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Research Article

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Nitazoxanide Adverse Effects on Biochemical Markers of Liver & Kidney Injury and Antioxidant Enzymes on Rats

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ABSTRACT

The current study has covered key issues related to the adverse effects of Nitazoxanide (18mg/kg, orally once daily). Blood samples were collected on the 1st, 7th, 14th and 21th days post-treatment. The results indicated that Nitazoxanide caused the elevation of the liver enzymes ALT, AST, ALP, creatinine and urea. Obvious decline was observed in all blood parameters with a respectable decline in CAT, SOD, GPX and a significant increase in MDA. Histopathological results of liver in 7th day showed markedly dilated congested central vein surrounded by the rows and cords of hepatocytes with clean cytoplasm due to the severe fatty changes. On 14th day, the dilated congested central vein with a mild fatty change of the hepatocyte was observed. Histopathological results on 7th day, showed the dilated congested vascular space between renal tissues and heavy aggregations of chronic inflammatory cells. On 14th day, the dilated congested vascular space and the mild aggregation of chronic inflammatory cell was observed.

Keywords: *Nitazoxanide, Parasitic diseases, Hematological, Biochemical parameters and Antioxidant enzymes*

INTRODUCTION

Nitazoxanide, is a new nitrothiazolebenzamide compound [1]. It is a broad-spectrum antiviral and anti-parasitic drug that is used in medicine for protection against various protozoal, helminthic and viral infections [2]. Nitazoxanide (NTZ) has a broad-spectrum activity against anaerobic bacteria and parasites. It has been shown that NTZ is a potent inhibitor of the pyruvate ferredoxin/ flavodoxinoxidoreductases (PFORs) of the anaerobic bacteria and parasites [3]. Nitazoxanide is absorbed from the GIT, with about one-third of the oral dose excreted in urine and two-thirds excreted in feces. In blood, Nitazoxanide is speedily hydrolyzed by plasma esterase into its desacetyl derivative, tizoxanide (desacetyl-nitazoxanide) [4]. Tizoxanide is the active metabolite in vivo, and the only measurable species in plasma. Following oral administration of Nitazoxanide, Tizoxanide has been found in plasma, urine, bile, and feces, and tizoxanideglucuronide has been found in plasma, urine, and bile [5]. Tizoxanide does not block cytochrome P450 enzymes in vitro and, therefore, no significant interaction has been expected when Nitazoxanide was administered at the same time with other agents that have been metabolised or blocked by cytochrome P450 enzymes. With the high plasma protein binding of tizoxanide, the care is secured when other drugs with high plasma protein binding (>99.9%) and small therapeutic index are used at the same time. It has been shown that Nitazoxanide did not appear to affect the kinetic of warfarin when the two drugs were administered concurrently [6]. Adverse effects of Nitazoxanide included abdominal pain, diarrhea, vomiting, and headache, Dizziness, somnolence, insomnia, tremor, and hypesthesia, increased creatinine, SGPT, pruritus, rash, and sweat, pale yellow

eye discoloration, epistaxis, rhinitis, lung disease, and pharyngitis, myalgia, leg cramps, and spontaneous bone fracture, tachycardia, syncope, and hypertension, anemia [7].

MATERIAL AND METHODS

Drugs and Chemicals

Nitazoxanide (ALINIA®) was supplied by Romark laboratories. Nitazoxanide was dissolved in normal saline.

Animals

The present study was carried out on 40 adult female albino rats weighing 150-200 g. All animals were protected under the observation for two weeks for the 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 two groups and each group contained 20 rats. The 1st group was left non treated, 2nd group was treated with Nitazoxanide (18mg/kg b.wt orally once a day), the dose was calculated according to [8].

Preparation of serum sample and tissue sampling

Rats were sacrificed one day post-treatment. Blood samples were collected: Blood was allowed to clot for 30 minutes at 25°C. Centrifuged at 3000 rpm for 10 min, and the serum was taken. The liver and the kidney of each rat were isolated for the histopathological evaluation.

Biochemical markers of liver and kidney injury

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and serum alkaline phosphatase (ALP) activities were evaluated [9].Creatinine was evaluated [10] and urea was measured [11].

Hepatic and nephrohistopathological evaluation

Liver and kidney tissues were histopathologically examined [12].

Biochemical markers of antioxidant activity

Determination of catalase activity (CAT), superoxidase dismutase activity (SOD), glutathione peroxidase activity (GPX) and malondialdehyde activity (MDA) were measured by the method according to the previous principles [13,14, 15].

Hematological testing

It included methologies and sources of error for blood cell counts, hematocrit and hemoglobin determinations, white blood cell count, platelet count, basophil, eosinophil and lymphocyte. Calculating indices (MCV, MCH and MCHC) were formulated according to [16].

Statistical analysis

The results were statistically analyzed by (ANOVA).

RESULTS

The effect of Nitazoxanide (18mg/kg, orally once daily) on biochemical markers of liver function of rats on the 1st, 7th,14th and 21st day post treatment was assessed.

Nitazoxanide in therapeutic dose resulted in a significant increase in ALT, AST, ALP level after one-day post treatment when compared with the control group, but this elevation decreased gradually by time.

Nitazoxanide in a therapeutic dose produced a significant decrease in total protein and albumin level after one-day post administration when compared with the control group.

Meanwhile, Nitazoxanide in therapeutic dose for 14 successive days to female rats induced a non- significant change in total bilirubin level after one day post administration when compared with the control group.

The effect of Nitazoxanide (18mg/kg, orally once daily) on biochemical markers of kidney function of rats on the 1st, 7th, 14^{th} and 21^{st} day post treatment was assessed.

The results suggested that the administration of Nitazoxanide in therapeutic dose for 14 successive days to female rats resulted in a significant increase in serum creatinine and urea level after one day post treatment when compared with the control group.

The effect of Nitazoxanide (18mg/kg, orally once daily) on biochemical markers of antioxidant enzymes of rats on the 1st, 7th,14th and 21^{st} day post treatment was assessed.

The results suggested that the administration of Nitazoxanide in therapeutic dose for 14 successive days on female rats resulted in a significant decrease in catalase, superoxide dismutase, and Glutathione peroxidase activity after one day post administration when compared with the control group.

The administration of Nitazoxanide in therapeutic dose for 14 successive days on female rats resulted in a significant increase in MDA activity after one day post treatment when compared with the control group.

Histopathological results on liver on the 7th day were measured.

Liver of Nitazoxanide-treated animals (7th day sacrifice) showed the dilated congested central vein surrounded by the rows and cords of hepatocytes with the clean cytoplasm due to the severe fatty change (A).

Histopathological results of Liver on the 14th day.

Liver of Nitazoxanide-treated animal (14th day sacrifice) showed the dilated congested central vein with the mild fatty change of the hepatocyte (B).

Histopathological results of kidney on the 7th day were assessed.

Kidney of Nitazoxanide-treated animal (7th day sacrifice) showed the dilated congested vascular space between the renal tissues and the heavy aggregation of the chronic inflammatory cells (C).

Histopathological results of Kidney on the 14th day were assessed.

Kidney of Nitazoxanide-treated animal (14th day sacrifice) showed the dilated congested vascular space and the mild aggregation of the chronic inflammatory cells (D).

Table 1. The effect of Nitazoxanide (18mg/kg, P.O. once daily) on the biochemical markers of liver injury on rats on the 1st, 7th, 14th and 21st day 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
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
14th day	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± 2.41B	68.1± 1.6	7.52± 0.23	4.55± 0.21	1.92± 0.13
21th day	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

Table 2. The effect of Nitazoxanide (18mg/kg, P.O. once daily) on the biochemical markers of kidney injury on rats on the 1st, 7th, 14th and 21st day post treatment.

		Creatinine	Urea
1st day	Control	0.94± 0.02	25.12± 1.48
	Nitazoxanide	3.40± 0.33	53.8± 2.45
7th day	Control	0.85± 0.18	20.9± 0.51
	Nitazoxanide	2.00± 0.13	32.1± 1.86
14th day	Control	0.89± 0.13	25.6± 2.04
	Nitazoxanide	1.14± 0.18	29.4± 2.48
21th day	Control	0.76± 0.08	19.6± 1.04
	Nitazoxanide	1.08± 0.21	22.4± 2.50

Table 3. The effect of Nitazoxanide (18mg/kg, P.O. once daily) on the biochemical markers of the antioxidant enzymes of rats on the 1st, 7th, 14th and 21st 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
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

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
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

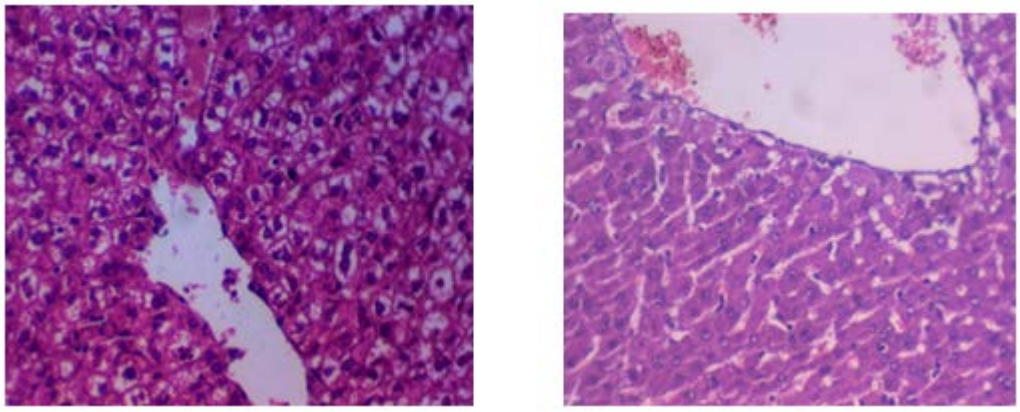

Figure 1. A: Liver of Nitazoxanide-treated animal (7th day sacrifice) showing the dilated congested central vein surrounded by rows and cords of hepatocytes with clean cytoplasm due to the severe fatty changes. B: Liver of Nitazoxanide-treated animal (14th day sacrifice) showing the dilated congested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

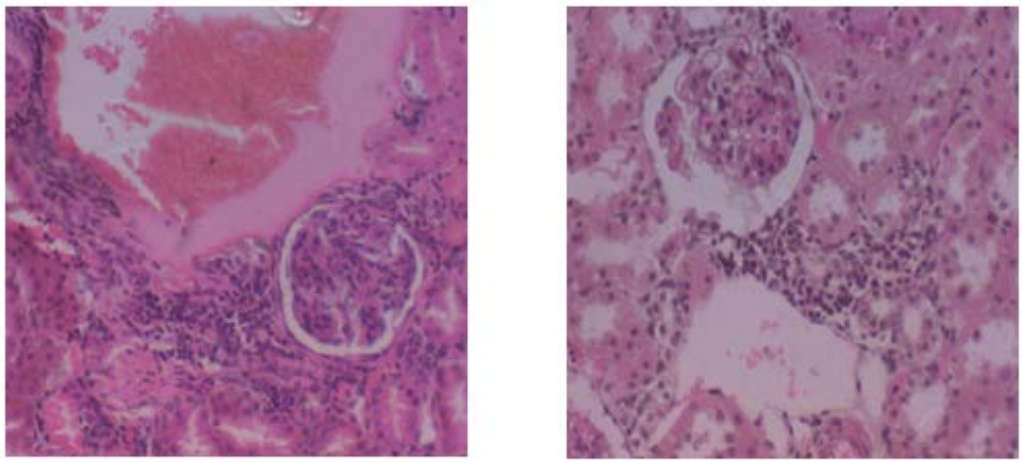

Figure 2. C: Kidney of Nitazoxanide-treated animal (7th day sacrifice) showing the dilated congested vascular space between renal Ztissue and heavy aggregation of the chronic inflammatory cells .D: Kidney of Nitazoxanide-treated animal (14th day sacrifice) showing the dilated congested vascular space and mild aggregation of the chronic inflammatory cells.

DISCUSSION

Liver plays an important role concerned with the biochemical activities and physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion, and storage; liver has a great capacity to detoxicate toxic substances and synthesize useful principles. Kidney is recognized as one of the most common target organs of toxicity of the drugs and environmental chemicals. Some drugs have exhibited widespread damage on the kidney with marked tubular damage [17].

In this study, Nitazoxanide was used in a dose of (18mg/kg,b.wt) once daily for successive 14 days. The results suggested that the administration of Nitazoxanide showed a significant decrease in Blood parameters with the increased corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC) compared with the control group.

The results were in harmony with the results reported by [7] who found that the effect of Nitazoxanide included anemia and leukopenia.

Nitazoxanide significantly produced hepatic and renal disturbance.

ALT, AST and ALP have been the known indicators for the assessment of the functional integrity of the liver cells [18].

Nitazoxanide in therapeutic dose resulted in a significant increase in ALT, AST, ALP level after one day post treatment when compared with the control group, but this elevation decreased gradually by time.

Nitazoxanide in a therapeutic dose produced a significant decrease in total protein and albumin level after one-day post administration when compared with the control group.

Meanwhile, Nitazoxanide in therapeutic dose for 14 successive days on female rats induced a non- significant change in the total bilirubin level after one day post administration when compared with the control group.

The results of this study were in harmony with the results reported by [7] who found that the effect of Nitazoxanide included increased SGPT and SGOT.

Histopathological results on the 7th day, showed markedly dilated congested central veins surrounded by rows and cords of hepatocytes with clean cytoplasm due to the severe fatty changes. On the 14th day, the dilated congested central vein with the mild fatty change of the hepatocyte was observed.

The results of this study were in harmony with the results reported by [7] who found that the effect of Nitazoxanide included the increased creatinine and serum urea.

Histopathological results on the 7th day showed the dilated congested vascular space between the renal tissues and heavy aggregation of the chronic inflammatory cells. On the 14th day, the dilated congested vascular space and the mild aggregation of the chronic inflammatory cells were observed.

The results suggested that the administration of Nitazoxanide in therapeutic dose for 14 successive days on female rats resulted in a significant increase in the serum creatinine and urea level after one day post treatment when compared with the control group.

The results of this study were in harmony with the results reported by [19], who found that the general mechanisms caused the renal disturbance including a change in glomerular hemodynamic, tubular cell toxicity, inflammation.

The results suggested that the administration of Nitazoxanide in therapeutic dose for 14 successive days on female rats resulted in a significant decrease in catalase, superoxide dismutase and Glutathione peroxidase activity after one day post administration when compared with the control group.

The administration of Nitazoxanide in therapeutic dose for 14 successive days on female rats resulted in a significant increase in MDA activity after one day post treatment when compared with the control group.

CONCLUSION

In conclusion, Nitazoxanide has some adverse effects on liver and kidney of rats. Therefore, Nitazoxanide should be used with caution by people with liver or kidney problems.

Conflict Of Interest

The authors declared no conflict of interest.

REFERENCES

1. Rossignol J F, and Cavier R. New derivative of 2 benzamide 5 nitrothiazoles .ChemAbstr 1975; 83-28216.
2. Sisson G, Goodwin A, and Raudonikiene A. Enzymes associated with reductive activation and action of nitazoxanide, nitrofurans, and metronidazole in Helicobacter pylori. Antimicrobl Agents Chemother 2002; 46(7), 2116-23.
3. Hoffman P S , Sisson G , Croxen M A, Welch K, Harman W D, Cremades N, Morash M G. Antiparasitic drug nitazoxanide inhibits the pyruvate oxidoreductases of Helicobacter pylori, selected anaerobic bacteria and parasites, and Campylobacter jejuni. Antimicrob Agents Chemother 2007;51(3), 868-876.
4. Broekhuysen J, Stockis A, Lins R L, Graeve J, Rossignol J F. Nitazoxanide: pharmacokinetics and metabolism in man. Int J ClinPharmacolTher 2000;38(8), 387-94.
5. Stockis A, Deroubaix X, Lins R, Jeanbaptiste B, Calderon P, Rossignol J F. Pharmacokinetics of nitazoxanide after single oral dose administration in 6 healthy volunteers. Int J ClinPharmacolTher 1996 ;34(8) 349-51.
6. Romark. Alinia (Nitazoxanide) tablets. (Nitazoxanide) for oral suspension. Romark Pharmaceuticals 2006; Nov 24.

7. Stockis A, Allemon A M, DeBruyn S, Gengler C, Rosillon D. Nitazoxanide pharmacokinetics and tolerability in man using single ascending oral doses. Int J ClinPharmacolTher 2002;40(5), 213-20.
8. Paget S, Parnes R. Evaluation of drug activities, pharmacometrics, ed. Laurance and Bacharach, Academic press, New York. 1964;.
9. Tietz , N W. Fundamentals of clinical chemistry, 2nd ed, W.B. Saunders Company, Philadelphia 1976; 873-944
10. Henry R J, Cannon D C, Winkelman J W. Clinical Chemistry: principles and Techniques.2nd ed 1974; 1354-1369.
11. Vassault A, Grafmeyer D, Naudin C, Dumont G, Bailly M, Henny J, Gerhardt M F, Georges P. Protocol for the validation of methods. Ann. Biol. Clin. 1986; 686-745.
12. Suvarna S K , Layton C , Bancroft J D. Bancroft,s theory and practice of histological technique 7th ED. Churchil living stone-elsevier, England 2013;.
13. Aebi H. Colourmetrical determination of Catalase activity. Methods Enzymol 1984; 121-126.
14. Nishikimi M, Roa N A , Yogi K. Measurement of superoxide dismutase. Bioch. Btoph. Res. Commun. 1972; 46:849-85.
15. Paglia D E, Valentine W N. Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase.J. Lab. Clin. Med. 1967; 70:158-169.
16. Jacob Filho W, Lima C C, Paunksnis M R R , Silva A A , Perilhao M S , Caldeira M, Bocalini D, de Souza R R . Reference database of hematological parameters for growing and aging rats. Aging Male. 2017; 11:1-4.
17. Barbier O, Jacquille G T, Tauc M, Cougnan M, Poujeol P. Effect of heavy metals on, and handling by kidney. Nephro physical 2005; 99(4):105-110
18. Adaramoye O A, Osaimoje D O, Akinsanye M A , Fafunso M A. Changes in antioxidant status and biochemical indices after acute administration of artemether, artemether-lumefantrine and halofantrine in rats. Basic. Clin. Pharmacol. Toxicol 2008; 102 (4):412-418
19. Ferguson M A, Vaigya V S ,Bonventre J V. Biomarkers of nephrotoxic acute kidney injury. Toxicology 2008; 245 (3): 182-193.