.66± 2.38	6.75± 0.10	20.04± 2.43

Histopathological results of Liver in 7th day

Liver of Nitazoxanide-treated animal (7th day sacrifice) showing dilated congested central vein surrounded by rows and cords of hepatocytes with clean cytoplasm due to severe fatty change (A). Liver of Nitazoxanide + Vit. E treated animal (7th day sacrifice) showing moderate fatty change of the hepatocyte (B).

Histopathological results of Liver in 14th day

Liver of Nitazoxanide-treated animal (14th day sacrifice) showing dilated congested central vein with mild fatty change of the hepatocyte (C). Liver of Nitazoxanide + Vit. E treated animal (14th day sacrifice) showing mild fatty change of the hepatocyte **(**D**).**

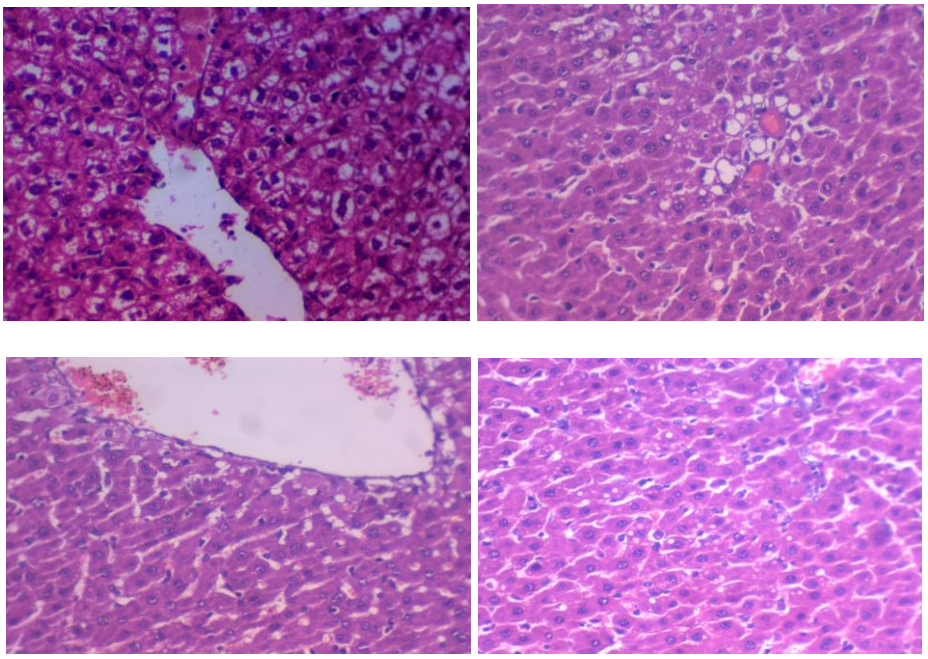

Figure. (1): A: Liver of Nitazoxanide-treated animal (7th day sacrifice) showing dilated congested central vein surrounded by rows and cords of hepatocytes with clean cytoplasm due to severe fatty change. B: Liver of Nitazoxanide + Vit. E treated animal (7th day sacrifice) showing moderate fatty change of the hepatocyte. C: Liver of Nitazoxanide-treated animal (14th day sacrifice) showing dilated congested central vein with mild fatty change of the hepatocyte. D: Liver of Nitazoxanide + Vit. E treated animal (14th day sacrifice) showing mild fatty change of the hepatocyte.

Histopathological results of Kidney in 7th day

Kidney of Nitazoxanide-treated animal (7th day sacrifice) showing dilated congested vascular space in between renal tissue and heavy aggregation of chronic inflammatory cell (E). Kidney of Nitazoxanide + Vit. E treated animal (7th day sacrifice) showing moderate aggregation of chronic inflammatory cell around glomerulus (F).

Histopathological results of Kidney in 14th day

Kidney of Nitazoxanide-treated animal (14th day sacrifice) showing dilated congested vascular space and mild aggregation of chronic inflammatory cell (G). Kidney of Nitazoxanide + Vit. E treated animal (14th day sacrifice) showing mild aggregation of chronic inflammatory cell around glomerulus(H).

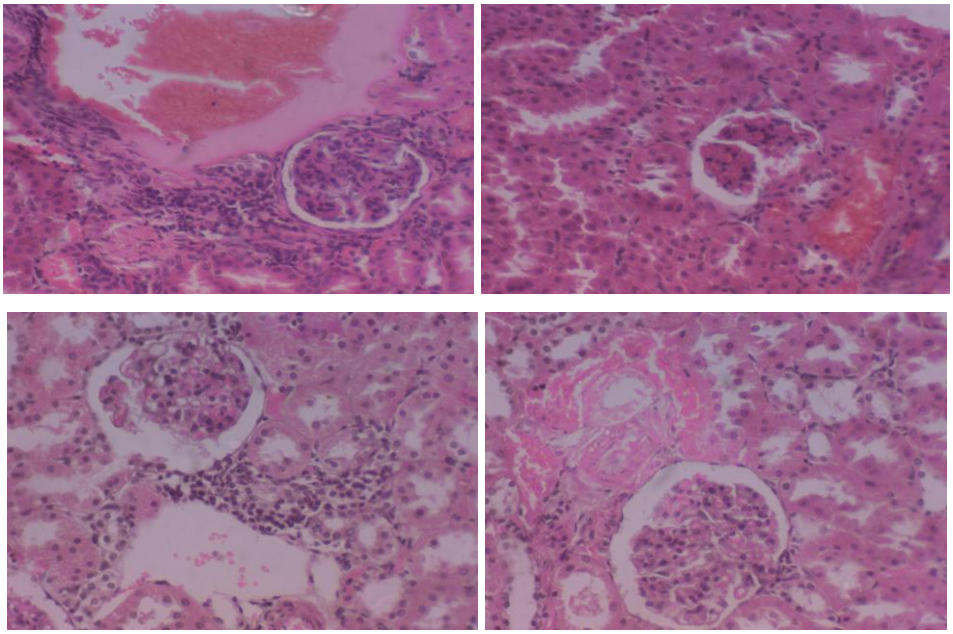

Figure. (2): E: Kidney of Nitazoxanide-treated animal (7th day sacrifice) showing dilated congested vascular space in between renal tissue and heavy aggregation of chronic inflammatory cell. F: Kidney of Nitazoxanide + Vit. E treated animal (7th day sacrifice) showing moderate aggregation of chronic inflammatory cell around glomerulus. G: Kidney of Nitazoxanide-treated animal (14th day sacrifice) showing dilated congested vascular space and mild aggregation of chronic inflammatory cell. H: Kidney of Nitazoxanide + Vit. E treated animal (14th day sacrifice) showing mild aggregation of chronic inflammatory cell around glomerulus.

DISCUSSION

Chemical agents are known to induce hepatic and renal disturbance in human. Chemical agent are screened daily for their hepatoprotective and nephroprotective properities, for example Vit.E. It is one of the most important antioxidant drugs due to its hepato-nephroprotective properties as reported by researches Pryor et al., 1993.[21] This study demonstrated the hepato-nephroprotective effect of Vit. E. Vit. E has hepatoprotective effect as it expresses important functions in the membranes; preventing ROS damage in polyunsaturated fatty acids as alipid soluble antioxidant and acting against damage caused to phospholipids as amembrane-stabilizing agent. Additionally, Vit.E is known to act by breaking the antioxidant chain that prevent ROS-produced cell membrane. It decreases lipid peroxidation and protects against liver injury. It decrease liver fibrosis, tumor necrosis factors, inflammation and hepatic porphyrin Bradford et al., 2003.[22] The previous observation explained the hepatoprotective effects of Vit.E observed in the present study.

Vit.E normalized levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, serum bilirubin, glutathione superoxide dismutase, malondialdehyde and improved histopathological changes in the liver induced by chemical agents in the control group. The possible pathway can be explained through the structure of Vit.E, the side chain in the 2-position facilitates the incorporation and retention of Vit.E in bio membranes so that the 6-position is optimal for scavenging free radicals and terminating lipid peroxidation. Furthermore, it's antioxidant activity is exhibited through protection of poly unsaturated fatty acid from oxidation by reactive oxygen species, stabilization of membrane and breaking of antioxidant chains that prevent reactive oxygen species damage to membranes. In the present study, Vit.E used in a dose (18mg/kg b.wt) once daily for 14 days to clarify the hepatic-nephroprotective effect on rats. We noticed that, there is arise in the activities of anti-oxidative stress enzymes (CAT, SOD and GPX) and significant decrease MDA activity.

Histopathological results in liver in 7th day showing moderate fatty change of the hepatocyte. In 14th day showing mild fatty change of the hepatocyte and the results according to kidney in 7th day showing moderate aggregation of chronic inflammatory cell around glomerulus. In 14th day showing mild aggregation of inflammatory cell aroung glomerulus than that observed by administration of Nitazoxanide.

The administration of Vit. E with Nitazoxanide showed an obvious decline in action of serum ALT, AST, ALP when contrasted with Nitazoxanide group. Sanchez-valle et al., 2012[23] reported the anti-oxidative therapy, mainly using natural and synthetic antioxidants, represents a reasonable therapeutic approach for the prevention and treatment of liver diseases due to the role of oxidative stress in contributing to initiation and progression of hepatic damage. Medina and Moreno-Otera, 2005[24] reported that the antioxidant therapy has been considered to have the possibility of beneficial effects in the management of these liver diseases; antioxidant have produced mixed results in a number of clinical trials of efficacy.

Parola et al., 1992[25] reported that antioxidant Vit.E retard hepatic fibrosis in biliary-obstructed rats. Oxidant stress may play arole in the pathogenesis of hepatic fibrosis in secondary biliary cirrhosis. Beytut et al., 2003[26] reported that the increased level of antioxidant enzymes, CAT, SOD, GPX resulted from administration of Vit.E might normalized the lipid peroxidation reaction and related biochemical changes which in turn protects the cells from the increased risk of peroxidative damage as aresult of administration of cytotoxic drugs.

Kagan, 1989[27] reported that one of the ways in which α-tocopherol was believed to stabilize membranes to form a complex with the membrane lipids components that have atendency to destabilize the bilayer structure theraby countering their effects and rendering the membrane more stable as also supported by the observed reduction of MDA and nitric oxide as well as increased glutathione. Another explanation of the action of Vit.E is the decrease of lipid peroxidation radical was suggested by Halliwell and Gutteridge, 2002[28] which suggested that administration of α-tocopherol averted oxidative damage, probably through its capacity to quickly and efficiently scavenge lipid peroxide radicals before they attack the membrane lipid. This ability might be related to the fact that lipid peroxyl radicals react more rapidly (by four orders of magnitude) with α-tocopherol than with membrane lipids.

CONCLUSION

It could be concluded that Nitazoxanide has hepatic and renal disturbance in rats; Vit. E has a protective effect against hepatic and renal disturbance, which may attribute to decrease the harmful effects of Nitazoxanide by inhibiting free radical formation and by restoration of the antioxidant systems. The combination of Vit.E and Nitazoxanide showed better results than Nitazoxanide alone.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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