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Original Article

SUPPRESSION OF N-NITROSODIETHYLAMINE INDUCED OXIDATIVE RENAL TOXICITY BY SULPHATEDPOLYSACCHARIDE AND AQUEOUS EXTRACT OF *ULVA LACTUCA* IN RATS

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ABSTRACT

Objective: The ancient tradition habits of consuming seaweeds in Asian and Middle East countries have been made a key part of their diet, and as an antioxidant, sulphated polysaccharides have made a large number of researches as one of the ocean's greatest treasures. Therefore, the scope of the present study was conducted to investigate the chemopreventive actions of sulphated polysaccharides and aqueous extract of *Ulva lactuca* on N-nitrosodiethylamine (NDEA) promoted by phenobarbital (PB) induced renal toxicity in male albino rats.

Methods: Adult male albino rats were divided into four groups. Group B received a single dose of NDEA intra peritonealy (200 mg/kg body weight) for 2, 12 and 24 weeks to induce renal toxicity. Groups (C& D) simultaneously received (50 mg/kg body weight) sulphated polysaccharides and aqueous extract of *U. lactuca*, respectively by oral gavage. Further, the B, C& D groups received PB (0.05%) in drinking water after two weeks of NDEA administration along the entire period of study. Saline (0.9%) treated control group (A) was also built-in. Several *In vivo* enzymatic antioxidant defense system like superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione-s-transferase (GST), myeloperoxidase (MPO), and non-enzymatic system like reduced glutathione (GSH) were evaluated to determine the reno-protective and antioxidant activity of *U. lactuca* extracts. The oxidative stress markers like lipid peroxidation (LPO) and nitric oxide (NOx) were also investigated as the products of NDEA metabolism.

Results: NDEA followed by PB provoked renal and nephrotoxicity was evident from the decreased activity of almost antioxidant enzymes (SOD, CAT, GST, GR and MPO) and increased oxidants (LPO & NOx) in renal tissues. A significant rise in the levels of malondialdehyde (MDA) throughout the entire period of study along with the striking decline of the above enzymic and non-enzymic antioxidants marks the severity of oxidative stress in NDEA-induced rats. However, concomitant treatment with sulphated polysaccharides restored the above antioxidant enzyme levels in the kidneys near to normal better than the aqueous extract, and exhibited a significant dose dependent protective effect against NDEA induced nephrotoxicity, which might be attributed to the antioxidant properties of the extracts.

Conclusion: The present study provides evidence, for the first time, that sulphated polysaccharides exert a chemoreceptive significant effect on NDEA-initiated nephrotoxicity through induction of apoptosis. In addition, the restoration effect of enzymic and non-enzymic antioxidant to their normal levels. The study justified the ethno-medicinal use of sulphated polysaccharide extract for protection against renal toxicity.

Keywords: *Ulva lactuca*, Renal toxicity, N-nitrosodiethylamine, Antioxidant enzymes, Apoptosis.

INTRODUCTION

N-nitrosodiethylamine (NDEA) is a potent environmental carcinogen, widely present in tobacco smoke, various foodstuffs, such as cured and fried meats and in a number of alcoholic beverages [1]. It is the most common screening model to characterized system of xenobiotic-induced hepato carcinogenesis, and is a common screening model to speculate the protective effects of drugs with antioxidant properties [2].

Nitrosamines have been initially gained a regulatory concern due to the generation of reactive oxygen and nitrogen species (ROS/RNS) that leading to oxidative stress, alter the antioxidative defense system in different organs and cellular injuries, which may be one of the factors in cancer etiologies [3-6]. The vast majority of tumors are suggested to the potential involvement of these compounds, and their precursors in human surroundings together with the possibility of their endogenous formation in the body [7, 8]. NDEA promoted by phenobarbital (PB) is frequently used to induce nephrotoxicity in rats [6]. and has been suggested to cause oxidative stress and cellular injury due to involvement of free radicals [9, 10].

Recently, considerable researches have been carried out looking for non-toxic naturally occurring or synthetic compounds to control cancer incidence. In this regard, several naturally occurring compounds isolated from marine algae have been tested with proved efficacy against several cancer models. The antioxidant activity of these isolated compounds is attributed in scavenging activity against superoxide and hydroxyl radicals [11,12]. The polysaccharide compound, isolated from green macro alga *U. lactuca*, has been widely recognized as effective tools to detect, and have a significant antiviral effect, reducing replication rates of a variety of strains of human and avian influenza viruses [13].

Moreover, it became rapidly apparent that the mechanisms of the pharmaceutical effect of biologically active polysaccharides on diseases have been frequently studied, and several isolated polysaccharides with various curative effects have been tested and recently applied in therapies.

Thus, the aim of the present study was to investigate the chemo preventive efficiency of sulphated polysaccharides and aqueous extract of *U. lactuca* against NDEA-induced renal carcinogenesis in rats. The protective effect of sulphated polysaccharides and aqueous extract on NDEA-induced renal toxicity was assessed by evaluating the enzymatic, non enzymatic antioxidants and DNA fragmentation assay.

MATERIALS AND METHODS

Drugs and chemicals

All the reagents, solvents and biochemicals used were of analytical grade, and were procured from Sigma Aldrich (St. Louis, MO) (Germany), Randox chemical company (Northern Ireland, UK), bio-Merieux chemical company (France), and Spinreact chemical company (Girona, Spain).

Seaweed collection and processing

The marine green algal species *U. lactuca* were collected in polythene bags from the intertidal regions of the Red Sea western coastal regions of Egypt, particularly from Marsa Alam(Lat. 25°04' 0.48'' N; Long. 34°54' 7.2'' E) and El-Qusair district (Lat.26°06' 54'' N; Long. 34°16' 58.08'' E). The seaweed was washed with sea water and fresh water to eliminate the contaminations, was then air dried in the shade, roughly cut and minced using a mechanical blender. The extraction process was carried out as follow:

The dried seaweed (100g) was rehydrated with 1:1 of distilled water and heated at 100°C for 1h, respectively. After centrifugation, each supernatant was precipitated with EtOH (3 volumes) and then freeze dried to give sulphated polysaccharide extracts. The precipitate was washed with distilled water and ethyl alcohol several times to eliminate salts and minerals and then dried (to get rid of any alcohol traces). The aqueous extract dose was prepared by addition 50mg of the powdered algae to 100 ml of distilled water and exposed to boiling for 15 minutes, then filtered and was orally given to the rats daily for 24 weeks. Polysaccharide sulphate was dissolved in boiling q-water and was orally administered for the entire time course.

Animal model

Adult male albino rats of Sprague-Dawley strain, initially weighing 90–100 gm, were purchased from the National Research Center, Dokki, Giza, Egypt and housed in a conventional animal facility. The animals were acclimatized under standard laboratory conditions of temperature 24±1°C, relative humidity 55±5% and a 12 hour photoperiod, and were housed in stainless steel cages (6-8 rats per cage) for 2 weeks before the initiation of the experiment. Further, animals were kept under observation for that period before commencement of the experiment to exclude any inter-current infections. During the entire period of study, the rats were supplied with a semi-purified rat pelleted basal diet and water *adlibitum*. The experimental animals were handled according to the Canadian Committee for Animals Use and Care guidelines [14].

Experimental regimen

The animals were randomly divided into four groups of 6 rats each as follows: Group A rats was served as saline treated vehicle control. Group B rats were administered a single necrogenic, intra peritoneal (i. p.) Injection of NDEA (Sigma-Aldrich, St. Louis, MO) (200 mg/kg body weight dissolved in 0.9% saline) at 9 weeks of age. Following 2 weeks of NDEA injection, i.e. after week 11, all the NDEA-initiated rats were supplied with phenobarbital (PB Sigma-Aldrich) in drinking water (0.05%) 6 days a week and continuing thereafter till the end of the study. Group C was given sulphated polysaccharide (50 mg/kg b.wt) concomitantly along with NDEA and PB for 24 weeks. Group D was given an aqueous extract of *U. lactuca* (50 mg/kg b.wt) concomitantly along with NDEA and PB for 24 weeks. The body weights of the animals from each group were recorded every week. The animals were maintained for 2, 12 and 24 weeks. At the end of the experimental regimen the animals were sacrificed under light ether anaesthesia at overnight fasting state. Trunk blood samples were collected from the jugular vein in non-heparinized tubes and allowed to coagulate at room temperature, then centrifuged at 3000rpm for 20 min. The non-haemolysed supernatant sera were immediately removed and stored at-20°C for subsequent biochemical analysis.

After sacrification and dissection, kidneys were immediately removed and rinsed in ice cold physiological saline (0.9%) and divided into two parts:

-1st part is kept in 10% neutral buffered formalin for histopathological investigation at histology unit, National Cancer Institute, Cairo, Egypt.

-2nd part is homogenized in phosphate buffer (10 mM, pH 7.4) which contained potassium chloride (1.15%KCl) and (1.15% EDTA) and centrifuged at 3000rpm for 20 min. The aliquots of the tissue homogenate were processed for the assessment of the following: Malondialdehyde content (MDA), reduced glutathione (GSH) level, catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione-S-transferase (GST) and nitric oxide (NO) activities.

Evaluation of oxidative stress and renal antioxidant status

Lipid peroxidation (LPO) was assayed according to the modified method of Preuss et al. (1998) [15], by measuring the level of malondialdehyde (MDA) formed in the kidney tissue. The activity of superoxide dismutase (SOD) was assayed according to the method of Marklund and Marklund (1974)[16] based on the amount of enzyme required to give 50% inhibition of rapid auto-oxidation of pyrogallol in aqueous solution. Total Reduced glutathione (GSH) was estimated according to the method of Beutler et al. (1963) [17]. This method was based on the reaction of reduced GSH with 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) to give a compound that measures and absorbs at 412 nm. The activity of Glutathione-S-transferase (GST) in the kidney tissue homogenate was assayed by the method described by Habig et al. (1974) [18]. One enzyme unit is defined as the amount of enzyme producing 1 mmol of CDNB-GSH conjugate/min that detected at 340 nm. Glutathione Reductase (GR)was assessed according to the method of Goldberg and Spooner (1983) [19] and its activity were assessed by the reduction of GSSG (oxidized glutathione) in the presence of NADPH. The activity of catalase (CAT) in the tissue homogenate was assayed as described by Cohen et al. (1970) [20]. The activity of Myloperoxidase (MPO) was assayed on the basis of Manoranjan and Mishra (1976) [21]and its activity were assessed in terms of purpurogallin formation.

Assessment of Nitric oxide (NO)

Nitric oxide is unstable in presence of molecular oxygen. With a half-life of approximately 3-5 seconds and is rapidly oxidized to nitrate and nitrite totally designated as NOx. The measurement of its level provides a reliable and quantitative estimate of nitric oxide output *In vivo* on the basis of Montgomery and Dymock (1961) method[22]. NO is produced in trace quantities by neurons, endothelial cells, platelets and neutrophils in response to homeostatic stimuli, but is scavenged rapidly (t½ = 4 sec).

$$\text{Nitrite concentration } (\mu\text{mol/L}) = A_{(\text{sample})}/A_{(\text{standard})} \times 50$$

Apoptotic DNA Fragmentation analysis

This assay was measured as described by Mahvash and Azra (2000)[23] method. Suspend 1 ml homogenate in 500 µl lysis buffer. Centrifuge at 10000rpm/20 min. Suspend 200µl of both supernatant and pellets separately in 200µl of TCA. Centrifuge at 5000rpm/10 min. Add both supernatant and pellets individually to 1 ml DPA. The supernatant is used for fragmented DNA. Boil in a water bath for 10 min and then cool in ice, then measure the formed blue colour at a wavelength (575-600 nm) against blank (DPA) on double beam spectrophotometer.

$$\text{Fragmented DNA (\%)} = (\text{ODs}/\text{ODs}+\text{ODp}) \times 100$$

Expression of results and statistical significance

Data were analysed statistically with computerized Graph Pad Prism program (Graph-Pad Prism 6.01 software for windows) using one-way analysis of variance (ANOVA) to test the significance of differences along the time, between treatments and the control. Tukey-Kramer multi-comparison test was also used to evaluate the changes among different time intervals within a variable using the error calculated from ANOVA. Significant differences among means were estimated at $P<0.05$ for all the values. The obtained results were presented as mean ± standard error of means (S. E. M.).

RESULTS

Table (1) shown that administration of NDEA and PB significantly declined the levels of renal MPO, GR and SOD by (-22.5%,-73.3% and-75.9%, $P<0.0001$) for MPO, (-52.5%,-70.7% and-80.1%, $P=0.0007$) for GR, and (-58.6%,-48% and-73.5%, $P<0.0001$) for SOD at the entire time points 2, 12 and 24 weeks NDEA post-dosing, respectively. However, sulphated polysaccharides significantly increased their levels by (29.1%, 118.9% and 128.8%) for MPO, (82%, 136.6% and 215.8%) for GR, and (217.5%, 116% and278.5%)

for SOD greater than the aqueous extract of *U. lactuca* at the entire time points, respectively. The catalase activity was declined only at 12 and 24 weeks of NDEA post-dosing by-64% and-70.6%, respectively. However, its activity rises up to 74.6±8.9 from 34.2±3.3U/mg tissue at 2 weeks. The aqueous extract seems to be more promised than sulphated polysaccharide particularly at 2 and 24 weeks by 21.4% & 376.1%, respectively. Most notable of these parameters, GST level decreased after 2 weeks from 5.33±0.09 to 4.18±0.2U/gm tissue, and then upregulated again at 12 and 24 weeks of NDEA-postdose by 2% and 9.45%, respectively. However, the aqueous extract of *U. lactuca* abrogates the decreased level of GST(4.18±0.2 to 5.74±0.7U/gm tissue) and (5.67±0.05 to 5.88±0.09U/gm tissue)better than polysaccharide sulphate at 2 and 24 weeks of NDEA post-dosing, respectively.

Table 1: Effect of oral administration of sulphated polysaccharide and aqueous extract of *U. lactuca*on renal concentrations of enzymatic antioxidant activities of nephrotoxic rats

Parameter	Time (week)	Group (A)	Group (B)	Group (C)	Group (D)	P_{value}
	2	0.626±0.04[a]	0.489±0.04[ab]	0.62±0.08[a]	0.49±0.07[a]	ns
MPO (U/mg)		--------	(-22.5)	(29.1)	(2)	--------
	12	1.39±0.1[a]	0.375±0.02[a]	0.814±0.09[a]	0.636±0.09[a]	0.0004
		--------	(-73.3)	(118.9)	(70.2)	--------
	24	2.45±1.5[a]	0.594±0.01[b]	1.35±0.09[b]	1.159±0.05[b]	ns
		--------	(-75.9)	(128.8)	(94.9)	--------
	2	402.5±0.6[a]	191.1±22.7[b]	347.8±12[b]	277±15.8[a]	<0.0001
GR		--------	(-52.5)	(82)	(44.9)	--------
(U/gm)	12	405.4±1[a]	118.4±0.9[a]	280.3±8[a]	260.8±6.5[a]	<0.0001
		--------	(-70.7)	(136.6)	(120.2)	--------
	24	410.4±0.5[b]	81.3±2.7[a]	257±12[a]	212.8±21.8[a]	<0.0001
		--------	(-80.1)	(215.8)	(161.5)	--------
	2	1.38±0.09[a]	0.57±0.07[ab]	1.81±0.07[a]	1.58±0.05[a]	<0.0001
SOD (U/gm)		--------	(-58.6)	(217.5)	(177.1)	--------
	12	1.56±0.8[a]	0.81±0.05[b]	1.75±0.06[a]	1.50±0.07[a]	<0.0001
		--------	(-48)	(116)	(85.1)	--------
	24	1.59±0.1[a]	0.42±0.08[a]	1.59±0.08[a]	1.39±0.06[a]	<0.0001
		--------	(-73.5)	(278.5)	(230.9)	--------
	2	34.27±3.3[a]	72.9±12.4[b]	74.64±8.9[a]	88.52±4.1[ab]	0.0009
CAT (U/mg)		--------	(112.7)	(2.3)	(21.4)	--------
	12	111.59±13.8[b]	36.82±7.2[ab]	110.26±6[b]	71.99±5.1[a]	0.001
		--------	(-64)	(199.4)	(95.5)	--------
	24	90.22±11.9[b]	26.44±2.4[a]	56.72±5.6[a]	125.9±16.6[b]	0.008
		--------	(-70.6)	(114.5)	(376.1)	--------
GST (U/gm)	2	5.33±0.09[ab]	4.18±0.2[a]	4.86±0.31[a]	5.74±0.3[a]	0.003
		--------	(-21.5)	(16.26)	(37.3)	--------
	12	5.83±0.11[b]	5.95±0.07[b]	5.94±0.01[b]	5.50±0.15[a]	ns
		--------	(2)	(-0.16)	(-7.5)	--------
	24	5.18±0.14[a]	5.67±0.05[b]	5.86±0.07[b]	5.88±0.09[a]	0.001
		--------	(9.4)	(3.3)	(3.7)	--------

-In the column, mean values with the same superscript letters are non-significant, P>0.01,otherwise are significant, P<0.01, -Data are expressed as mean±S. E. M (n=6), -% changes are calculated by comparing NDEA-intoxicated group with normal one and intoxicated treated groups with NDEA-intoxicated group, -(A) normal group, (B) NDEA+PB, (C) NDEA+PB+sulphated polysaccharide, (D) NDEA+PB+aqueous extract of *U. lactuca*.

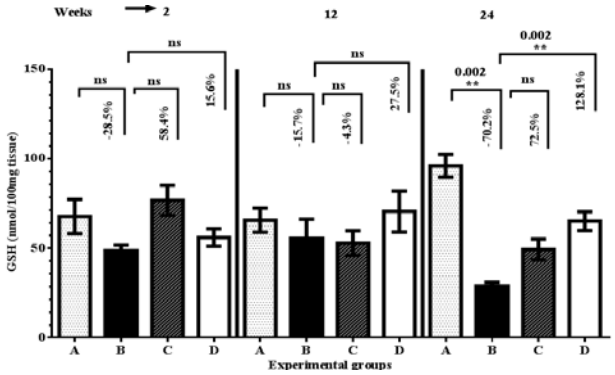

Fig. 1: Effect of sulphated polysaccharides and aqueous extract of *U. lactuca* on the renal GSH level.(A) normal group, (B) NDEA+PB, (C) NDEA+PB+sulphated polysaccharide, (D) NDEA+PB+aqueous extract of *U. lactuca*

Administration of NDEA and PB (group B) decreased the levels of renal GSH from 67.8±9.5 to 48.5±3.3, 65.7±6.7 to 55.3±10.9 and96.1±6.3 to 28.6±2.3 nmol/100mg tissue at 2, 12 and 24 weeks of NDEA post-dose, respectively. However, sulphate polysaccharide supplements significantly up regulated GSH level by 58.4% and 72.5% (76.8±8.3 vs 48.5±3.3 and 49.3±5.8 vs 28.6±2.3 nmol/100 mg tissue at 2 and 24 weeks of NDEA post-dose, respectively, so it seems to be better than aqueous extract of *U. lactuca*, although the later up regulated the GSH level at 12 and 24 weeks greater than the former by 27.6%.

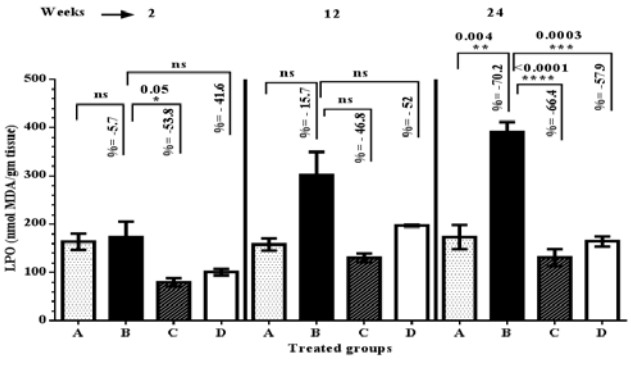

Fig. 2: Evaluation of MDA formation in renal tissues of treated rats

Administration of NDEA and PB significantly increased renal MDA levels in the group (B) rats along the entire periods of study. The MDA values of NDEA-treated group (B) in comparison to normal were(301.6±47.8 vs 166.6±16.8 and 390.5±20.9 vs 173.1±24.7 nmol MDA/gm tissue) at 12 and 24 weeks of NDEA post-dose), respectively. Otherwise, administration of sulphated polysaccharide has been shown a decline in MDA by-53.8%,-46.8% and-66.4% ((79.9±8.9 vs 173.1±32.3, 160.4±17.6 vs 301.6±47.8 and 130.9±17.6 vs 390.5±20.9 nmol MDA/gm, (P= ns, 0.02 and<0.0001)) at 2, 12 and 24 weeks, respectively. These findings indicate that sulphated polysaccharides seem to be more efficient than aqueous extract of *U. lactuca.*

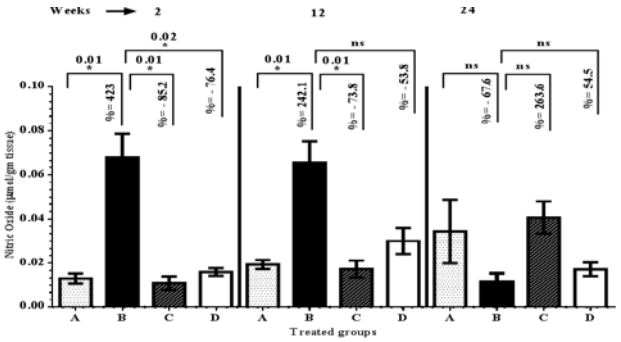

Fig. 3: Evaluation of Nitric Oxide (NO) bioavailability in renal tissues

Nitric oxide levels were significantly increased in renal tissues of NDEA-intoxicated rats (0.068±0.01 and 0.065±0.009 µmol/gm tissue, P<0.01, respectively) at 2 and 12 weeks post-dose, and then non-significantly declined again at 24 weeks). Otherwise, administration of sulphated polysaccharides significantly (P<0.01) decreased NO level by-85.2% and 73.8% (0.01±0.007 vs 0.068±0.01 and 0.017±0.003 vs 0.065±0.009 µmol/gm) at 2 and 12 weeks NDEA post-dose, respectively.

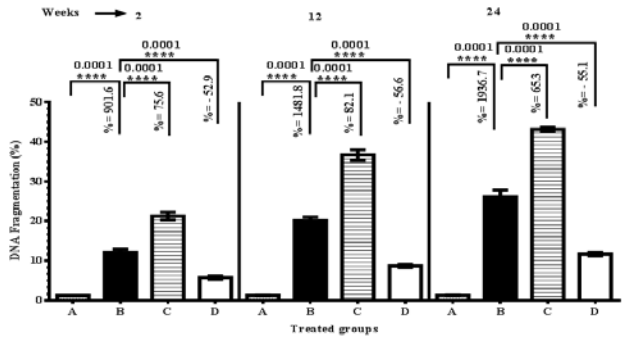

Fig. 4: Qualitative assay of DNA fragmentation test

(Degradation of nuclear DNA into nucleosomal units is one of the hallmarks of apoptotic cell death. It occurs in response to various apoptotic stimuli in a wide variety of cell types).

Based on fig. (4) we can conclusively say that rats-NDEA dosing causes moderate significant increase in apoptotic-DNA damage of renal cells vin comparison to normal rats. Furthermore, the adverse effect of NDEA and the tested agents on renal DNA damage-based apoptosis of various groups at different time points (2, 12 and 24 weeks) are illustrated. Data are showing that normal rats induced moderately non-significant increase of renal apoptotic-DNA damage a long time, while significant (P<0.0001) effect for intoxicated treated ones. The deleterious effect of NDEA caused moderately significant (P<0.0001)increases of apoptotic-DNA damage as a reverse action of the cell after 2, 12 and 24 weeks, respectively, as compared with their normal group. Otherwise, NDEA-intoxicated treated groups with sulphated polysaccharide seem to be more efficient than the other treatment in the abrogation of DNA to be transcribed and arrest the cell before entering mitosis by elevation of apoptosis by 75.6, 82.1 and 65.32% at the selected time points of study.

DISCUSSION

It was well known that generation of reactive oxygen species following metabolic activation of derived compounds from nitrosamines could be an important cause of their harmful toxicity, which capable of initiating damage in the cells [5,6,24,25]. Excessive cellular ROS production and deficiency of an antioxidant defense system might cause several pathological stresses to the cells and tissues of various organs such as liver, kidney, intestine, spleen, etc. These reactive species have been shown to induce DNA damage [26]. Undoubtedly, the kidney is considered a highly energetic organ and is a vulnerable organ in being susceptible to toxicity induction on the basis of chemical interference, and therefore relies on oxidative phosphorylation of ATP production. Thus, their functions would suffer from the reactive oxygen species.

Cellular ROS are continuously generated as a result of NDEA and other xenobiotics administration; causing oxidative stress that seriously damaged the biological systems through several reactions such as altering biochemical compounds, chromosomal instability, eroding cell membranes and creating mutation, which are involved in multisteps of carcinogenesis, i.e. initiation, promotion and progression [27]. There has been scientific evidences reported that LPO plays an important role in carcinogenesis induced by NDEA [28], so consider the most important marker of oxidative stress because it is the source of ROS generation [29]. MDA has long been used as a biomarker of LPO and oxidative damage, and the high levels of MDA reflect the enhancement of the LPO [30]. Interestingly, the current study has been shown that the level of MDA was significantly elevated after NDEA and PB administration along the entire periods of study (2, 12 and 24 weeks). However, the rats treated with sulphated polysaccharides displayed a manifest restoration in the level of MDA compared with that of NDEA-PB group and seems to be more efficient than aqueous extract of *U. lactuca*. The observed reduction in the level of LPO in the sulphated polysaccharide-treated rats was presumably attributed to the increase of antioxidative capabilities of these polysaccharides. Our study also provides insight attenuation and renoprotection by sulphated polysaccharide and aqueous extract of *U. lactuca* on the toxicity of NDEA and PB, where the nitric oxide was significantly elevated at 2 and 12 weeks of NDEA post-dose, due to generation of oxygen free radicals, which in turn react with nitric oxide to produce peroxinitrite. Peroxinitrite is a potent oxidant that further decreases nitric oxide bioavailability [31,32]and results in more tissue injury, which supports the current study, especially at 24 weeks of NDEA post-dose, but this attenuation might relate to the sulphated polysaccharides greater than the aqueous extract.

In view of the severity of diseases caused by environmental and occupational exposures to variety of carcinogens, the objective of preventing cellular damage induced by ROS has drawn attention. Data obtained from various animal model studies indicated that the body has a lot of antioxidative defense system, including the non-enzymatic (mainly GSH) and enzymatic antioxidant defenses including (MPO, SOD, GR, GST, and CAT). In response to oxidative stress, GSH plays a major role in maintaining the normal reduced state of cells and abrogate the harmful effects of oxidative damage [33]. In addition, an active debate, GSH can effectively remove free radicals and other reactive species through non-enzymatic and enzymatic process via conjugation with GPx and GST [34]. GST locates in cytosol and has been widely recognized as phase II metabolizing enzyme that plays a crucial role in detoxification and excretion of xenobiotics[3]. In this regard, GST catalyzes the conjugation of the necleophilic pool (-SH) of GSH with different electrophiles and xenobiotics to form mercaptures[35,36]leading to elimination of xenobiotic-GSH conjugate [37]. In this reaction, the GSH eventually oxidized into GSSG, which can be reduced to GSH by

GR with the consumption of NADPH molecules to adjunct the control of peroxides and other free radicals [38]. In addition to GSH and GSH-related antioxidative enzymes, other important antioxidant enzymes like SOD and CAT also confers a considerable degree of protection and so takes a major role in the antioxidant defense system. Bishop et al. [39] studies observed that SOD can catalyze the dismutation of two superoxide radicals to H_2O_2 and O_2, which supported by CAT enzyme in transforming the peroxides to water, and thereby providing protection against ROS.

Sulphated polysaccharide of *U. lactuca* had a powerful antioxidative defense system according to the other results from scavenging activity of DPPH and superoxide radicals, and so minimized the intracellular oxidative stress by protection of lipid oxidation in rats bearing cancer in both liver and kidney. In this regard, we suggest that sulphated polysaccharides have immunomodulatory activities due to induction of considerable amounts of cytokine production [40,41]. José et al. [42] stated that polysaccharides have the ability to increase the glucose uptake in the cells responsible for the fuels for the oxidative phosphorylation, and thereby upregulate the levels of NADPH/NADP+ and enhancing the activity of CAT. Interestingly, CAT activity in the current study at 2, 12 and 24 weeks of NDEA-postdose was significantly restored upon sulphated polysaccharides and aqueous extract of *U. lactuca* administration.

The aqueous extract of *U. lactuca* seemed to be more efficient than sulphated polysaccharides, particularly at 12 and 24 weeks of NDEA-postdose, which might attribute to the total flavonoids and terpenoids in the aqueous phase. With the increased levels of NADPH using polysaccharides, GR activity also improves, and thereby increasing the levels of GSH that act as the substrate for GPx[42]. In the present study, NDEA resulted in a significant decrease in the level of renal GSH at 2 and 24 weeks of NDEA-post dose as well as the activities of antioxidation enzymes, including MPO, GR, CAT, SOD and GST along most of the entire study. The reduction in the activities of these enzymes may be resulted from excessive LPO during NDEA metabolism [28]. However, the effects of NDEA and PB were partially counteracted by sulphated polysaccharides and aqueous extract of *U. lactuca*, which suggests that elevation of almost antioxidant and non-antioxidant defense systems along the entire periods of study particularly GSH, which might be one of the important reno protective mechanism for polysaccharides against NDEA-induced renal toxicity.

Apoptosis is well known active process of programmed cell death that acts under control of molecular mechanisms and need energy to proceed [43,44]. Elmore [45] stated that apoptosis was used to describe the morphology of a distinct form of cell death and promotes the elimination of renal epithelial cells that describe acute and chronic kidney diseases. In the present study, DNA fragmentation was assessed to identify cells undergoing apoptosis. In this regard, the results had shown a very low order of DNA fragmentation in NDEA-exposed renal rat cells indicating apoptosis evasion. Upon sulphated polysaccharide treatment at 2, 12 and 24 weeks of NDEA-postdose, it showed a significant increases of DNA fragment formation greater than the aqueous extract of *U. lactuca*, which provides substantial evidence and simultaneously feasible in cell death induction by apoptosis and a subsequent retardation of transforming renal cells in NDEA-treated rats. Our data showing apoptosis induction during the experimental hepato carcinogenesis and nephrotoxicity in rats by sulphated polysaccharide phytoconstituents of different rare sugars like iduronic acid and aldobiouronic acid had previously identified [46].

CONCLUSION

In conclusion, the results of this study suggest a significant role of oxidative stress, and vasoregulatory nitric oxide in the pathogenesis of NDEA-induced nephrotoxicity. In addition, it could be inferred that renal toxicity, which was induced by NDEA and PB was effectively inhibited by sulphated polysaccharides better than the aqueous extract of *U. lactuca* except for CAT. Therefore, further studies are warranted to test high doses of ulvan sulphated polysaccharide alone or in combination with other antioxidants and/or anti-inflammatory drugs to prevent NDEA-induced renal toxicity.

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DISCLOSURE STATEMENT

The authors have declared no conflict of interest

ETHICAL STANDARD

The present study does not contain clinical studies or patient data.

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