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Research Article
Ovothiol-A Ameliorates Renal Injury Induced by Bile Duct Ligation in Rats (Biological, Quantum-Chemical and Molecular Docking Study)

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Abstract

Background and Objective: Cholemic nephropathy is determined as Cholestasis-induced kidney damage. Ovothiols extracted from marine animals especially sea urchins display awesome antioxidant characteristics due to the thiol group's peculiar position on the imidazole part of histidine. This study investigated the protective effect of Ovothiol-A contra kidney dysfunction induced by bile duct ligation in rats. The chemical properties of Ovothiol-A are estimated by Quantum chemical approaches (DFT B3LYP/6-311G++), Frontier orbitals, MEP and Molecular docking. **Materials and Methods:** Thirty rats (male) were randomly allocated into three groups: BDL, Sham and BDL + 500 mg kg^{-1} Ovothiol-A. All rats were treated for 7 days. (Sham group and BDL group were administered with distilled water, another group was administered with Ovothiol-A). Oxidative stress biomarkers and kidney function tests were then evaluated in all groups of rats. Histopathological examination of the kidney was also evaluated. **Results:** Ovothiol-A decreased concentrations of urea, creatinine, uric acid, MDA, NO and increased the levels of CAT, GSH, SOD and GST. Microscopic examination of the kidneys of Ovothiol-A-treated rats revealed normal structure without noticeable pathological alterations. **Conclusion:** Ovothiol-A is a potent antioxidant substance that protects the kidney against toxicity results from BDL in rats.

Key words: Bile duct ligation, cholestasis, natural product, Ovothiol-A, renal injury, intrahepatic bulk, molecular docking

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

AKI which referred to acute kidney injury is a rife complication in patients who suffered from the last stage of hepatic injury which is an extremely dangerous condition[1]. Patient with obstructive jaundice has a higher risk of AKI[2]. Chronic liver disease is perilous and is one of the prime causes of death all over the world. Cholestasis is a lower flow of bile leading to the accumulation of intrahepatic bulk of the bile acids and other venomous compounds with the progression of pathology of the liver, including injury of hepatocellular cells and fibrosis[3]. Fibrosis is a presumptive response to continued injury of tissue and is associated with most chronic inflammatory diseases. Inflammation or injury of the liver occurred by hepatitis or alcohol or drugs, or impaired metabolism exhort apoptosis of hepatocytes then the hepatocytes apoptosis exhorts myofibroblast for regeneration and angiogenesis. Once the inflammation persists, liver regeneration stops and extracellular matrix proteins replace hepatocytes, thence fibrosis occurs[4]. The liver and the kidney are two main organs having a twofold role. Abstraction of many potentially venomous xenobiotics, including endogenous metabolites, toxins and drugs is a master role. In almost all clinical conditions patients who suffer from liver cirrhosis may hit kidney (renal) failure. BDL which is Bile duct ligation is a renowned technique for causing cholestatic injury and periportal biliary fibrosis. The bile duct's obstruction evokes increasing mild inflammation, biliary pressure and secretion of cytokine by biliary epithelial cells, consequently obstetrics cholestasis. These results in the proliferation of biliary epithelial cells, an increment in the expression of the fibrogenic marker and cumulation of T-cells and B-cells in portal tracts generating ROS and liver damage[5]. Cholestasis is the frailty of bile flowing due to biliary canal occlusion or deterioration of bile acid sucking, conjugation, or secretion. Bile duct ligation is set apart by increment systemic oxidative stress and damage to central organs, like the intestine, brain, kidney, liver and heart[6]. It was mentioned that ARF which referred to acute renal failure coming as a result of common bile duct occlusion remains prime clinical trouble[2]. Tubular epithelial injury represents a remarkable reason for kidney failure in patients suffering cholestasis or any advanced liver disease[7]. The need to evolve more efficacious products and benefits for humanity to remedy or overcome almost all of the confirmed drugs' side effects has induced a gigantic interest from the nutraceutical and pharmaceutical industrial field to discover newfangled natural substances derived from less-explored resources. In recent years, there has been a surge in interest in the marine environment which is distinguished by higher biodiversity in comparison to earth[8]. Nowadays several natural products come from marine sources, having a large number of approved pharmaceutical drugs that are now in clinical use[9]. There is an insistent need to evolve antifibrotic treatments that can prohibit or even in verse kidney dysfunction caused as a result of liver diseases as it represents a worldwide health problem linked with high mortality and morbidity. Sea urchin is a conventional paradigm organism in developmental biology and it contains plentiful pharmaceutical products that can be extracted from it. Ovothiols extracted from marine animals especially sea urchins display awesome antioxidant characteristics due to the thiol group's peculiar position on the imidazole[10].

In sea urchins, Ovothiol-A's role has been related to the detoxification of hydrogen peroxide generated during fertilization[11,12]. Ovothiol-A, purified by sea urchin eggs, was an effective treatment for in vitro liver fibrosis and induced autophagy in the hepatocarcinoma cell line of humans[13]. So the present study aims for investigating the protective effect of Ovothiol-A contra kidney dysfunction induced by bile duct ligation in Wistar rats.

MATERIALS AND METHODS

Study area: The experiment was performed at Cairo University (Egypt) between January-March, 2022.

Chemicals and reagents: All solvents and chemicals were obtained from the Aldrich chemical company and they were of good analytical grade.

Molecular DFT calculation of Ovothiol disulfide (OTDS): DFT which referred to Density Functional Theory calculations was done to investigate the equilibrium geometry of Ovothiol disulfide OTDS at the B3LYP/6-311G++ (dp) level of theory using the Gaussian 09 program[14].

Computational protein-ligand docking simulation: Docking calculations were achieved using MOA2019 software[15]. The optimized structure of OTDS was taken from the output of Gaussian09 calculations and was changed to PDB file format. The crystal structure of the receptor OXIDOREDUCTASE/3NRZ (PDB ID: 3NRZ) protein was obtained from the protein data bank (http://www.rcsb.org/pdb).

Ovothiol-A isolation from the eggs of sea urchins: Ovothiol-A was isolated according to Russo *et al.*[13] methods Firstly, KCl was injected referred to as potassium chloride (about 1 mL) into sea urchin and waited for seconds then the sea urchin

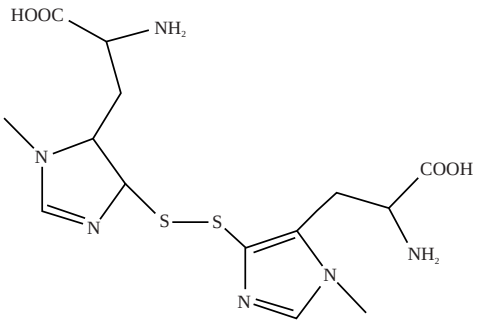

Fig. 1: Chemical structure of Ovothiol-A disulfide

was put on the surface of a beaker full of seawater. Collect the eggs of sea urchins, centrifuge at 2000 RPM for 10 min and discard the supernatant. Eggs of sea urchin were ground in the solution of 20 mL 1 M HCl with 80 mL ethanol, then stirred for 12 hrs. The homogenate was centrifuged at 6000 rpm for 15 min at 4°C. The supernatant was collected and the ethanol was evaporated using a rotatory evaporator. The lipids were removed from the solution by ethyl ether (50 mL). The solution is freed from peroxide by passage over an alumina column. The acidic solution was loaded onto a Dowex 50WX2, column (1×22 cm). Water, 0.1 M, 0.5 M and 4M HCl were used as illusions. Fractions were collected showing the UV spectrum characteristic of ovothiol, evaporated to a small volume and oxidized to Ovothiol disulfide (Fig. 1) in the presence of air at pH 8 for 4 hrs. The solution pH was adjusted at 2, then re-chromatographed on the same Dowex column. After dryness in an oven at 40°C colourless, glassy solid crystals were formed.

Experimental animals: Thirty Wistar Albino rats (males only) (Rattus norvegicus) (100-120 g) were used in the experiment. The rats were selected from similar age groups (±1 week) and weight (±2 g). Animals were placed in boxes made of polycarbonate with tops of steel wire and bedded with wood shavings. They were supplied with a standard laboratory diet, libitum and water. The rats were preserved under steady circumstances of habitation and handling. The Cairo University-approved procedures and experimental protocols were used in this study by the Faculty of Sciences Institutional Animal Care and Use Committee (IACUC) (Egypt). All the experimental procedures were done under the international guidelines for the care and use of laboratory animals.

Induction of liver fibrosis: The bile duct ligation technique was carried out following the Vogel and Vogel method[16]. A midline cut was formed in the abdomen, exposing the muscular layers and the linea alba, which was subsequently slit over a length consistent with the skin slit. The duodenum was brought down to reveal the common bile duct. Two ligates were applied to the duct and a cut was performed between them. An abdominal slit was formed without a ligature in sham-operated animals.

Experimental design: Thirty Wistar rats (male) were randomly allocated into three groups (10/group) as follows:

- **Sham:** Had a slot in their abdomen with no ligated bile duct, then administrated distilled water for a sequel 7 days
- **BDL:** Had a slot in their abdomen with the ligated bile duct, then administrated distilled water for a sequel 7 days
- **Ovothiol-A:** Had a slot in their abdomen with the ligated bile duct, then administrated Ovothiol-A (500 mg kg^{-1} p.o.)[17] for a sequel 7 days

Animal handling: At the end of the experimental period, all rats were euthanized from sodium pentobarbital overdose. Blood samples were collected in suitable centrifuge tubes. The kidney was taken away and promptly blotted using filter paper for removing the remnant of blood after the kidney was divided into two parts. The first part was stored at -80°C for the determination of oxidative stress markers in the kidney. While the other part(the second one) was fixed in 10% formal saline for histopathological examination.

Biochemical parameters: Concentrations of some biochemical parameters like uric acid, urea and creatinine contents (kidney function) were determined using Bio-diagnostic test kits.

Determination of oxidative stress parameters: In ice-cold 0.1 mol L^{-1} Tris-HCl buffers with a pH of 7.4, kidney tissue was homogenized (10% w/v). At 40°C, the homogenate was centrifuged at 4000 rpm for 15 min, then the supernatant was then utilized for oxidative stress tests. MDA referred to Malondialdehyde, CAT referred to catalase, GSH referred to reduced glutathione, GST referred to glutathione-S-transferase and SOD referred to superoxide dismutase were determined following the manufactures instructions using Biodiagnostic kits (Giza, Egypt).

Histopathological examination: For Histopathological examination using a light microscope, in 10% formal saline, kidney tissue will be fixed. They will be stained using hematoxylin and eosin.

Statistical analysis: All data was analyzed by ANOVA which referred to the analysis of variance followed by the Duncan multiple range test when the F-test was significant ($p<0.05$). All analyses were achieved using SPSS which referred to Statistical Package for Social Sciences software on a PC-compatible computer.

RESULTS

Molecular DFT calculation of Ovothiol disulfide (OTDS): The optimized structures of OTDS as the lowest energy configurations as shown in Fig. 2. The dipole moment and the natural charges obtained from NBO which are referred to as Natural Bond Orbital Analysis are shown on the active sites of oxygen, nitrogen and sulfur atoms.

The carbonyl groups $C_{11} = O_2$ and $C_{14} = O_3$ have bond lengths of 1.205 and 1.212 Å, respectively. While the C_{11}-O_1 and C_{14}-O_4 single bonds have larger bond lengths of 1.351 and 1.351 Å, respectively. The O_4-H_2O bond length is larger than O1-H14 by 0.038 Å because of the formation of a hydrogen bond between N_2 and H_2O. The hydrogen bond length N_2·····H_2O is 1.790 Å. The angle O_4-H_2O·····N_2 is 160.8°. The S-S bond length is 2.186 Å, Table 1.

The MEP surface, Fig. 3, is to find the positive (blue colour) and negative (red colour, excess electrons) charged electrostatic potential in the molecule.

The computed total energy of Ovothiol disulfide (OTDS) is -1971.75 Hartree, HOMO which referred to the highest occupied molecular orbital energy is -6.1060 eV, LUMO which referred to the lowest unoccupied molecular orbital energy is -2.0338 eV and the dipole moment of OTDS is 4.0441 Debye. The more negative value of total energy indicated that OTDS is stable. Also, the energy gap (Fig. 4):

$$E_g = E_{LUMO}\text{-}E_{HOMO} = 4.0722 \text{ eV}$$

Reactivity studies: Many reactivity descriptors derived from the HOMO and LUMO energies like electrophilicity index (ω)ionization potential (I), Electronegativity (χ), electron affinity (A), chemical potential (μ), softness (S) and hardness (η), used for study the reactivity in chemical reactions, Table 2.

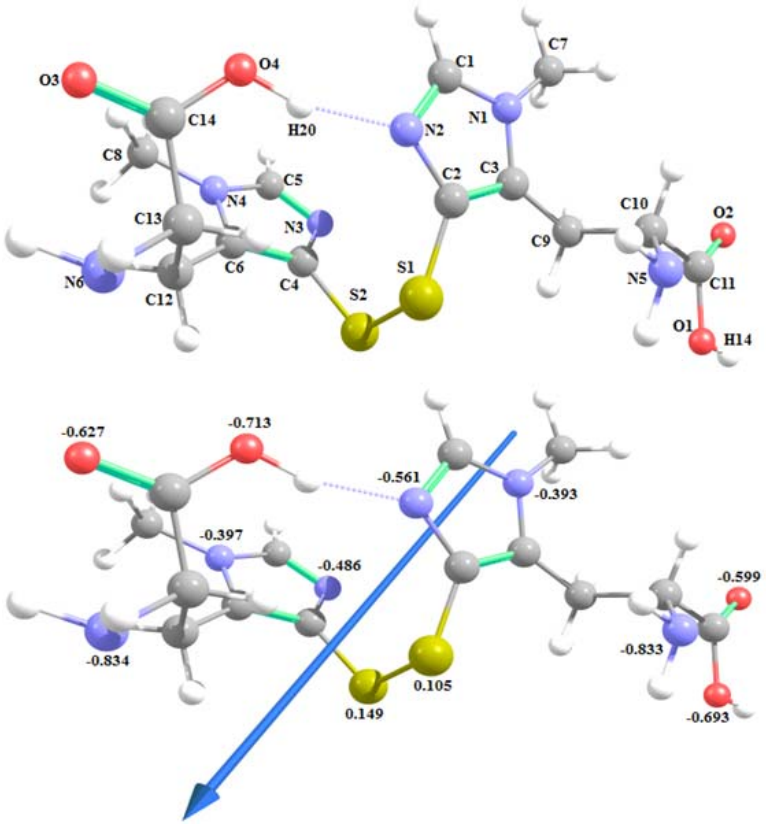

Fig. 2: Optimized structure of Ovothiol disulfide (OTDS), the vector of the dipole moment and the natural charges on atoms by density function b3lyp/6-311g++ (dp)

Table 1: Selected optimized bond lengths (Å) and bond angles (°) of Ovothiol disulfide (OTDS)

Type of bond	Bond length (Å)	Type of bond	Bond length (Å)
$C_{11} = O_2$	1.205	$C_{14} = O_3$	1.212
C_{11}-O_1	1.351	C_{14}-O_4	1.351
O_1-H_{14}	0.969	O_4-H_2O	0.997
N_2····H_{20}	1.790		
N_1-C_1	1.364	N_4-C_5	1.371
N_1-C_3	1.383	N_4-C_6	1.378
N_1-C_7	1.459	N_4- C_8	1.461
N_2-C_1	1.313	N_3-C_5	1.309
N_2-C_2	1.382	N_3-C_4	1.379
N_5-C_{10}	1.454	N_6-C_{13}	1.460
S_1- S_2	2.186		
S_1-C_2	1.754	S_2-C_4	1.755
Type of angle	Angle (°)	Type of angle	Angle (°)
C_2-S_1-S_2	103.1	C_4-S_2-S_1	105.1
O_1-C_{11}-O_2	123.1	O_3-C_{14}-O_4	120.7
O_4-H_2O····N_2	160.8		
C_1-N_2-H_2O	119.5	C_{14}-O_4-H_2O	115.8

Table 2: Electronic reactivity descriptors of Ovothiol disulfide (OTDS)

Parameters	Values
Ionization potential I = -EHOMO	6.106
Electron affinity A = -ELUMO	2.0338
Electro-negativity $\chi = (I + A)/2$	4.0699
Chemical hardness $\eta = (I - A)/2$	2.0361
Chemical softness $S = 1/2\eta$	0.2456
Chemical potential $\mu = -\chi$	-4.0699
Electrophilicity $\omega = \mu 2/2\eta$	4.0676

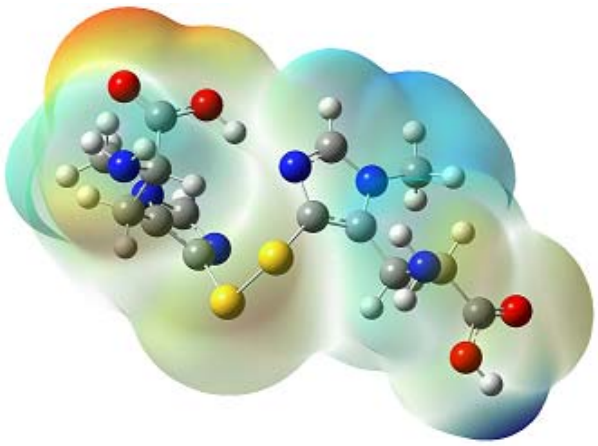

Fig. 3: MEP which referred to the molecular electrostatic potential surface of OTDS b3lyp/6-311g++ (dp)

Molecular docking interaction between OTDS with OXIDOREDUCTASE/3NRZ (PDB ID: 3NRZ): In our present study, the binding free energy of OTDS with the active sites of the receptor of OXIDOREDUCTASE/3NRZ (PDB ID: 3NRZ) is found to be -10.4 kcal mol^{-1}, (Table 3). The more negative the binding energy the stronger interaction. Figure 5 showed a 2d plot of the interaction between OTDS with oxidoreductase. Figure 6a shows the superimposition of the docked OTDS with oxidoreductase (Fig. 6), while Fig. 6b represented the binding patterns of the OTDS in the active site of oxidoreductase.

Kidney function markers: Table 4 revealed that serum uric acid, serum urea and serum creatinine levels increased significantly ($p<0.5$) in BDL rats than Sham group. Otherwise, the administration with Ovothiol-A orally caused a significant decrease ($p<0.5$) in serum urea, serum creatinine and serum uric acid levels.

Oxidative stress markers: Table 5 showed that, NO and MDA levels increased significantly in BDL rats than Sham group and GSH, CAT, SOD and GST levels decrease ($p<0.5$). Otherwise, administration of Ovothiol-A orally caused a significant decrease ($p<0.5$) in MDA and NO levels and a significant increase in GSH, GST, SOD and CAT levels.

Histopathological examination: The kidney of the control group presented the renal cortex and medulla with normal structure, in which the cortex contained numerous renal corpuscles and proximal and distal convoluted tubules (Fig. 7a). The renal medulla was formed by collecting ducts and renal tubules. The spleen of the control group displayed normal histology covered by a capsule of smooth muscle and elastic fibres. The kidney of BDL rats suffered from congestion (Fig. 7b) in some tissue sections and the multifocal area of degeneration and necrosis in the cortical tubules characterized by karyorrhexis, nuclear pyknosis and cellular sloughing (Fig. 7c-d). While the examination of kidney tissue sections of Ovothiol-A treated groups revealed normal structure without noticeable pathological alterations (Fig. 7e).

Table 3: Docking interaction data calculations of OTDS with OXIDOREDUCTASE/3NRZ (PDB ID: 3NRZ)

	Receptor	Interaction	Distance (Å)*	E (kcal mol^{-1})
N_{17}	OE1 GLU 699	H-donor	3.04 (2.14)	-0.8
O_{19}	OE1 GLU 699	H-donor	2.78 (2.04)	-8.0
S_{13}	CE LYS 1304	H-acceptor	3.63 (2.22)	-0.5
O_{25}	ND2 ASN 1307	H-acceptor	3.12 (2.59)	-1.1

*Lengths of H-bonds are in brackets

Table 4: Protective effect of Ovothiol-A on kidney functions of bile duct ligated (BDL) rats

Parameters (mg dL^{-1})	Sham	BDL	Ovothiol-A
Creatinine	0.79±0.17^{a}	1.89±0.21^{b}	1.10±0.07^{a}
Uric acid	1.22±0.36^{a}	2.94±0.53^{b}	1.81±0.05^{a}
Urea	19.74±0.47^{a}	34.21±1.42^{c}	26.53±1.80^{b}

Values are given as Mean±SE for 10 rats in each group and each value not sharing a common letter superscript is significantly different (p<0.05)

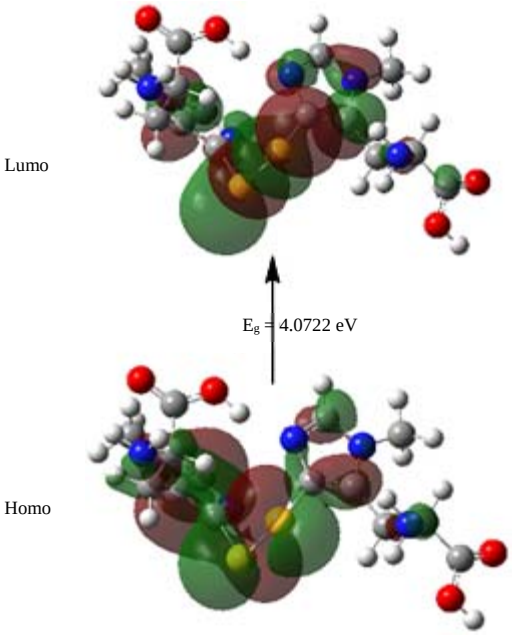

Fig. 4: HOMO and LUMO charge density maps of OTDS, b3lyp/6-311g++ (dp)

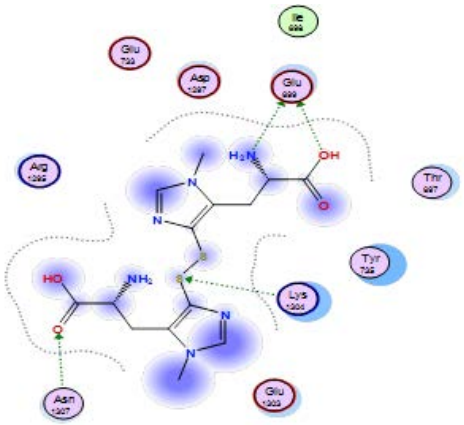

Fig. 5: 2d plot of the interaction between OTDS with oxidoreductase/3nrz (pdb id: 3nrz)
Dotted curves are referred to as hydrophobic interactions with amino acid residues

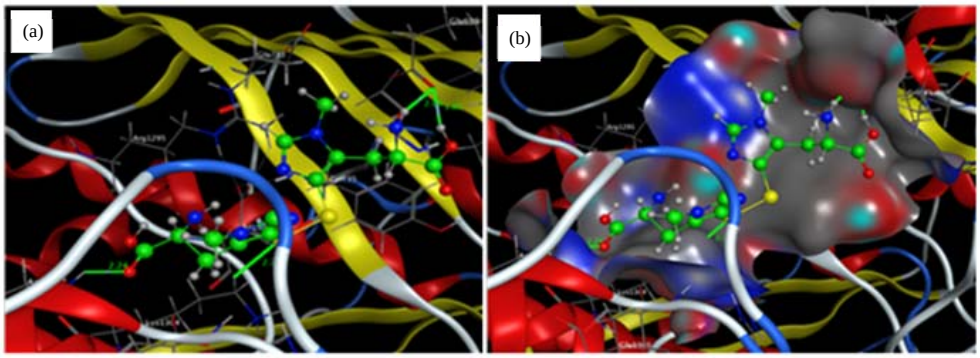

Fig. 6(a-b): Molecular docking simulation studies of the interaction between OTDS with oxidoreductase/3nrz (pdb id: 3nrz), (a) Superimposition of OTDS with oxidoreductase and (b) Binding patterns of the OTDS in the active site of oxidoreductase

Docked conformation of the compound is shown in ball and stick representation

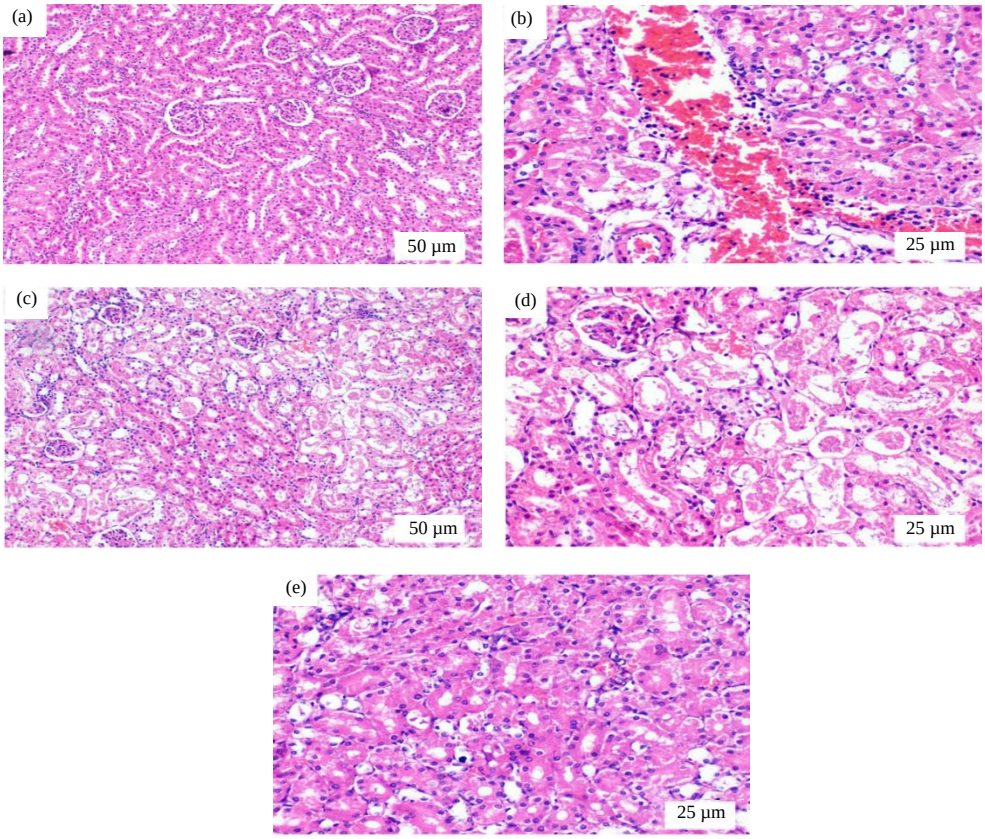

Fig. 7(a-e): Kidneys sections of rat from the control group, (a) BDL group (b, c, d) and (e) Ovothiol-A treated group

Table 5: Protective effect of Ovothiol-A on oxidative stress parameters on the kidney of bile duct ligated rats

Parameters	Sham	BDL	Ovothiol-A
MDA (nmol g^{-1} tissue)	1.68±0.14^{a}	3.03±0.18^{c}	2.55±0.04^{b}
NO (µmol g^{-1} tissue)	314.91±43.33^{a}	613.31±34.47^{c}	447.42±52.25^{b}
GSH (mg g^{-1} tissue)	1.18±0.04^{b}	0.82±0.02^{a}	1.09±0.06^{b}
CAT (U/min)	308.92±28.02^{c}	156.94±49.01^{a}	213.66±13.16^{b}
GST (µmol g^{-1} tissue)	0.28±0.04^{c}	0.17±0.02^{a}	0.21±0.01^{b}
SOD (U g^{-1} tissue)	30.31±6.69^{c}	14.92±1.03^{a}	20.28±1.41^{b}

Values are given as Mean±SE for 10 rats in each group and each value not sharing a common letter superscript is significantly different ($p<0.05$)

DISCUSSION

The kidney and liver are essential organs that provide a variety of functions. One of its primary functions is to eliminate a wide range of potentially poisonous xenobiotics[18]. Prolonged cholestasis, featured by retention of bile compound, could cause renal (kidney) damage, which sometimes leads to kidney failure[19]. In the current study, cholestasis impaired kidney function which increases urea, creatinine and uric acid. Kidneys are the most affected extrahepatic organs in cholestasis[20,21]. Cholestasis-induced kidney damage is known as CN which is referred to as cholemic nephropathy[22]. kidney tissue exposure to a crest concentration of those cytotoxic molecules (hydrophobic bile salts and bilirubin) is proposed to be engaged in CN's pathogenesis[23]. Though the liver is predominantly responsible for bilirubin's excretion, the kidney has a secondary role in that process. If the bilirubin level super passes, it can pile in the renal cells[24]. Piling up of the cytotoxic bile acids during cholestasis is engaged in the pathogenesis of acute oxidative stress in the liver and other organs like kidneys[25]. MDA which is referred to as Malondialdehyde is a secondary product of oxidative stress formed during the peroxidation of lipid. In our present study, MDA levels increased in BDL rats. MDA level increased because of reactive oxygen species (ROS) toxicity in rats after BDL[20]. The increment of MDA concentration results from tissue damage and failure of the antioxidant defence mechanism to prevent the generation of excessive ROS[26]. Also releasing of NO plays a fundamental part in the pathophysiology of cholestasis-induced organ damage[27]. Furthermore, BDL rats showed depression in the antioxidant system including GSH, SGT, CAT and SOD which represents dysfunction of the renal antioxidant system by oxidative stress[28]. Acute biliary obstruction is linked with the development of oxidative stress and renal impairment. Organs other than the liver, like the kidney suffer from damage as a systemic response to cholestasis[29]. On the other side, rats treated with Ovothiol-A showed improvement in renal function parameters, the antioxidant system as well as renal histology. Antioxidant properties of Ovothiol-A resulting from the unusual position of the thiol group on the imidazole ring of histidine[11]. Restoration of antioxidant system healthy and decrease MDA and NO levels after administration of Ovothiol-A represent the antioxidant and protective activity of it against cholestasis. Furthermore, the repression of xanthine oxidase would be an approach for treating disorders related to excess uric acid and ROS[30]. Thus Docking's study confirms the binding of Ovothiol-A strongly with xanthine oxidase and inhibits it, which results in a decrease in uric acid concentration and oxidative stress.

CONCLUSION

Current research revealed that Ovothiol-A is a potent antioxidant substance that has a protective effect on kidneys against the toxic effect of cholestasis. The physiological mechanisms of Ovothiol-A action include inhibition of xanthine oxidase, prevention of uric acid accumulation, scavenging of free radical and reactive oxygen and nitrogen species resulting in improving kidney functions and structure.

SIGNIFICANCE STATEMENT

This study discovers the protective effect of Ovothiol-A against kidney dysfunction that can be beneficial for improving kidney function in cholestatic rats. This study will help the researcher to uncover the critical kidney dysfunction results from cholestasis that many researchers were not able to explore. Thus, a new theory on this Ovothiol-A may be arrived at.

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