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Synergistic Effect of antioxidants combinations (Vit A, C, E and selenium) (Antox drug) against hepatorenal toxicity induced by Sorafenib in male Albino rats.

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ABSTRACT

The present study was assessed to eluicidate the protective role of the antioxidants antox against Sorafenib renal toxicity in adult male albino rats. One hundred and twenty mature male albino rats divided into 4 equal group, were used in this study. The 1st group was kept as normal control group, the 2nd group was given anti-cancer drug sorafenib orally and daily for successive 2 weeks in adose of (10 mg /kg b.wt.),the 3rd group was administered the antioxidant drug antox orally and daily for 2 weeks in adose of (10 mg /kg b.wt.), the 4th group was given the two drugs together in their recommended doses. Blood samples were collected from 5 rats of each group at 1st,3rd days and 1st,2nd,3rd and 4th weeks post end of treatment and were subjected to biochemical assays including: Liver enzymes (AST and ALT),total protein , albumin, Alkaline phosphatase (ALP), Gamma-glutamyl transferase (γ-GT),albumin, creatinine,uric acid and urea . Treatment with sorafenib alone resulted in a significant increase in ALT, AST, ALP, γ-GT, creatinine ,uric acid and urea. The activity of total protein ,albumin are significantly decreased in sorafenib treated group compared to control group. Treatment for two weeks with antox after sorafenib elicited a significant decrease in ALT, AST, ALP, γ-GT, creatinine ,uric acid and urea as well as a significant increase in serum total protein and albumin revealed that sorafenib treated group showed histopathological alterations in liver and kidney structure compared with control and other treated groups.

Keywords: Sorafenib, Antox, hepatic functions, kidney functions, histopathology.

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INTRODUCTION

Sorafenib tosylate is a white to yellowish or brownish solid with a molecular weight of 637 g/mole. Sorafenib inhibits tumour growth by blocking the activity of serine/threonine and receptor tyrosine kinases located in both the tumour cell (c-Raf, b-Raf, V600E b-Raf, KIT, and Flt3) and in the tumour vasculature (c-Raf, VEGFR2, VEGFR3, and PDGFR-beta) [1]. In spite of the extensive literature in the last years around sorafenib effects, the specific molecular pathways involved in sorafenib-induced tumor cell death remain unclear and caspase-dependent and independent mechanisms, as well as endoplasmic reticulum stress have been proposed [2]. In vitro studies have shown that under baseline culture conditions, sorafenib is a poor apoptotic inducer in HCC cells unless this drug is used at high doses. However, it greatly potentiates the apoptotic effects of other therapeutic drugs, such as TRAIL, BCL-XL inhibitors, rapamycin, or MEK/ERK inhibitors [3].

Antioxidant vitamins are the most important free radical scavengers in extracellular fluids, trapping radicals in the aqueous phase and protect biomembranes from peroxidative damage [4].

Antox is an antioxidant contains three supplementary nutritional vitamins A, C and E with trace element selenium which is essential for normal metabolic reactions [5].

Antioxidant containing drug (Antox) inhibits free radical generation in the small intestine, which acts as a contributing factor to the rejection process [6]. Vitamins are ideal antioxidants to increase tissue protection from oxidative stress due to their effective and safe dietary administration in a wide range of concentrations without harmful side effects [7].

Vitamin C is well known as an antioxidant; which act as an electron donor to protect the body from radicals and pollutants [8]. Vitamin C is also known to act as free radical trap and as cofactor in the synthesis of biologically antioxidant materials such as glutathione [9]. Vitamin E is a potential antioxidant and a liposoluble antioxidant present in biological membranes and inhibits free radical formation in biological system [10] .So, it's deficiency causes changes in membrane properties underlies many diseases [11].

Selenium (Se) is an essential trace element for mammalian cells. It has regulatory functions in cell growth, cellular death and modulates signals transduction in various cells [12]. Se regulates immune function; it may provide important health benefits to people whose oxidative stress loads are high, such as those with inflammatory or infectious diseases [13]. Selenium as an essential constituent of Glutathione -peroxidase enzyme (GSH-Px) plays an important role in scavenging reactive oxygen species (ROS). It is known that ROS and GSH are closely involved in Se metabolism and bioactivity of various cells [14].

This study aimed to evaluate the possible synergistic effect of the antioxidants antox and its ability to protect against hepato-renal toxicity induced by anti-cancer drug sorafenib, through studying the effect on liver and kidney functions parameters with histopathological structure alterations.

MATERIALS AND METHODS

Chemicals:

Sorafenib (Nexavar, 200® mg) obtained from Bayer Healthcare (Leverkusen, Germany) was used. Pills were ground in a tissue mill. The resulting powder was mixed with distilled water and applied via gavage to rats by stomach tube. Antox :Arab Company for Pharmaceuticals & Medicinal Plants (Mepaco-Medifood)– Egypt.A dietary Supplement, Contains the three main antioxidant vitamins A, C and E together with very important rare element, selenium.The tablets were crushed and suspended in 0.5% CMC in distilled water.

Experimental Animals:

One hundred and twenty mature male albino rats, obtained from Animal Breeding Unit, Faculty of Vet. Med. Zagazig University were used in this study. Their weights ranged between (150-180 gm b.wt.) They were housed in wire cages with natural ventilation, illumination and allowed free water and standard pelleted diet ad-libitum and acclimated for 2 weeks before experimentation. Animal experiments were performed after

following the European Community Directive (86/609/EEC) and national rules in accordance with the 8th edition of NIH Guidelines for the Use of Animals.

Experimental Design:

The rats were randomly divided into 4 equal groups, each of 30 rats as follows:1st control group was administrated 1 mL dist.water .2nd group Sorafenib group was given sorafenib (10 mg/Kg) [15] orally for successive two weeks. 3rd Antox group was administrated Antox drug (10 mg/Kg) [16] for successive two weeks.4th group was given a combination of sorafenib drug and followed by Antox drug for the same periods.

Blood collection and hepatorenal biomarkers evaluation:

Two blood samples were collected from 5 rats of each group at 1st and 3rd days and 1st, 2nd, 3rd and 4th weeks post end of treatment. First sample without anticoagulant for preparation of serum, blood was collected and allowed to clot and serum was separated by centrifugation at 3000 rpm and kept at -20°C for measuring biochemical parameters. Serum was harvested and preserved at –80°C until kidney biomarkers estimated. The serum activity of alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were determined according to (Tietz, 1976). Alkaline phosphatase activity was determined colorimetrically according to Belfield and Goldberg (1971). Serum γ–GT activity was determined according to the method described by Beleta and Gella (1990). Serum total proteins was determined according to Henry (1964). Serum albumin, Globulin, Creatinine and uric acid were determined by commercial kits. The urea level was estimated according to Patton and Crouch [17].

Histological evaluation

Samples of liver and kidney were taken from all rat groups and fixed in 10% formal saline for 24 hrs. The sections were stained by eosin and hematoxylin for examination through the light microscope and using a digital camera for photographing **[18].**

Statistical analysis:

The obtained data were analyzed and graphically represented using the statistical package for social science ***(SPSS,* version 16),** for obtaining [Mean value ± standard error]. The results were statistically analyzed by using one-way ANOVA test. Subsequent multiple comparisons between the different groups were analyzed by Duncan's multiple comparison tests ***(Duncan, 1955)***. values at ($P < 0.05$) were considered significant **[20]**.

RESULTS

Effect on hepatic function biomarkers:

(Table.1,2) illustrated that oral administration of sorafenib (10 mg/ kg) to normal rats daily for successive two weeks afforded a marked elevation in serum ALT and AST activities when compared with control and other treated groups.While antox treated group showed significant and non-significant changes in ALT level compared with normal control and sorafenib + antox treated group, while, induced a significant decrease when given alone when compared with sorafenib treated group. Whereas, the combined treatment with sorafenib and antox induced a significant increase in serum ALT and AST levels when compared with control and significant decrease when compared with sorafenib treated rats.

The obtained results in (table.3) demonstrated a significant increase in serum Gamma-glutamyl transferase (γ-GT), total bilirubin and serum indirect bilirubin levels activity in sorafenib exposed group along the course of study when compared with normal control group and other treated groups.Oral administration of antox to sorafenib intoxicated rats showed a significant increase and decrease in serum (γ-GT) activity when compared with control and sorafenib treated group respectively. However, antox treated group elicited non-significant change compared with control rats.

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Table (1): Effect of Sorafenib (10 mg/kg.b.wt.), Antox (10 mg/kg.b.wt.) and Sorafenib + Antox (10 mg/kg.b.wt.), on the activities of serum ALT in male albino rats. Mean ± S.E (n=5)

Parameter / Experimental group	ALT *(U/l)*					
	1st day	3rd day	1st week	2nd week	3rd week	4th week
Control	55.38±4.8 [a b]	45.38±3.53 [d]	45.34±3.14 [c]	49.04±5.63 [c]	62.38±4.43 [c]	62.04±4.22 [c]
Sorafenib	62.90±4.90 [a]	154.03±6.36 [a]	181.40±2.41 [a]	208.77±8.87 [a]	217.43±9.59 [a]	204.77±5.38 [a]
Antox	67.40±2.44 [a]	71.97±1.74 [c]	80.77±2.55 [b]	86.53±3.13 [b]	66.73±6.47 [c]	86.53±5.09 [b]
Sorafenib & antox	57.13±4.15 [ab]	88.30±6.17 [b]	77.60±4.40 [b]	79.93±1.69 [b]	86.00±3.82 [b]	85.13±1.47 [b]

Means within the same column in each category carrying different litters are significant at (P≤0.05) using Duncan's multiple range test.

Table (2): Effect of Sorafenib (10 mg/kg.b.wt.), Antox (10 mg/kg.b.wt.) and Sorafenib+Antox (10 mg/kg.b.wt.) on the activities of serum AST in male albino rats. Mean ± S.E (n=5)

Parameter / Experimental group	AST *(U/l)*					
	1st day	3rd day	1st week	2nd week	3rd week	4th week
Control	107.28 ± 3.09 [d]	131.71 ± 1.18 [c]	105.01 ± 3.77[d]	89.80 ± 8.68 [d]	127.40 ± 10.55[d]	96.47 ± 3.27 [d]
Sorafenib	161.80 ± 2.03 [b]	359.80 ± 4.64 [a]	423.73 ± 3.34 [a]	420.27 ± 9.03 [a]	453.60 ± 9.32 [b]	289.07 ± 3.96 [a]
Antox	170.00 ± 2.26 [a]	136.00 ± 9.00 [c]	174.00 ± 6.09 [c]	155.00 ± 5.95 [c]	560.07 ±25.56 [a]	148.07 ± 4.86 [c]
Sorafenib & antox	133.07 ± 0.47 [c]	217.60 ± 3.49 [b]	310.07 ± 7.03 [b]	217.53 ± 12.25[b]	235.93 ± 12.15 [c]	186.43 ± 12.07 [b]

Means within the same column in each category carrying different litters are significant at (P≤0.05) using Duncan's multiple range test.

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Table (3): Effect of Sorafenib (10 mg/kg.b.wt.), Antox (10 mg/kg.b.wt.) and Sorafenib +Antox (10 mg/kg.b.wt.) on the activities of serum ALP in male albino rats. Mean ± S.E (n=5)

Parameter / Experimental group	ALP *(U/l)*					
	1st day	3rd Day	1st week	2nd week	3rd week	4th week
Control	67.43 ± 4.85 b	55.13 ± 0.91c	66.57 ± 4.95c	64.90 ± 2.91c	65.90 ± 3.72c	57.57 ± 4.82c
Sorafenib	72.50 ± 5.15 ab	89.55 ± 6.94ab	228.34 ± 8.06a	217.46 ± 12.18a	240.62 ± 11.94a	114.10 ± 7.99ab
Antox	64.97 ± 3.53 b	104.10 ± 5.34a	64.67 ± 2.38c	61.58 ± 6.35c	59.29 ± 6.95c	125.56 ± 8.10a
Sorafenib & antox	82.07 ± 1.94 a	84.90 ± 4.88b	156.13 ± 0.63b	153.84 ± 8.71b	170.25 ± 8.47b	132.36 ± 6.32a

Means within the same column in each category carrying different litters are significant at (P≤0.05) using Duncan's multiple range test.

Table (3): Effect of Sorafenib (10 mg/kg.b.wt.), Antox (10 mg/kg.b.wt.) and Sorafenib +Antox (10 mg/kg.b.wt.) on the activities of serum γ-GT in male albino rats. Mean ± S.E (n=5)

Parameter / Experimental group	γ-GT *(U/l)*					
	1st Day	3rd day	1st week	2nd Week	3rd week	4th week
Control	45.47 ± 3.44 b	53.14± 3.93c	52.14± 4.58b	50.47± 3.01c	50.80± 3.31c	51.80± 2.60c
Sorafenib	58.35± 2.33 a	137.74± 6.75a	125.64± 4.81a	185.34± 6.95a	283.18± 8.49a	171.20± 8.49a
Antox	51.34± 3.86 ab	52.21± 5.61c	49.62± 4.31b	57.00± 1.66c	47.36± 5.10c	53.98± 4.30c
Sorafenib & antox	58.04± 3.35 a	85.56± 5.98b	123.72± 9.35a	156.33± 9.28ab	162.74± 1.54b	157.70± 7.82ab

Means within the same column in each category carrying different litters are significant at (P≤0.05) using Duncan's multiple range test.

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Table (4): Effect of Sorafenib (10 mg/kg.b.wt.), Antox (10 mg/kg.b.wt.)and Sorafenib +Antox (10 mg/kg.b.wt.) on serum total proteins in male albino rats. Mean ± S.E (n=5)

Parameter / Experimental group	Serum total proteins *(g/dl)*					
	1st Day	3rd Day	1stweek	2ndweek	3rdweek	4thweek
Control	5.77 ± 0.27^{a}	5.67 ± 0.25^{a}	5.77 ± 0.27^{a}	6.10 ± 0.20^{a}	6.44 ± 0.30^{a}	6.11 ± 0.27^{a}
Sorafenib	5.70 ± 0.16^{a}	3.32 ± 0.15^{c}	4.52 ± 0.20^{b}	3.68 ± 0.27^{c}	3.62 ± 0.14^{c}	4.01 ± 0.14^{c}
Antox	6.29 ± 0.15^{a}	5.96 ± 0.19^{a}	5.25 ± 0.14^{a}	6.03 ± 0.32^{a}	6.95 ± 0.22^{a}	5.85 ± 0.26^{a}
Sorafenib & antox	5.77 ± 0.26^{a}	4.49 ± 0.16^{b}	4.59 ± 0.15^{b}	5.40 ± 0.12^{ab}	5.07 ± 0.15^{b}	4.68 ± 0.22^{b}

Means within the same column in each category carrying different litters are significant at (P≤0.05) using Duncan's multiple range test.

Table (5): Effect of Sorafenib (10 mg/kg.b.wt.), Antox (10 mg/kg.b.wt.) and Sorafenib +Antox (10 mg/kg.b.wt.) on serum albumin in male albino rats. Mean ± S.E(n=5)

Parameter / Experimental group	Albumin *(g/dl)*					
	1st day	3rd day	1st week	2nd week	3rd week	4th week
Control	3.38 ± 0.10^{a}	3.71 ± 0.14^{a}	4.22 ± 0.13^{a}	3.99 ± 0.13^{a}	3.79 ± 0.07^{a}	3.86± 0.01^{a}
Sorafenib	3.25 ± 0.18^{a}	2.84 ± 0.04^{b}	1.55 ± 0.17^{c}	2.56 ± 0.22^{bc}	2.64± 0.15^{b}	3.13± 0.22^{b}
Antox	3.52 ± 0.09^{a}	3.45 ± 0.03^{ab}	3.18 ± 0.25^{b}	2.23 ± 0.19^{c}	3.95± 0.29^{a}	3.69± 0.14^{a}
Sorafenib & antox	3.05 ± 0.12^{a}	2.97 ± 0.20^{b}	2.94 ± 0.18^{b}	2.94 ± 0.12^{b}	2.80± 0.30^{b}	3.22± 0.15^{b}

Means within the same column in each category carrying different litters are significant at (P≤0.05) using Duncan's multiple range test.

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Table (6): Effect of Sorafenib (10 mg/kg.b.wt.), Antox (10 mg/kg.b.wt.) and Sorafenib+Antox (10 mg/kg.b.wt.) on serum globulins in male albino rats. Mean±S.E(n=5).

Parameter / Experimental group	Globulins *(g/dl)*					
	1st day	3rd day	1st week	2nd week	3rd week	4th Week
Control	3.39 ± 0.25 ^{a}	1.96 ± 0.16 ^{a}	1.55 ± 0.22 ^{b}	2.12 ± 0.33^{a}	2.65 ± 0.15 ^{a}	2.25± 0.16 ^{a}
Sorafenib	2.45 ± 0.24^{b}	1.48 ± 0.19 ^{b}	2.81 ± 0.28 ^{a}	1.11 ± 0.25 ^{c}	0.98 ± 0.19 ^{d}	0.88± 0.11^{c}
Antox	2.77 ± 0.15 ^{b}	1.51 ± 0.20 ^{ab}	2.07 ± 0.32 ^{b}	1.80 ± 0.31^{ab}	2.00 ± 0.12^{b}	2.16± 0.13^{a}
Sorafenib & antox	2.62 ± 0.22^{b}	1.53 ± 0.23^{ab}	1.65 ± 0.26^{bc}	2.46 ± 0.27^{a}	2.26 ± 0.25^{ab}	1.47± 0.11^{b}

Means within the same column in each category carrying different litters are significant at (P≤0.05) using Duncan's multiple range test.

Table (7): Effect of Sorafenib (10 mg/kg.b.wt.), Antox (10 mg/kg.b.wt.) and Sorafenib + Antox (10 mg/kg.b.wt.) on urea level in male albino rats. Mean ± S.E (n=5)

Parameter / Experimental group	Urea*(mg/dl)*					
	1st Day	3rd day	1st week	2ndweek	3rdweek	4th week
Control	15.35 ± 1.13 ^{a}	12.64 ± 0.81^{c}	16.58 ± 1.52^{c}	14.17 ± 1.20^{c}	13.26 ± 0.48^{c}	16.38 ± 0.41^{c}
Sorafenib	16.75 ± 1.62 ^{a}	22.89 ± 0.89^{a}	35.15 ± 1.00^{a}	41.22 ±3.21^{a}	33.26 ± 1.48^{a}	32.93 ± 2.54^{a}
Antox	13.41 ± 0.18 ^{a}	13.03 ± 0.61^{c}	17.53 ± 1.57^{c}	18.41 ± 0.97^{c}	16.75 ± 1.39^{c}	14.87 ± 0.72^{c}
Sorafenib & antox	13.10 ± 0.53 ^{a}	18.01 ± 1.30^{ab}	28.68 ± 1.89^{b}	35.79 ± 2.62^{ab}	25.25 ± 1.08^{b}	26.05 ± 0.80^{ab}

Means within the same column in each category carrying different litters are significant at (P≤0.05) using Duncan's multiple range test.

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Table (8): Effect of Sorafenib (10 mg/kg.b.wt.), Antox (10 mg/kg.b.wt.) and Sorafenib+Antox (10 mg/kg.b.wt.) on serum creatinine level in male albino rats. Mean ± S.E(n=5)

Parameter / Experimental group	Creatinine *(mg/dl)*					
	1st day	3rd day	1st week	2ndweek	3rdweek	4thweek
Control	0.60 ± 0.12 [a]	0.51 ± 0.20[c]	0.73 ± 0.06[c]	0.53 ± 0.10[c]	0.61 ± 0.09[c]	0.51 ± 0.09[c]
Sorafenib	0.44 ± 0.20 [a]	0.97 ± 0.12[a]	1.67 ± 0.23[a]	1.78 ± 0.20[a]	1.93 ± 0.27[a]	1.84 ± 0.31[a]
Antox	0.43 ± 0.19 [a]	0.41 ± 0.05[c]	0.56 ± 0.06[c]	0.55 ± 0.04[c]	0.51 ± 0.11[c]	0.65 ± 0.04[c]
Sorafenib & antox	0.41 ± 0.20 [a]	0.82 ± 0.10[ab]	1.09 ± 0.09[b]	0.99 ± 0.06[b]	1.17 ± 0.13[b]	1.14 ± 0.08[b]

Means within the same column in each category carrying different litters are significant at (P≤0.05) using Duncan's multiple range test.

Table (9): Effect of Sorafenib (10 mg/kg.b.wt.), Antox (10 mg/kg.b.wt.) and Sorafenib +Antox (10 mg/kg.b.wt.) on uric acid level in male albino rats. Mean ± S.E (n=5)

Parameter / Experimental group	Uric acid *(mg/dl)*					
	1st day	3rd day	1st week	2ndweek	3rdweek	4th Week
Control	2.98±0.08 [a]	2.78±0.22[a]	2.39±0.21[bc]	2.31±0.19[a]	3.79±0.84[a]	4.33±0.22[a]
Sorafenib	1.20±0.06 [b]	2.99±0.25[a]	3.15±0.15[a]	2.36±0.15[a]	3.79±0.54[a]	4.48±0.08[a]
Antox	1.54±0.08 [d]	2.14±0.26[b]	2.0±0.26[bc]	2.31±0.39[a]	1.32±0.22[b]	4.10±0.21[a]
Sorafenib & antox	2.45 ±0.03 [a]	3.05±0.28[a]	2.80±0.34 [ab]	2.66±0.29[a]	1.18±0.20[b]	1.49±0.59[b]

Means within the same column in each category carrying different litters are significant at (P≤0.05) using Duncan's multiple range test.

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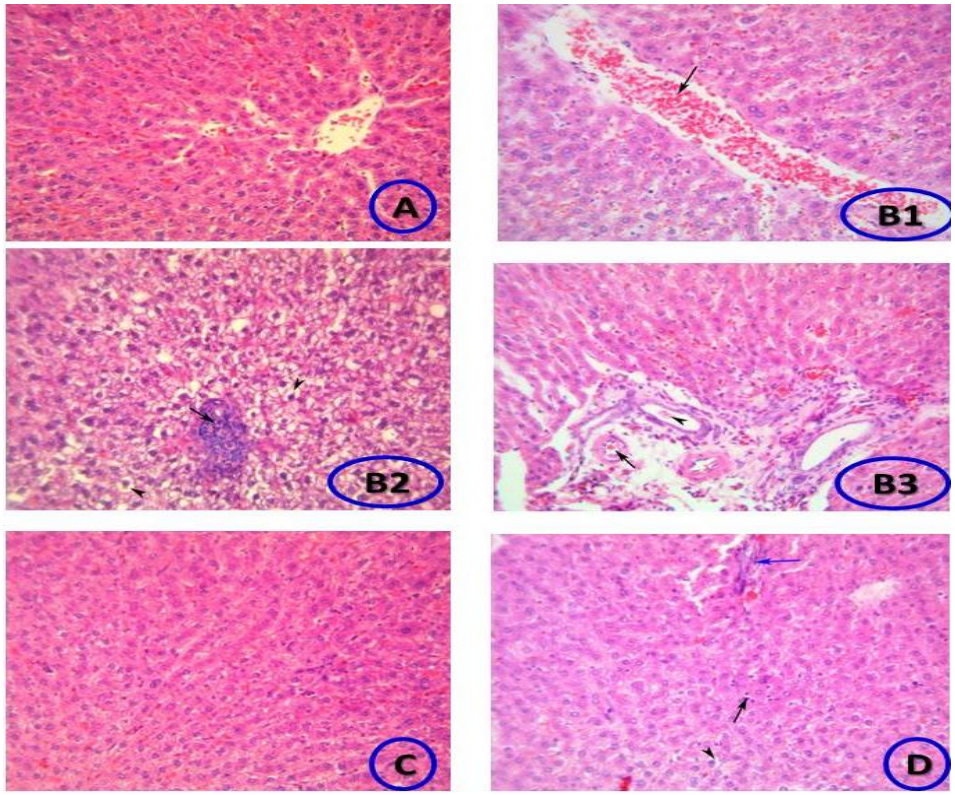

Fig (1): Histopathological slides of the liver stained with hematoxylin and eosin in (A) Control group: The liver of normal control rats showed normal histomorphological appearance (X400). (B1-3): Sorafenib-treated rat, showing vascular congestion (arrow), showing dilated sinusoids (arrow), and hydropic degeneration of numerous hepatocytes (arrow head) and showing severe hydropic degeneration of hepatocytes (arrow head), with focal mononuclear cell aggregation (arrow) (X400). (C): Antox-treated rat, group showing normal histological structure without any pathological alterations (X400). (D): Sorafenib+Antox-treated rat, showing mild vacuolation of some hepatocytes (arrow head), proliferated Von Kupffer cells (black arrow), beside slight proliferation of fibrous tissue (blue arrow)(X400).

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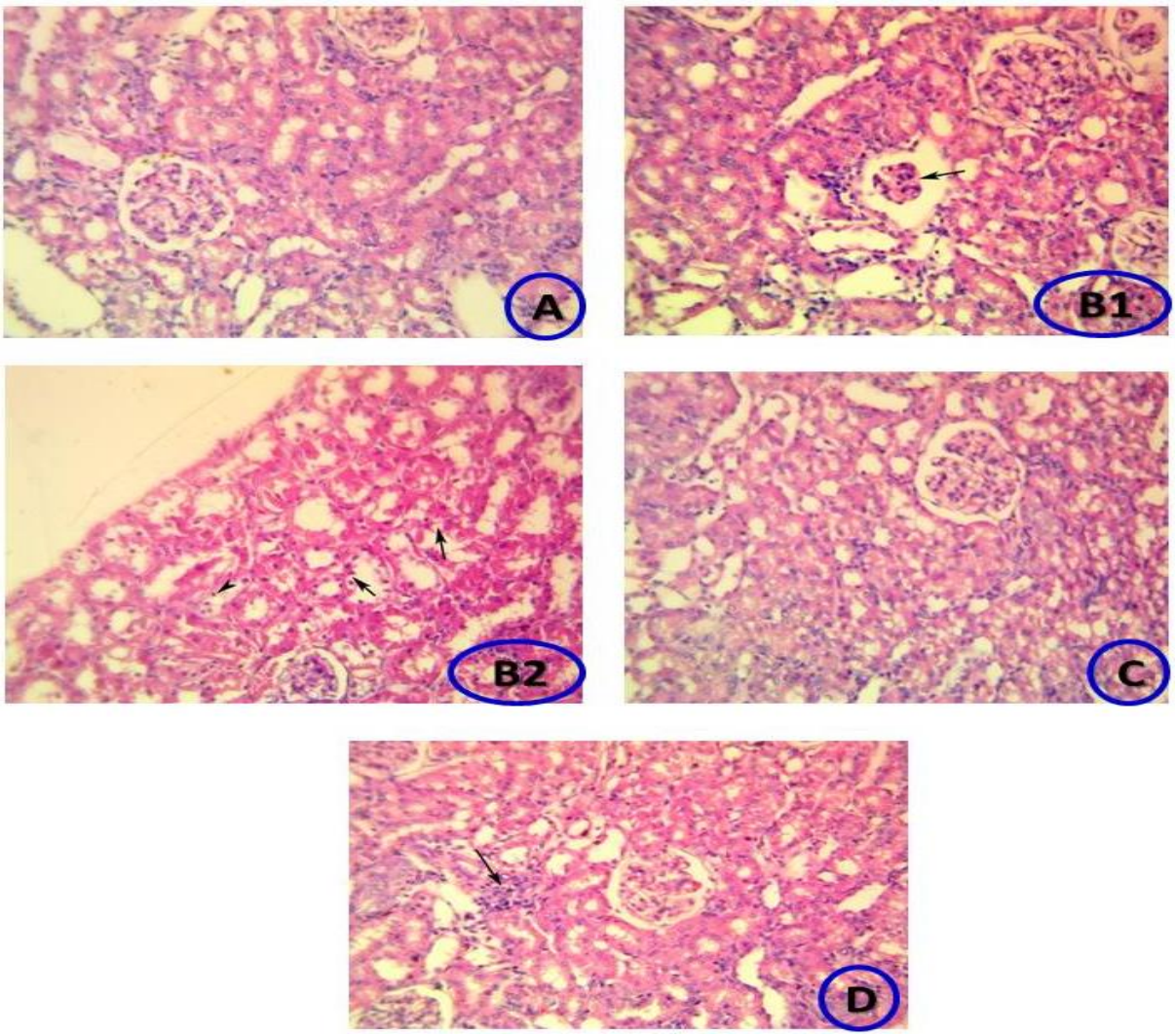

Fig (2): (A) control group: showing normal histological structure. (renal cortex with normal Malpigian corpuscle, proximal and distal convoluted tubules)(X400). (B_{1-2}): Sorafenib-treated rat: showing collapsed glomerulus (arrow) and vacuolation of the epithelial lining of the renal tubules (arrow), with pyknosis of the nuclei (arrow head) (X400).(C)Antox-treated rat: showing normal histological structure without any pathological alterations (X 400). (D) Sorafenib + Antox-treated rat: showing mild focal mononuclear cell aggregation, (X 400).

Table (4,5 and 6) demonstrated that treatment of healthy rats with sorafenib exhibited a significant decrease in total protein , albumin,globulin levels along the entire period of the study except 1st day which showed non significant change. Meanwhile antox treated group afforded non significant change when compared with control along the course of the study. On the other hand sorafenib+antox treated group exhibited non-significant change when compared with control rats.

Effect on renal function biomarkers:

The obtained data presented in (table.7,8 and 9) showed that, sorafenib treated rats showed a significant increase in serum creatinine,uric acid and urea levels along the course of the study except post 1st day which showed non-significant increase as compared with normal control group and other treated groups.While antox treated group revealed non-significant change in serum creatinine, uric acid and urea levels when compared with control. Whereas, combined treatment with sorafenib and antox showed significant increase in creatinine level as compared with control and significant decrease when compared with sorafenib treated group.

Effect on liver histopathology:

Treatment of male rats with sorafenib , Antox and/or their combinations showing the following The liver of normal control rats showed normal histomorphological appearance (Fig. 1A). The liver of sorafenib treated group showed vascular congestion , dilated sinusoid, hydropic degeneration of numerous hepatocytes,some sections showed severe hydropic degeneration with focal mononuclear cell aggregation, congested central veins with marked portal leukocytic infiltration and leukocytic infiltration with the presence of eosinophilic material in the lumen of some bile ducts (Fig.1B1-3). The liver of antox treated group showed normal histological structure without any pathological alteration (Fig. 1C). The liver of Sorafenib + Antox treated groups showed mild congestion of the central veins and portal blood vessels with slight portal leukocytic infiltration, mild vacuolation of some hepatocytes, proliferation of von kupffer cells, beside slight proliferation of fibrous tissue, focal proliferation of von kupffer cells and tiny necrotic foci replaced by mononuclear cells (Fig.1D).

The kidney of control rat, showing normal histological structure (Fig.2A). The kidney of sorafenib treated rats showed collapsed glomeruli, focal mononuclear cell aggregation, vacuolation of the epithelial lining of the renal tubules with pyknosis of the nuclei (Fig. 2B(1-2). The kidney of sorafenib + antox treated group revealed mild focal mononuclear cell aggregation (Fig. 2C). The Kidney of Sorafenib+Antox-treated rat, showing mild focal mononuclear cell aggregation (Fig. 2D).

DISCUSSION

The present study showed that treatment with antox in combination with sorafenib offered considerable hepato-renal protection as evidenced from biochemical parameters and histopathological sections.

The study showed that treatment with antox in combination with sorafenib offered considerable protection to liver as evidenced from the levels of biochemical parameters (ALT, AST, ALP, GGT, total Proteins, albumin and globulins, in the serum). Moreover, the effect of antox on kidney function parameters, heart function parameters, as well as on oxidative/antioxidative parameters and comet test assay were also studied in this work. These results were supported by the liver, kidney, heart and spleen histopathological changes.

Liver diseases are considered to be a serious health problem, as the liver is an important organ for the detoxification and deposition of endogenous and exogenous substances. Steroids, vaccines and antiviral agents, which have been employed as therapies for liver diseases, have potential adverse effects, especially when administered for long terms. Therefore, herbal, natural products and traditional medicines with improved effectiveness and safety profiles are needed as a substitute for chemical therapeutics. It has been reported that a number of herbal and natural products have been shown to protect against liver injury and possess one or a combination of antioxidant, anti-fibrotic, immune modulatory and antiviral activities [21].

Conventional or synthetic drugs used for liver diseases are sometimes inadequate and can have serious adverse effects. Therefore, there is a worldwide trend to go back to traditional medicinal plants and natural products. Many natural products of herbal origin are now in use for the treatment of liver ailments [22].

The enzymes ALT, AST and ALP are important enzymes that are often employed in assessing liver injury [23].Sturgill and Lambert [24] reported also that serum activities of ALT and AST are the most commonly used biochemical markers of liver injuries. Another study demonstrated that sorafenib with higher doses aggravated liver injury [25] and this result came in parallel with the results of our study.

Chronic administration of sorafenib developed a significant hepatic damage which was observed by the substantial increase in the activities of serum ALT, AST and ALP. The recorded increase in the present study was in harmony with that previously obtained by Manna et al. [26]; Narendra et al.[27] ; Bhushan et al. [28] and Ibiang et al. [29].Antox ameliorated the toxic effects of sorafenib and this was supported by Oz et al., (2004) who reported that antox is an antioxidant drug used in therapy of different liver diseases.

GGT is a glycosylated protein that is partially embedded in the outer surface of the plasma membrane at the N-terminal transmembrane domain. GGT is an enzyme normally present in the serum and on the outer surface of numerous cell types Dominici et al., [30]. Serum GGT is especially active in the proximal renal tubule, pancreas and intestine, but primarily in the liver. In most cases, serum GGT levels are examined for the diagnosis of liver, gallbladder and biliary tract diseases [31] , especially in alcoholic liver disease [32].

The liver synthesizes not only the protein it needs but also produces numerous export proteins. Among the later, serum albumin is the most important one. Export proteins are synthesized on polyribosomes bound to the rough endoplasmic reticulum of the hepatocyte, in contrast, proteins destined for intracellular use are synthesized on free rather than bound polyribosomes [33]. Immunoglobulin is synthesized by immunocytes and hyperglobulinemia is found in hepatocellular disorders, appearing as an inflammatory reaction of liver [34].

The obtained results in this study revealed that oral administration of sorafenib (10 ml/kg) resulted in a marked decrease of serum total protein, albumin and globulins. Pointing to a decline in the liver synthetic function caused by sorafenib induced fibrosis. Whereas, protection with antox afforded a non-significant and significant increase in serum total proteins compared with normal and sorafenib groups respectively. This result was in agreement with those demonstrated by Omotuyl et al., [35] who mentioned that total protein showed a significant decrease in the test groups of rats which were subjected to continuous oral administration of cyflthrin (pyrethroid) for 15 week [36]. The result in this study showed a significant decrease in albumin due to administration of sorafenib and this result agree with Saxena and Saxena [37] they reported that stress due to exposure to a pesticide cypermethrin may influence albumin synthesis. The changing levels of serum albumin, thus, provide valuable indices of severity progress and prognosis in hepatic disease. Decreased albumin in serum indicated hepatocellular origin as in liver diseases.The total protein and albumin also showed a significant improvement and our results were reinforced with Hassanin et al. [16] they obtained the same result when treated male rats with antox along with arsenic.

Hyperbilirubinemia is a very sensitive indicator to substantiate the functional integrity of the liver and the severity of necrosis which increases the binding, conjugating and excretory capacity of hepatocytes that is proportional to the erythrocyte degeneration [38]. The liver damage induced by sorafenib in this study resulted in a significant elevation in serum total and direct bilirubin. Because in cases of liver injury due to hepatotoxin, there is a defect in excretion of bile by the liver which is reflected by an increase in bile level in the serum [39].

Our results coincides with Arroyo et al.[40] they attributed the increase in creatinine to the occurrence of hepatorenal syndrome (HRS) which was observed in patients with advanced liver failure (acute or chronic). They stated that (HRS) type 1 is characterized by a rapid decline in renal function, defined as a doubling of serum creatinine to a level > 2.5 mg/dl or halving a creatinine clearance to <20 ml/ min. within 2 weeks. The clinical presentation is that of acute renal failure, whereas, in type 2 (HRS) renal function deteriorates more slowly, with serum creatinine increasing to >1.5 mg/dl or a creatinine clearance of <40 ml/min. The clinical presentation is that of stable renal failure in a patient with refractory ascitis.

The observed results revealed also that the administration of antox (10 ml/kg b.wt.) daily for 2 weeks to rats suffering from cellular toxicity induced by anti-cancer drug sorafenib caused a significant decrease in serum urea and creatinine compared with sorafenib non-treated rats. These results agree with that recorded by Bokemeyer et al. [41] and Gaedeke et al.[42]. They observed that silibin prevented cisplatin –induced glomerular and tubular nephrotoxicity in rats as measured by (BUN), creatinine and fibronectin and histopathological changes in renal tubules. The antioxidative and scavenging free radicals and inhibiting lipid peroxidation might be probably the cause of decreased urea and creatinine concentration.

The significant decrease in urea, uric acid and creatinine in rats treated with either antox compared with sorafenib treated group seems conceivable to be due to their antioxidative and antiperoxidative effect as previously mentioned. Antox is an antioxidant drug composed of selenium, vitamin A acetate, ascorbic acid and vitamin E, and this agree with Oz et al.[43] who reported that antox was used in therapy of various liver diseases and kidney injury.

CONCLUSION

The present study indicates that administration of antox has the ability to reduce effects of sorafineb hepato-renal intoxication under experimental conditions. These signs of therapeutic effects might be correlated with the scavenging effect of sorafineb either individually or in presence of other antioxidant like (vitamins C, A and E in antox) and/or the enhancing ability for the antioxidant defense system.

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