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Modulatory Impact of Ebselen against Diethylnitrosamine Initiated Hepatocarcinogenesis in Rats

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ABSTRACT

Seleno-compounds have gained a significant consideration due to their efficiency as chemotherapeutic agents. The aim of the current work was to elucidate the impact of "Ebselen" on Diethylnitrosamine-initiated hepatocarcinogenesis in male albino rats. Hepatocellular carcinoma was initiated with oral supplementation of diethylnitrosamine (DEN] (15mg⁄ kg body-weight) for 2 months. Ebselen (5mg⁄ kg body-weight) was orally given to rats for 2 months after DEN-administration. Biochemical analysis includes assessment of liver functions (ALT&AST activities), kidney functions (urea and creatinine), determination of reduced glutathione (GSH) and malondialdehyde (MDA) contents in liver tissues [oxidative stress markers], alfa-fetoprotein (AFP) (tumor marker), besides Bcl_2 expression and caspase-3 activities (apoptotic markers). The level of some essential trace elements (iron, zinc, copper, calcium and manganese) was also measured. The results revealed the powerful capacity of ebselen in minimizing the changes induced by DEN verified by significant increment in the level of hepatic antioxidant GSH accompanied by a marked decline in MDA concentration (marker of lipid peroxidation). Also, ebselen-treatment decreases ALT & AST activities, urea and creatinine concentrations. The increase of AFP, Bcl_2 and the decrease of caspase-3 were less marked. The increase of Fe, Ca and Cu, the decrease of Zn and Mn were improved, compared to DEN-group. Histopathological examinations of hepatocyte cells were in accordance with the biochemical results confirming the potential role of ebselen in minimizing DNE-induced damage. Ebselen-treatment improved the harmful impacts initiated by DEN as it is an effective antioxidant and scavenger of free toxic radicals.

INTRODUCTION

Liver cancer is the first type of liver diseases in the world [1, 2]. Various researches have been performed to get the underlying molecular components for liver cancer, as well as finding potential treatment assays. Several trials have centered on creating or finding successful new chemoprevention procedures [3, 4]. Agents leading to liver cancer include acute viral diseases (HBV or HCV), aflatoxin and alcohol [5, 6], besides over-weight, diabetes, and cirrhosis [7].

Nitroso compounds were recognized since 1937 as carcinogenic. Diethylnitrosamine (DEN) was considered one of the strongest carcinogenic inducers in human beings [8]. It initiates hepatocellular carcinoma by the formation of alkyl DNA adducts, which produces genome precariousness, and inevitably leads to changes of preneoplastic or neoplastic cells, which has been considered as the key step in DEN-initiated cancer [9, 10]. DEN is recognized to induce irritations within the nuclear enzymes included in deoxyribonucleic acid (DNA) and is regularly utilized as a carcinogen to initiate liver tumor in experimental models.

Until now, there is no confirmed chemical or surgical treatment to prevent or limit the spread of cancer. So, more studies have been coordinated towards the strategies of chemoprevention by natural and non-natural products to diminish the spread of the disease. Chemo-prevention interfere with the characteristics of carcinogenesis as tumor cell development, restrain initiation of tumor and diminishes its severity [11]. Selenium could be a crucial

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component in human body with a preventive effect of a few forms of tumor [12], as well as restraint of inflammations, cardiac illness in addition to directing flow of the blood pressure [13]., selenium also plays a critical role in numerous metabolic pathways as antioxidant [14]. Seleno-compounds have the capacity to initiate cancer cell apoptosis via distinctive pathways according to the cellular type and depending on the chemical structure of the compounds [15]. Ebselen (2-phenyl-1,2-benzisoselenazol-3 [2H]-one) is a selenium-containing heterocytic cycle recognized to bound covalently to thiols to make seleno-disulfides responsible for its pharmacological impacts. Ebselen exhibits multi-pharmacological activities including impacts on apoptosis illness, cell differentiation, immune regulation, and neurodegenerative disease, with anti-bacterial, anti-fungal activity, detoxifying and anti-cancer activities [16]. Ebselen is a multifunctional component, so it is under clinical examinations and experimental investigations to elucidate its chemotherapeutic effect on several diseases as carcinogenesis. The current work aims to investigate the possible efficiency of Ebselen against DEN-initiated hepatocyte carcinoma by measuring oxidative stress markers, serum liver enzymes, kidney functions, AFP values, apoptosis markers and trace elements besides, histopathological investigations.

MATERIALS AND METHODS

Chemicals

Diethylnitrosamine (DEN) ($C_4H_{10}N_2O$ molecular weight 102.1350) was dissolved in a saline solution before administration [17] and Ebselen ($C_{13}H_9NOSe$ molar mass 274.17666 g/mole) was dissolved in distilled water before treatment [18]. All chemicals were obtained and purchased from Sigma–Aldrich, St. Louis, MO Company.

Experimental design

Forty male *Wistar* male albino rats (120–150 g) were left for one week to adapt to the surrounding conditions before starting the experiment. Animals were kept under standard conditions of ventilation, temperature, light, and humidity and were allowed free access to a standard pellet diet containing all the necessary nutritive elements. Food and water were available *ad libitum*. Rats were randomly divided into four groups as follows:

G I, untreated control; G II, rats were administrated with DEN orally at a dose of 15 mg⁄Kg body-weight for 2 months; G III, rats were treated with ebselen orally at a dose of 5 mg/Kg body-weight for 2 months; G IV, rats were administrated DEN as mentioned in GII, then treated with ebselen as mentioned in GIII. At the end of the experimental period [4 months] all animals were sacrificed by decapitation, and blood samples were collected in dry tubes, left for 1 hour at 25^0C, then centrifuged at 5000 rpm for 15 min to obtain the serum for the analysis of liver enzymes AST and ALT and kidney function parameters urea and creatinine. Liver samples were rapidly excised washed in ice-cold saline and dried. Liver tissues were separated into 2 parts: the 1st part was fixed in 10% formalin for histology analysis, and the 2nd part was homogenated in a saline solution for the determination of GSH, MDA, AFP, Bcl_2, caspase-3 and trace elements.

Biochemical procedures

The level of malondialdehyde (MDA) was determined as a product of lipid peroxidation in liver homogenate according to Yagi [19]. Reduced glutathione content (GSH) was determined according to Beutler *et al.*, [20]. AFP and caspase-3 were evaluated using ELISA kits. Expression of Bcl_2 by real time PCR. The activity of liver enzymes aminotransferase (ALT and AST) was evaluated according to Reitman & Frankel [21] and urea and creatinine were evaluated according to Fawcett & Scott [22]. Essential trace elements were evaluated by Atomic Absorbance Spectrophotometry according to Christian [23].

Histopathological examination

Autopsy examinations were taken from the liver of rats in the different groups, and were fixed in 10% formalin for 24 h. The samples were washed with tap water, and then serial dilutions of liquor (methyl, ethyl, and absolute ethyl) were utilized for dehydration. The samples were cleared in xylene and embedded in paraffin at 56°C in a hot air oven for 24 h. Paraffin-bees wax tissue blocks were arranged for segmenting at 4 µm with a sliding microtome. The tissue sections were collected on glass slides, deparaffinized, and were after that recolored or stained with hematoxylin and eosin [24] and examined utilizing light microscopy.

Statistical analysis

The values are displayed as mean ± SE. Data were analyzed utilizing one-way analysis of variance [ANOVA]. The level of significance between mean values was set at $p \leq 0.05$. All analyses were performed utilizing SPSS software.

RESULTS

Biochemical results

The current study examined the hepato-protective role of ebselen against the harmful impact of DEN on liver tissues. It was found that treatment of rats with ebselen (5 mg⁄ kg body-weight) for 2 months has not induced any changes or anomalies in the biochemical parameters. On the other hand, the oral administration of DEN to rats (15 mg⁄ kg body-weight) for 2 months has markedly increased the activity of serum liver enzymes ALT and AST by 170% and 159% ($p< 0.001$), respectively (Table 1); as well as intoxication of rats with DEN significantly increased serum levels of urea and creatinine by 43% and 53% ($p<0.01$) respectively, compared to the control group (Table 1). It was also observed that rats treated with ebselen after intoxication with DEN showed a significant improvement in the elevation of liver enzyme activities, and urea and creatinine levels, compared to DEN group (Table 1).

Regarding oxidative stress markers, the results showed that administration of DEN to rats induced a significant increase in the level of liver MDA by 52% $p<0.01$) (associated with a significant decrease in the content of reduced GSH by -33% ($p<0.05$), compared to the control group (Table 2). The expression of BCl_2 in the liver increased by 46% ($p<0.05$) and the activity of caspase-3 decreased by -60% ($p<0.01$), compared to the control group (Table 2). In addition, administration of DEN to rats provoked an increase in the content of the tumor marker AFP by 233% ($p<0.001$) (Table 2). Ebselen treatment after DEN-administration has significantly attenuated the increase of lipid peroxides and the decrease in the content of the antioxidant GSH, compared with the DEN-group. Furthermore, a significant improvement in the expression of BCl_2, the activity of caspase-3 and the level of AFP were noticed compared to DEN-group (Table 2).

The current results showed also that DEN-intoxication initiated a significant elevation in Fe, Ca and Cu levels by 204%, 65%, and 1664% ($p<0.001$) respectively as compared with the control levels (Table 3). Furthermore, DEN caused a significant reduction in the levels of Zn and Mn by -58%, and -85% ($p<0.001$), respectively when compared to the normal levels (Table 3). Ebselen treatment after the administration of DEN has significantly attenuated the variations in the levels of trace elements, compared to DEN group (Table 3).

Table (1): The activities of aminotransferases (AST and ALT), and the content of urea and creatinine in the serum of rats after different treatments

Parameters / Groups	ALT (U/L)	AST (U/L)	Urea (mg/L)	Creatinine (mg/L)
Control				
Mean ± S.E.	32.53±1.11	43.40±3.28	27.19±0.33	0.49±0.24
Ebselen				
Mean ± S.E.	35.38±2.71	38.02±2.9	23.58±0.95	0.45±0.10
Change %	9%	-12%	-13%	-8%
P1	>0.05	>0.05	>0.05	>0.05
DEN				
Mean ± S.E.	87.77±2.52	112.36±2.41	38.79±0.67	0.75±0.02
Change %	170%	159%	43%	53%
P1	<0.001	<0.001	<0.01	<0.01
DEN + Ebselen				
Mean ± S.E.	47.80±1.98	61.46±1.33	30.23±1.76	0.57±0.02
Change%	47%	42%	11%	16%
P1	<0.01	<0.01	>0.05	<0.05
P2	<0.001	<0.001	<0.05	<0.001

Data are expressed as mean ± SE. n = 8 for each group. P1: significance vs control group. P2: significance vs DEN group. P>0.05: Not significant. P<0.05: significant. P<0.01 highly significant. P<0.001: very highly significant.

Table (2): The level of malondialdehyde (MDA), reduced glutathione (GSH), Bcl_2, caspase-3 and alfa fetoprotein (AFP) in the liver of rats after different treatments

Parameters / Groups	MDA nmol/g tissue	GSH mg/ g tissue	Bcl-2 ng/g tissue	Caspase-3 ng/g tissue	AFP g/ml
Control Mean ± S.E.	58.40±1.64	48.52±1.79	6.22±0.32	22.50±0.88	21.43±0.14
Ebselen Mean ± S.E. Change % P1	60.91±1.48 4% >0.05	50.25±3.70 4% >0.05	6.00±0.41 4% >0.05	23.43±0.98 4% >0.05	20.04±0.59 6% >0.05
DEN Mean ± S.E. Change % P1	88.94±3.72 52% <0.01	32.29±1.45 -33% <0.05	9.05±0.22 46% <0.05	8.89±0.32 -60% <0.01	71.35±2.66 233% <0.001
DEN+ Ebselen Mean ± S.E. Change% P1 P2	68.50±2.14 17% <0.05 <0.001	39.72±2.17 -18% <0.05 <0.05	7.58±0.30 22% <0.05 <0.05	17.46±0.76 -22% <0.05 <0.05	36.00±1.65 68% <0.001 <0.001

Legends as table 1

Table (3): The level of essential trace elements in the liver of rats after diffe7rent treatments

Parameters / Groups	Iron µg/g tissue	Calcium µg/g tissue	Zinc µg/g tissue	Copper µg/g tissue	Manganese µg/g tissue
Control Mean ± S.E.	4.69± 0.47	8.39± 0.21	1.44±0.20	0.14±0.002	1.23±0.01
Ebselen Mean ± S.E. Change % P1	4.20± 0.74 -10% >0.05	8.33 ±0.30 -1% >0.05	1.45±0.32 7% >0.05	0.15±0.003 7% >0.05	1.22±0.03 -0.8% >0.05
DEN Mean ± S.E. Change % P1	14.28±0.82 204% <0.001	13.87±0.51 65% <0.001	0.61±0.20 -58% <0.001	2.47±0.002 1664% <0.001	0.18±0.02 -85% <0.001
DEN+ Ebselen Mean ± S.E. Change% P1 P2	7.24± 0.43 54% <0.001 <0.001	9.31 ±0.16 11% >0.05 <0.05	1.45±0.20 7% >0.05 <0.001	0.14±0.006 0% >0.05 <0.001	1.19±0.06 -3% >0.05 <0.001

Legends as table 1

Histological examinations

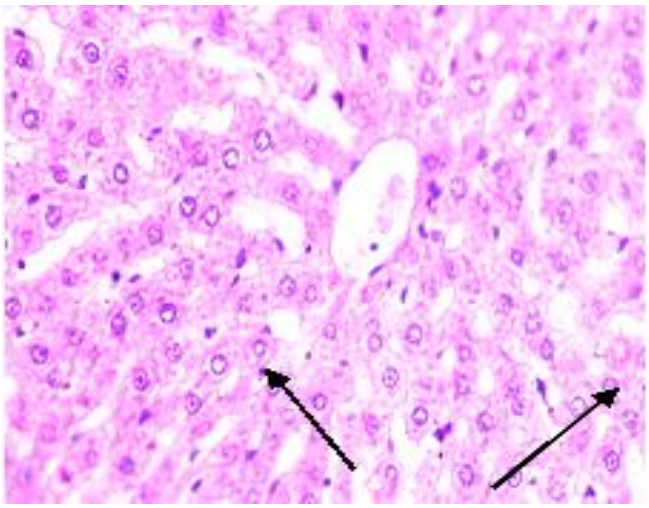
The most widely used grading system in HCC is the Edmondson and Steiner system. Grade I consists of small tumor cells, arranged in trabeculae, with abundant cytoplasm and minimal nuclear irregularity that are almost indistinguishable from normal liver tissue. Grade II tumors have prominent nucleoli, hyperchromatism and some degree of nuclear irregularity. Grade III tumors appear more pleomorphism than grade II and have angulated nuclei. Grade IV tumors have a prominent pleomorphism and often anaplastic giant cells [25]. The pathological response of HCC to therapy was graded as: 100% complete tumor necrosis, 51%-99% Partial tumor necrosis and <50% poor tumor necrosis [26].

Liver sections of both control and treated with ebselen groups showed normal hepatic lobules which consisted of polygonal cells arranged in cords with prominent round nuclei and eosinophilic cytoplasm vertical to central vein. Sinusoids are lined by a discontinuous layer of fenestrated endothelial cells with fine arrangement of Kupffer cells. The portal area revealed a normal histological structure (Fig. 1 A&B)

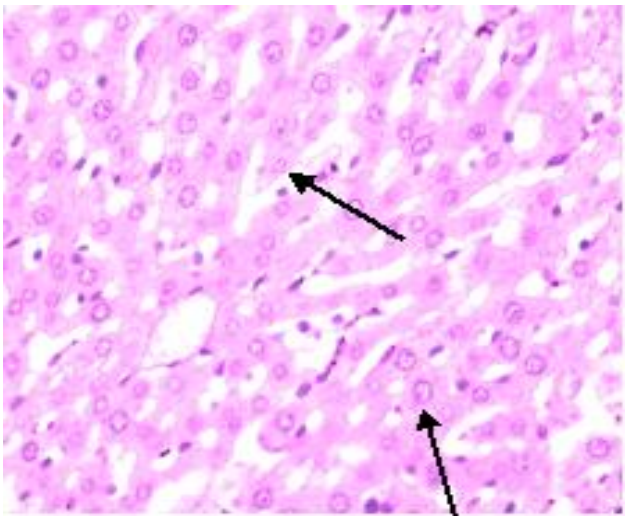
Histopathological changes of liver tissue sections of hepatocellular carcinoma- initiated rat group by DEN revealed a bridging fibrous connective tissue multiplication which is connected the central vein with portal area associated with disorganization of hepatic cords (Fig. 2A), also hyperplasia of bile duct with mononuclear cells accumulation basically lymphocytes and macrophages in the portal area was noticed (Fig. 2B). The neoplastic cells showed polyhedral to round hepatocytes with thick, centrally located vesicular nuclei (Fig. 2C). Tumors have prominent nucleoli, hyperchromatism and a few degrees of nuclear abnormality, deeply basophilic scanty cytoplasm and frequent mitotic figures Grade II. The hepatic lobule showed necrobiotic changes of hepatocytes with hyperplasia of Kupffer cells. Perivascular oedema and leukocytic conglomeration were also noticed (Fig 2D).

Carcinogenic rats group treated by ebselen after DEN revealed massive necrobiotic changes of hepatic carcinoma cells with hyperplasia of Kupffer cells. Partial [51%-99%] tumor necrosis was shown (Fig 3A). The necrotic hepatocytes showed nuclear pyknosis and deeply eosinophilic cytoplasm with leukocytic infiltration lymphocytes and macrophages. Numerous numbers of binucleated cells and numerous karyomegaly with peripheral condensation of its chromatin were observed (Fig 3B).

Another view showed mild degenerative changes of neoplastic cells including cellular swelling and few numbers of apoptotic cells associated with bridging fibrosis (Fig 3C). Pleomorphism of nuclei with clearly numerous karyomegaly with peripheral condensation of its chromatin and narrowing of hepatic sinusoids, hyperplasia of Kupffer cells and bile duct were noticed (Fig 3D).

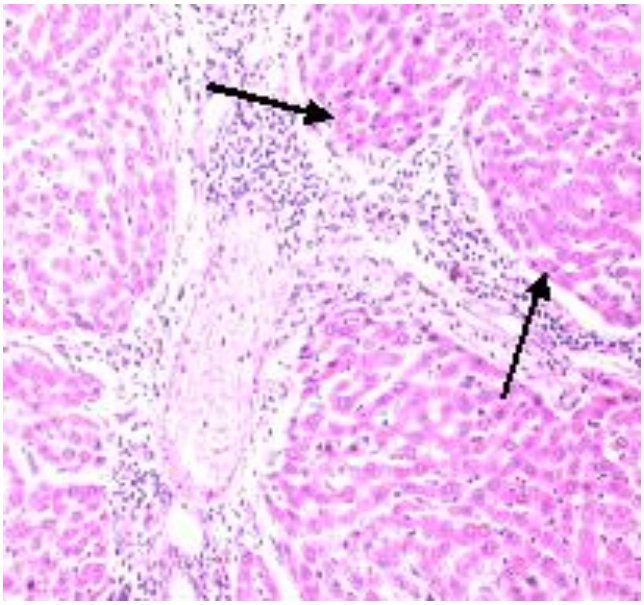


A- Normal histological structure of hepatic lobules **arrows.** [H&E x400]

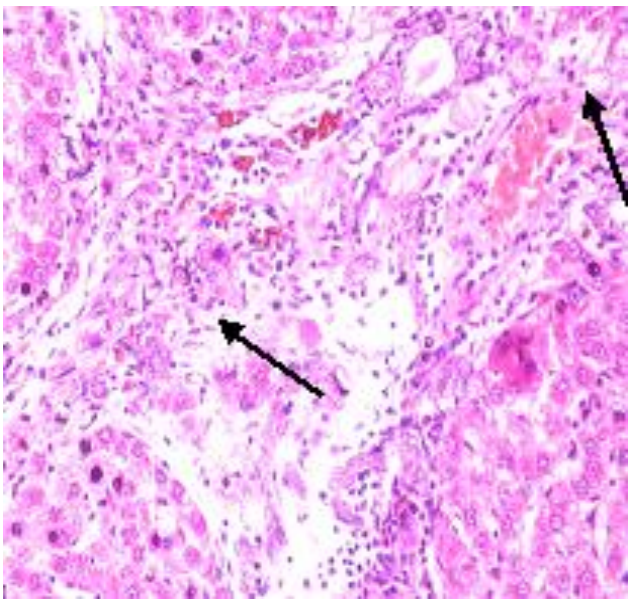


B- Normal large polygonal cells with prominent round nuclei and eosinophilic cytoplasm **arrows.** [H&E x400]

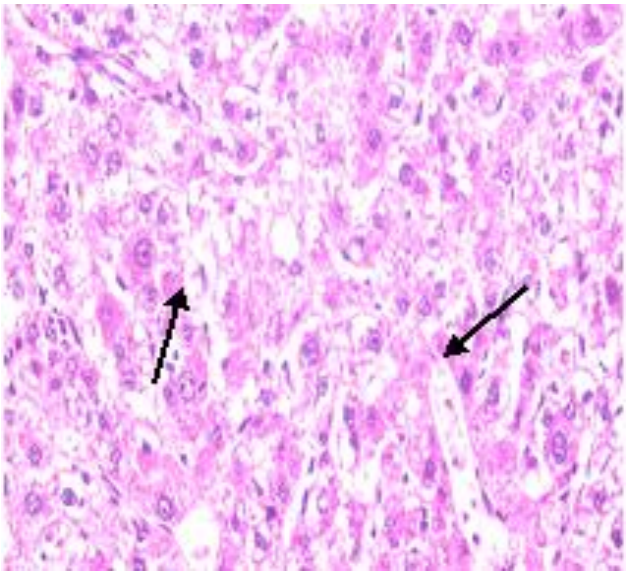
Fig. (1): Photomicrograph of hepatic tissue sections in control and ebselen-treated rats



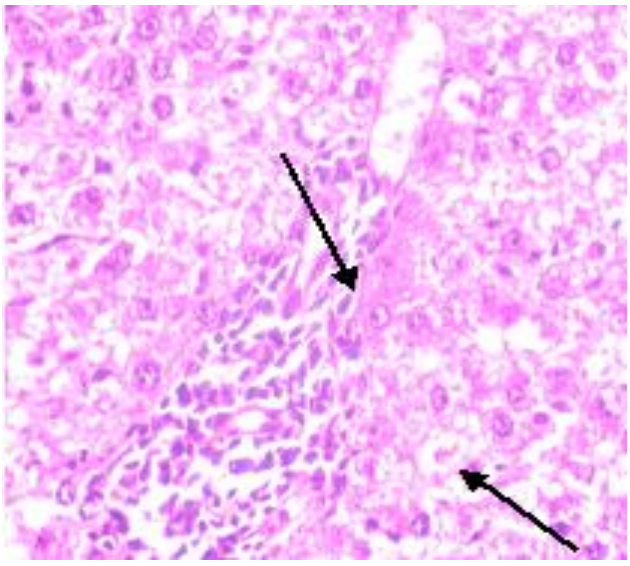
A- Bridging fibrous connective tissue proliferation and mononuclear cells infiltration mainly lymphocytes and macrophages **arrow** [H&E x200]



B- Hyperplasia of bile duct with mononuclear cells aggregation mainly lymphocytes and macrophages in the portal area **arrow** [H&E x200]

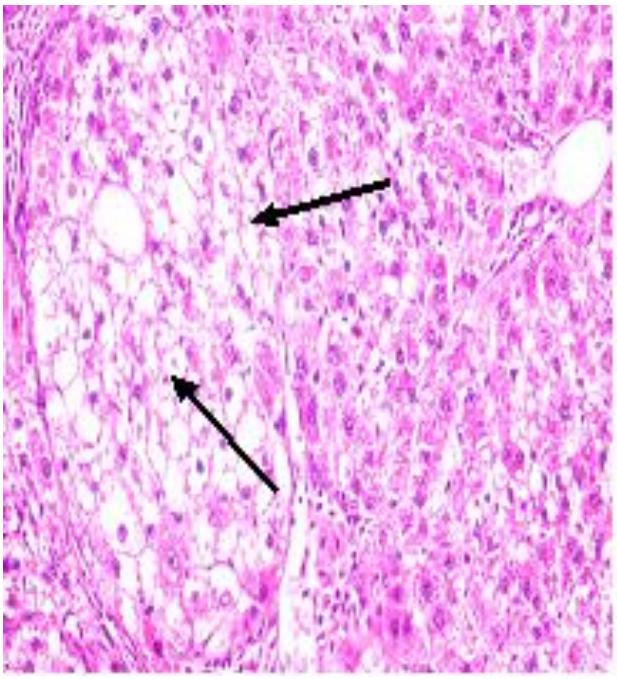


C- Polyhedral to round hepatocytes with dense, centrally located vesicular nuclei **arrow** [H&E x400]

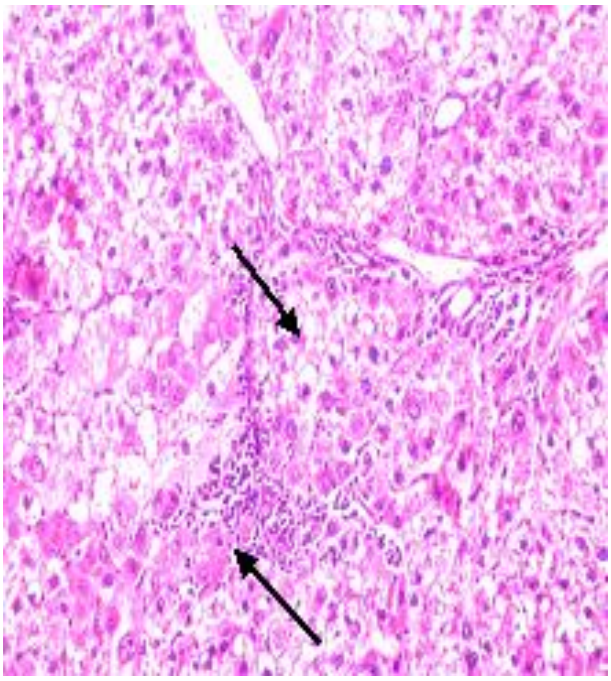


D- Perivascular oedema and leukocytic aggregation **arrow** [H&E x400]

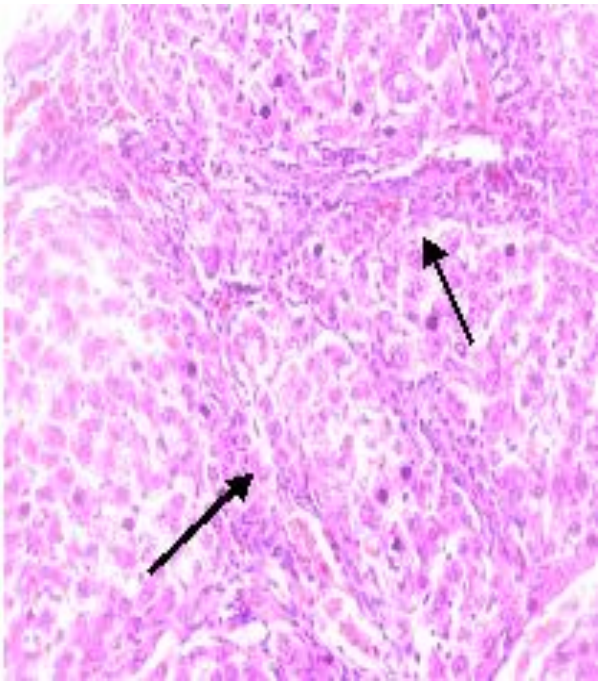
Fig. (2): Photomicrograph of hepatic tissue sections in DEN-administered rats



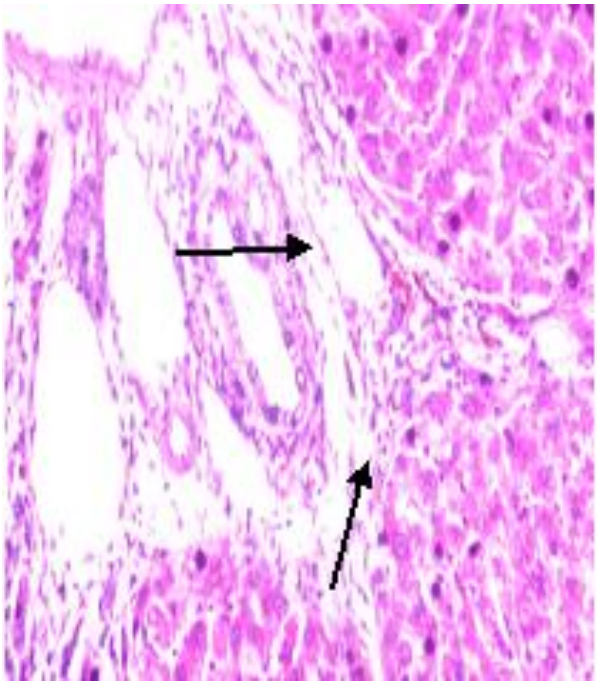
A- **P**artial 51%-99% tumor necrosis and hyperplasia of Kupffer cells. **arrow** [H&E x400]



B- Necrotic hepatocytes characterized by nuclear pyknosis and deeply eosinophilic cytoplasm with leukocytic infiltration lymphocytes and macrophages **arrow** [H&E x400]



C- Few numbers of apoptotic cells accompanied with bridging fibrosis **arrow** [H&E x400]



D- Narrowing of hepatic sinusoids, hyperplasia of Kupffer cells and bile duct **arrow** [H&E x400]

Fig. (3): Photomicrograph of hepatic tissue sections in rats treated by ebselen after DEN administration

DISCUSSION

Compelling studies have proposed that ebselen has the capacity to protect against oxidative stress or cytotoxicity due to its antioxidant properties [27]. The objective of the current work was to elucidate the influence of ebselen on DEN initiated hepatocellular carcinoma in rats with regard to oxidative stress markers, apoptotic markers, and some trace metals. Oral administration of DEN to rats (15 mg/kg body-weight) for 2 months has initiated a marked hepatotoxicity verified by significant modifications in liver enzymes (ALT and AST) as previously mentioned by Fahmy et al., [28] who reported that DEN induced toxic free radicals in hepatic tissue which leads to rupture of cells and layers causing release of these proteins from hepatocellular cytoplasm into the blood stream and initiated hepatocellular degeneration.

Furthermore, the current work revealed increment of serum urea and creatinine concentrations in rat models after DEN administration as compared to the control values. The results are in agreement with the previous findings reported by Qiarefuhan et al. [29] who illustrated that DEN, one of the foremost strong carcinogenic agents, generates in the body an excess of reactive oxygen species resulting in oxidative damage and cellular injury. DEN metabolized by cytochrome p450 initiates lipid peroxidation of cellular membranes, cause tissue rupture and renal dysfunction.

It is well known that DEN is a powerful carcinogenic compound that undergoes metabolic changes by cytochrome P450 enzymes, which cause an oxidative stress, driving to cytotoxicity and carcinogenicity [30]. The oxidative stress plays a central part in DEN initiated hepatotoxicity and generation of liver tumours [31] by causing DNA injury, and genetic instability. In the current study, concerning the oxidative stress markers after DEN administration, it was found that the level of liver MDA was significantly increased. This finding is in agreement with the previous reports by Joshi et al. [32] and Makalakshmi et al. [33]. This increase could be attributed to the production of an excess of free toxic radicals produced during the metabolism of DEN, and the interaction of free radicals with the polyunsaturated fatty acids in cellular membrane causing oxidative cell injury [34].

Additionally, the present work showed that the everyday administration of DEN for 2 months essentially diminished the content of liver GSH as compared to the control levels which corroborates previous studies, as DEN generates toxic free radicals causing cell death. Also, the decrease of GSH may be due to DEN initiated diminishing of Se levels. In this context, Lin et al. [35] reported diminished Se levels in patients with hepatocellular carcinoma. The depletion of GSH could be attributed to its increased utilization to detoxify the generated free radicals.

The results of the present work revealed that administration of DEN to rats at a dose of 15mg/Kg body-weight for 2 months induced a significant increment in AFP levels indicating hepato-toxicity. The results corroborate the previous findings by Taha et al. [36], who reported elevated serum AFP levels and confirmed that DEN may be a tumor initiator or a carcinogen operator. In the same line, Singh *et al.* [37] demonstrates that AFP plays an essential role in the control of tumor improvement, cell separation and in duplication of human hepatoma cells.

Apoptosis, also called programmed cell death, is a coordinated process that involves the activation of a group of cysteine proteases called “caspases” and a complex cascade of cytoplasmic and nuclear events that leads to molecular alterations and death of the cell [38]. Bcl2 are major anti-apoptotic proteins, that restrain apoptosis and thus improve cancer cell survival [39]. Bcl_2 is present at low levels in normal cells whereas is highly expressed in the tumor cells. In this line, Roberts & Huang [40] recorded high significant expressions of Bcl2 in cancer patients. In the current study, administration of DEN has induced a decrease in the activity of caspase-3 and an increase in the expression of Bcl_2 which substantiate that as in all tumors, apoptosis is suppressed in heptocarcinogenesis [41]. Animals treated with DEN initiated deactivation of caspase 3 and suppressed apoptosis which is a marker of DEN induced carcinogenicity and disabled apoptosis in harmed hepatocyte cells [39].

It is well known that DEN acts as a powerful carcinogenic compound that undergoes metabolic changes by cytochrome P450 enzymes, to produce responsive electrophiles which cause oxidative stress, driving to cytotoxicity and carcinogenicity [30]. Oxidative stress plays a central part in DEN initiated hepatotoxicity and generation of liver tumours [31] by causing DNA injury, and genetic instability.

There is an incredible effort towards decreasing the damage of DEN all over the world and to protect against its cytotoxicity and carcinogenicity. Among chemopreventive compounds which can provide defense against DEN through induction of the antioxidant system, ebselen is known as a potential chemopreventive agent against carcinogenesis.

The results of the current work indicate a positive and therapeutic effect of ebselen as follows; it is known that ebselen, a lipid-soluble organoselenium compound, exhibits glutathione peroxidase activity [GPx] and is considered as remarkable scavenger of reactive oxygen species [ROS] such as peroxynitrite [PN]. The rate of the reaction between ebselen and free radicals has been shown to be about three orders of magnitude higher than that of naturally occurring small molecules, such as cysteine, methionine and ascorbate. It is also known that ebselen effectively protect against lipid peroxidation induced by transition metal ions. However, the mechanism by which ebselen exerts its antioxidant activity and the importance of the cyclic selenazole moiety are still not well-understood [42].

In our view, the present work proved that the treatment with ebselen could attenuate DEN induced carcinogenesis where, ebselen treated rats showed a significant decrease in the liver and kidney functions in rats after administration of DEN, which could be referred to the ability of ebselen to reduce the toxicity in liver and kidney. Further, ebselen could reduce MDA levels with increasing in GSH content. Alternatively, biochemical improvement results occurred following ebselen treated on liver MDA and GSH contents as seleno-compounds have radioprotective effects on many organs were previously demonstrated [43]. As a component of GPx, it can scavenge intracellular free radicals directly or indirectly *via* its stimulatory actions on antioxidant enzymes activity and inhibitory actions on pro-oxidative enzymes activity [44].

Ebselen treated DEN intoxicated rats showed an increase in caspase-3 and a decrease in Bcl2. These findings are in agreement with Santofimia-Castano [45] and co-workers who reported that ebselen induced apoptosis in cancer cells by elevation in mitochondrial free radical production or as well as, ebselen has powerful cytotoxic activity against many different cancer cell lines. Meanwhile, Yang *et al.* [18] reported that ebselen may initiate apoptosis of cancer cells through a mechanism that involves intracellular thiol depletion and mitochondrial permeability transition.

Different genetic screening assays have proved that ebselen inhibits the activity of the divalent metal transporter 1 [DMT1] [45, 46]. DMT1, a proton-coupled metal-ion transport protein expressed in neurons is recognized to actively transport several different divalent cations such as Fe^{2+}, Zn^{2+} and Cu^{2+} [47]. Interestingly, ebselen has been known to be specific for inhibiting uptake of iron but not manganese [48]. It has been proposed that ebselen might influence the activity of specific factors included in the intracellular targeting of Fe. Further, it has been demonstrated that ebselen declined iron influx, reduced iron-initiated oxidant free radical production, caused diminishing in ferrous ions-initiated activation of cyclin-dependent kinase 5 [CDK5] and glycogen synthase kinase 3 beta [GSK3β] in human neuroblastoma [45]. However, ebselen not only interferes with Fe metabolism in cells, but also affects the intracellular calcium homeostasis. One study report that ebselen hampers an agonist-triggered increase in intracellular calcium by inhibiting inositol 1,4,5-trisphosphate [IP_3]-induced calcium release [49].

Ebselen has also been appeared to extend free cytosolic Ca^{2+} concentrations in rodent hippocampal astrocytes [50]. Moreover, ebselen has been found to corrupt mitochondrial work by stimulating Ca^{2+} discharge from mitochondria [51]. As calcium is included in a few signaling pathways, these comes about recommended that change of calcium homeostasis by ebselen might influence the downstream pathways. For illustration, ebselen restrains the kinase activity of in part decontaminated Ca^{2+}- and phospholipid-dependent protein kinase C [52]. Taken together, these think about demonstrate that ebselen targets iron and calcium homeostasis in the cells by balancing the transporter and capacity of these ions [53].

The abovementioned results are consistent with the result of the current work. The biochemical parameters were further confirmed by histopathological examinations of liver tissue. Histopathological examination of liver section of DEN treated rats showed that mononuclear cells aggregation (lymphocytes and macrophages) in portal area, fibrous connective tissue connected the control vein with portal area. In addition, polyhydral neoplastic cells and tumor have prominent hyperchromatism, nuclear irregularity, and hyperplasia of Kupffer cells. This is in agreement with [54, 55, 56]. Histopathological improvement was observed in the treatment with ebselen, where, showed binucleation of hepatocytes with frequent number of apoptotic bodies and clearly numerous karyomegaly with peripheral

condensation of its chromatin. Histologically, ebselen was able to improve most of the noticed histopathological alterations found in liver tissues after exposure to DEN. Additionally, the early preneoplastic alterations noticed in hepatic tissues during the induction of hepatocellular carcinoma were decreased and the remaining is some sort of inflammation, which is expected be recovered subsequently.

CONCLUSION

Ebselen compound is capable to handle distinctive forms of liver damage beginning from the inflammation, through fibrosis and even cancer which is considered a new hope for patients with liver diseases to get safe and effective treatment.

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