doi.org/10.36721/PJPS.2022.35.2.REG.595-603.1

In silico, *in vivo* enzymatic, non-enzymatic toxicity and antioxidant activity of a heterocyclic compound: 5-Benzyl -1, 3, 4-oxadiazole-2-thiol, a potential drug candidate

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Abstract: The present study aimed to investigate the enzymetic, non-enzymetic toxicity and antioxidant potential of a drug candidate 5-Benzyl-1,3,4-Oxadiazole-2-Thiol(OXPA) using computational tools and in vivo models. The binding pattern of it, with different toxicity/oxidative enzymes was predicted using software pkCSM, Protox- II, LAZAR, Mcule 1-Click Docking 3D-Ligand binding Site and best score obtained used as an evaluating criterion. After acute oral toxicity, *in vivo*. antioxidant and hepato protective activity was investigated on male wistar rats, segregated into four groups as control (NS), toxic (INH-RIF), standard (Silymerin) and sample (OXPA, 100mg/Kg) for 21days. Level of antioxidant enzymes / histopathology and serum biochemical parameters in liver and blood of treated rats was assessed by using scientific tools. In silico study reveal no profound toxicity parameters however, LD50 found to be 560mg/Kg while in vivo study declared it safe till 1000mg/Kg, as having no toxicity symptoms. Molecular interaction score with GTH reductase, s-transferase and significant in vivo antioxidant effect on catalase, SOD, TBARS enzymes and histopathological assessment, declare OXPA a good antioxidant having significant ($P< 0.05$) hepato protective activity. Results of in silico, in vivo studies declare the propensity of 5-Benzyl-1, 3, 4-oxadiazole-2-thiol as potential antioxidant, for further investigations as a drug.

Keywords: Antioxidant, acute oral toxicity, molecular docking, *enzymetic* and *non enzymetic*, 5-Benzyl-1, 3, 4-oxadiazole-2-thiol (OXPA)

INTRODUCTION

The new molecules are subjected to study their pharmacodynamics using diverse types of models, including both computer-based and lab-based. Computer-based or *in silico* studies have speeded up the process of the drug discovery and development by providing shortcuts to handle a large amount of data and point out targets and molecules which are helpful in making decisions as quickly as possible in every facet (Ferreira et al., 2015). For this physicochemical, biological and toxic data of the candidate compound can be retrieved from different databanks. Until now, molecular docking has been proved very efficient tool for novel drug discovery for targeting protein having a specified role in disease management. Various software are available by which a new compound can be investigated for the prediction of ligand binding with enzymes/disease-targets, toxicity and pharmacokinetics. The ligand's 3D structure and enzyme interactions in terms of energetically most favorable pose are evaluated/scored using different software and protein data bank repository. The best docked conformation is considered as a supporting data for further evaluation to develop a novel drug candidate and needs to be confirmed by lab-based experiments (Sousa et al., 2006). Finally, the data obtained in such studies are confirmed using specific *in vitro, ex vivo* and *in vivo* models.

Although, to evaluate the toxicity of the compound different *in vivo* tests are performed but their use is limited due to ethical consideration, time and financial constraints. Therefore, *in silico* toxicology plays a vital role in the drug development process after assessing the safety/toxicity profile of the molecule using different *computer based* descriptors, algorithms and data banks (Rowe, 2010; Deeb and Goodarzi, 2012). The capability and applicability of these computational methods to analyze, simulate, visualize and for the prediction of the toxicity profile are increasing day-by-day.

Oxadiazole ring containing compounds are reported to have many applications in industry, agriculture and in pharmacy (Dua *et al*., 2011). Biologically, this heterocyclic system has provided many active compounds (de Oliveira *et al.,* 2012; Mohana and Kumar, 2013), and many drugs comprising such system are in the late phase of clinical trials. Present study focused on one of the new derivative of oxadiazole named 5-benzyl,1,3,4-

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oxadiazole-2-thiol (fig. 1) synthesized by Rehman *et al*., 2013, considering the importance of its *in vitro* biological studies, its spectrophotometric method was developed to assess the stability (Qamar *et al*., 2018). Three major functional entities such as a thiol group (position 2), benzyl group (at position 5) and oxadiazole ring present in OXPA are further expected to make it a drug-candidate possessing several biological activities. The pleiotropic *in vitro* antioxidant profile of the compound (Qamar *et al*., 2019) necessitates to investigate its safety profile for further *in vivo* studies. After considering the above facts, the present study was designed to investigate OXPA for acute oral toxicity, *enzymetic* and *non enzymetic* antioxidant and hepato protective profile by using *in silico* predictions and *in vivo* methods.

MATERIALS AND METHODS

The chemicals used in the present study included n-butanol, chloroform (Merck, Germany), dimethyl sulfoxide (Panreac Quimica, SAU), ethylene diamine tetra acetic acid (Daejung, Korea), hydroxylamine hydrochloride (BDH, England), potassium chloride (BDH), phosphoric acid (E. Merck), silymarin (Xiangtan Jiayeyuan Biological Technology Co. Ltd., China) and normal saline (MediPak, Pakistan) were procured from the local market. tween 40 (BDH), For the determination of liver function markers commercially available kits (Span Diagnostics Ltd.) were procured from the local market. OXPA was received as gift from Government College University, Lahore. Pakistan.

In silico studies

Computational tools/software used in the present study included the Mcule Property Calculator (2016 mcule.com), AutoDock Vina, pkCSM (University of Cambridge), Swiss Target Prediction (Swiss Institute of Institute of Pharmacy, Lahore College for Women University, Lahore. Bioinformatics- 2013), pre ADMET (2005-2015 BMDRC), PROTOX and LAZAR (*In silico* Toxicology, Gmbh 2004-2011). Biological activities were predicted using the 1-Click Docking Mcule, Swiss Target Prediction and 3-DLigand Binding Site.

The data in the form of Simplified Molecular Input Line Entry System (SMILES) and molecular formula were entered in the software. The SMILES and molecular formula of the compound are (Sc2nnc (Cc1ccccc1) o2 and $C_9H_8N_2OS$, respectively.

In vivo studies

Preparation of solutions

The stock solutions of OXPA and the standard drugs were prepared in DMSO to get the final concentration 1 mg/mL. Then, a range of working solutions was prepared by diluting the stock solutions with buffer/1%Tween 40 as needed for a particular type of assay.

Animals and study protocol

Acute oral toxicity

OXPA was investigated for acute oral toxicity using the OECD 423(2001) guidelines. Briefly, five adult-healthy-non-pregnant female rats (150±20 g) were housed for one week under standard laboratory conditions to acclimatize with 12 h light and dark cycles at the Animal House, University College of Pharmacy, University of the Punjab, Lahore, Pakistan,. Breeder feed No. 14 (Hi-Tech Pvt. Limited, Lahore) and tap water was provided *ad libitum.* A single dose (1000 mg/kg/body weight) of the compound suspended in water was administered orally. The behaviour of the animal was continuously observed for 30 min and then periodically till 24h. The animal survived with no apparent signs and symptoms of toxicity. Then the dose was administered to remaining four animals which were observed once daily for 14 days to note appearance and disappearance of signs of toxicity. The major observations included mortality, body weight, toxic symptoms, behavioural changes. Other observations included changes in skin and fur, eyes, mucous membrane (nasal), respiration, heart rate, autonomic system (salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation) and nervous system (ptosis, drowsiness, gait, tremors and convulsion).

Antioxidant activity

Twenty-four, male rats (*Wistar)*, weight (250±20 g) were allowed to acclimatize for one week at room temperature with 12h light and dark Standard rodent diet was provided and water was supplied *ad libitum*. The animals were randomly segregated into 4 groups (n=6) and treated as: group-I (control, untreated/uncompromised, received vehicle), group-II (toxic, 100mg/kg p.o a mixture isoniazid and rifampicin, 1:1, *V/V*), group-III (positive control, toxic dose and 200 mg/kg p.o silymarin) and group-IV (OXPA-treated, toxic dose and 100 mg/kg p.o OXPA). The treatment was continued for 21 days and 24 h following the last dose, the blood was obtained from cardiac puncture under diethyl ether anesthesia. The serum was separated to find out oxidative stress and liver function biomarkers. Afterwards, the liver tissues which were washed with normal saline and used to prepare liver homogenate for biochemical analysis and for histopathological studies. The study was performed as per approved protocol of the Animal Ethics Committee, University of the Punjab, Lahore, Pakistan.

Preparation of liver homogenates

Liver homogenates (5%, *W/V*) of all the samples were prepared according to method followed by Hussain *et al*. (2010). For this purpose, liver tissue 150mg was cut off with scraper in a sterile petri-dish on ice pack and minced with forceps, then this minced tissue was transferred in glass test tube and 3mL of (0.1M) KCl solution was added. The mixture of each sample was then homogenized for 5 min by using tissue homogenizer and

these homogenates were preserved in the ice box till centrifugation in the refrigerated centrifuge machine at 2500 rpm for 10 min and temperature was controlled (10°C). The supernatants were separated from homogenate solution and transferred into separate labelled Eppendorf's carefully and these samples were preserved in the chiller (-80°C) until analysis for performing antioxidant activity.

Determination of catalase activity

Supernatant of liver homogenate (0.2mL), 1mL of hydrogen peroxide (30mM) and 2mL of phosphate buffer (pH 7.4) were mixed. A blank was prepared by liver homogenate supernatant with 0.2mL potassium chloride solution (0.15M). The absorbance of the prepared reaction mixture was measured at 240nm against the blank. The catalase activity was measured in µg/mg/min as described by Aebi (1984) and modified by (Hadwan and Abed, 2016).

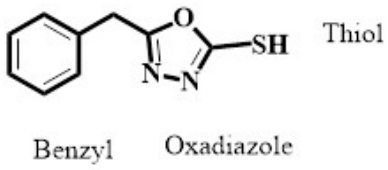

Fig. 1: Chemical structure of 5-Benzyl -1, 3, 4-Oxadiazole -2-Thiol (OXPA)

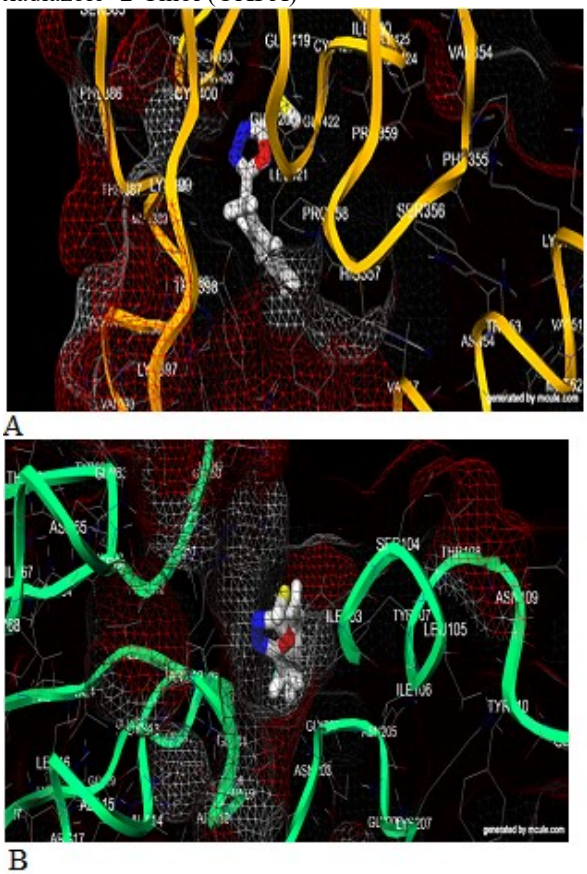

Fig. 2: Binding poses of OXPA and glutathione reductase (A) and glutathione transferase (B).

Determination of superoxide dismutase (SOD) activity

One milliliter sodium carbonate (50mM), 0.4mL of nitro blue tetrazolium chloride (24mM) and 0.2mL of ethylene diamine tetra acetic acid (0.1mM) solutions were mixed with 0.5mL of liver homogenate supernatant. The absorbance of the reaction mixture was noted at 560 nm at 0 min without adding hydroxyl amine hydrochloride against the blank containing water in place of the supernatant. After adding 0.4mL of hydroxylamine hydrochloride (1mM), absorbance was measured after 5 min (Beauchamp and Fridovich, 1971). SOD activity (nM/mg of protein) was determined by applying the Eq. 1 (Kakkar *et al.*, 1984)

$$\text{SOD activity (nM/mg)} = \left(\frac{V}{v} - 1\right) \times \text{dilution factor} \qquad \text{(Eq. 1)}$$

Where V is absorbance of sample without adding supernatant of liver homogenates and v is absorbance of mixture after adding hydroxylamine hydrochloride.

Determination of thiobarbituric acid reactive substances (TBARS)

Liver homogenate supernatant (0.5mL), 3mL phosphoric acid (1%) and 1mL 0.6% aqueous thiobarbituric acid were mixed and boiled on water bath for 45 min. After cooling, 4mL of n-butanol was mixed and mixture was allowed to separate into two phases. Absorbance of the upper phase was noted at 535 and 520 nm against butanol as a blank (Uchiyama and Mihara, 1978). TBARS were determined using molar extinction coefficient of MDA-TBA complex (155000nmol/mg protein) applying the Eq. 2.

$$\text{TBARS value} = \frac{A_{535} - A_{520}}{155000} \times 10^6 \qquad \text{(Eq. 2)}$$

Histopathological assessment

Liver sample fixed in formaline –saline solution was repeatedly washed in alcohol, embedded in paraffin and cut 4-5µm sized sections, stained with Hematoxylin-eosin and photo microscopic evaluation was performed by using 40,100 and 400 objectives.

Biochemical analysis for liver function markers

Serum was analyzed for various biochemical parameters like serum bilirubin, ALT, AST, alkaline phosphatase, total protein and albumin content.

STATISTICAL ANALYSIS

Each sample was analyzed in triplicate and the values were expressed as mean ±SEM (n=6), Statistical analysis was performed by using One-way analysis of variance (ANOVA) followed by Post hoc multiple comparison test. Values were considered statistically significant at $P < 0.05$ by using the software SPSS Version 20.0

Table 1: Predicted toxicity profile of 5-Benzyl-1, 3, 4- oxadiazole-2-thiol (OXPA)

Types of toxicity	pkCSM	Protox II	LAZAR
Predicted LD50 (mg/kg), Toxicity class	-	560, 4	-
AMES toxicity	No	-	-
Maximum tolerated dose (log mg/kg/day)	0.86	-	-
hERG I inhibitor	No	-	-
hERG II inhibitor	No	-	-
Oral rat acute toxicity (mol/kg)	2.27	-	NC
Oral rat chronic toxicity (LOAEL) log mg/kg/day	1.97	-	-
Skin sensitization	No	-	-
T. pyriformis toxicity log µg/L	0.74	-	-
Minnow toxicity log mM	1.21	-	-
Mutagenicity(Salmonella typhimurium)	-	-	M
Immuno toxicity, cytotoxicity, mutagenicity	-	Inactive	-

*NC (Non-carcinogenic); M (Mutagenic); - (No parameter available)

Table 2: Interaction of 5-Benzyl-1,3,4-oxadiazole-2-Thiol with toxicity enzymes by PROTOX

Toxicity enzymes	Target	Prediction	Probability
Tox21-*NRSP	Aryl hydrocarbon Receptor (AhR)	Inactive	0.55
Tox21- NRSP	Androgen Receptor (AR)	Inactive	0.97
Tox21- NRSP	Androgen Receptor Ligand Binding Domain (AR-LBD)	Inactive	0.96
Tox21- NRSP	Aromatase	Inactive	0.89
Tox21- NRSP	Estrogen Receptor Alpha (ER)	Inactive	0.79
Tox21- NRSP	Estrogen Receptor Ligand Binding Domain (ER-LBD)	Inactive	0.97
Tox21- NRSP	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	Inactive	0.83
Tox21-*SRP	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	Inactive	0.78
Tox21-NRSP	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	Inactive	0.83
Tox21- SRP	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	Inactive	0.78
Tox21- SRP	Heat shock factor response element (HSE)	Inactive	0.78
Tox21- SRP	Mitochondrial Membrane Potential (MMP)	Inactive	0.75
Tox21-SRP	Phosphoprotein (Tumor Suppressor) p53	Inactive	0.91

*NRSP (Nuclear receptor signalling pathways); SRP (Stress response pathways)

Table 3: Binding score and amino acids involved in binding of OXPA with antioxidant targets

Enzyme	Organism	Score	Amino acids
Glutathione reductase (1xan)	*Homosapien*	-5.1	Ile9(0.5),Gly10(0.01),Gly11(0.06),Gly12(0.02), Ser13(0),Gly14(0), Gly15(0.01),Val32(0.26), Glu33(0),Ser34(0),His35(0.01),Lys36(0.23), Gly39(0.01),Thr40(0),Cys41(0),Val44(0.28), Gly45(0.23),Cys46(0.12),Lys49(0),Gly111(0.03), His112(0),Ala113(0),Ala138(0.05),Thr139(0.01), Gly140(0.07),Gly141(0.03),Ser160(0.05), Tyr180(0.01),Ile181(0.61),Arg274(0.29),Leu281 (0.17),Gly313(0.05),Asp314(0),Val315(0.34), Leu320(0.03), Leu321(0.01),Thr322(0), Pro323(0.02), Ala325(0.57), Phe355(0.71)
Glutathione transferase (18gs)	*Homosapien*	-6.1	Not found

Table 4: *In vivo* antioxidant activity of liver homogenate in 5-Benzyl-1, 3, 4-oxadiazole-2-thiol treated rats and other different groups (n=6, SD)

Parameter Group	Catalase activity (µg/mg/min)	Superoxide dismutase (µg/mg)	TBARS (nM/mg protein)
Group-I	55.93±7.45	4.89±0.74	2.28±0.70
Group-II	20.74±6.36	1.21±0.36	5.81±0.74
Group-III	51.64±6.24	4.40±0.61	2.29±0.80
Group-IV	*51.51±5.59	*4.19±0.45	3.15±0.61

All values are expressed as mean ± SD, (control, group I), (INH-RIF, group II), (Silymarin+INH-RIF, Group III), (OXPA+INH-RIF, group IV),* between the group, within the group ($p < 0.05$)

Table 5: Liver function markers in 5-Benzyl-1, 3, 4-oxadiazole-2-thiol treated rats and other different groups (n=6, SD)

Group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Bilirubin (mg/dL)	Total protein (g/dL)	Albumin (g/dL)
Group-I	71.67±12.69	141.5±10.10	191.33±16.11	0.47±0.19	7.67±0.26	4.04±0.35
Group-II	107.83±9.33	202.83±13.76	354.83±14.20	1.17±0.36	5.68±.52	2.93±0.35
Group-III	73.67±6.74*	155.17±25.56*	199.67±16.68*	0.50±0.18*	7.41±0.41*	3.61±0.38*
Group-IV	74.17±12.77*	146.17±17.86*	206.83±7.03*	0.6±0.14*	7.52±0.23*	3.56±0.22*

Each group contains rats, * (significantly different from the toxic group $P<0.05$)

RESULTS

Acute oral toxicity

The toxicity profile of the compound, predicted by using three software is given in table 1. Half maximal lethal dose (LD_{50}) was predicted to be 560 mg/kg/day, and the lowest observed adverse effect dose (LOAEL) was found to be 1.97 log mg/kg/day. Acute oral toxicity studies against *T. pyriformis* and flathead Minnow Fish indicated that the compound was not toxic as the predicted value was higher than the limit (>-0.5 log µg/L).

The maximum recommended tolerated dose (MRTD) of the compound was found to be high than the limit (less than or equal to 0.447 log mg/kg/day). The compound was also found to have no skin sensitization and cardiotoxicity (medium risk to hERG; human ether-a-go-go gene), so was found to have no skin sensitization and cardiotoxicity. However, it was found to be on border-line as hepatotoxic and carcinogenic in pkCSM and PROTOX software, while it was inactive for immune toxicity, cytotoxicity and mutagenicity.

PROTOX software predicted that OXPA possibly did not bind to the common toxicity targets to produce serious effects described in table 2.

Antioxidant enzymes

The interaction of OXPA with the antioxidant targets is given in table 3. The compound showed binding affinity with antioxidant enzymes. The binding poses of OXPA-enzymes are given in fig. 2. These results indicate that the compound may have good interaction with the enzymes so may have reducing potential.

In vivo activity

Acute oral toxicity

The compound has not shown any signs and symptoms of toxicity at a dose of 1000 mg/kg. The dose of the compound was selected based on *in silico* toxicity profile (LD_{50}=560 mg/kg). These results indicate that lethal dose of OXPA is higher than the limit dose. These results confirmed the toxicity, predicted computationally. As per OECD guidelines No. 425 (OECD, 2014) the median lethal dose (LD_{50}) of the compound falls under class four values. Hence, the compound was subjected to *in vivo* antioxidant activity.

Antioxidant activity

Rat liver-homogenate was used to determine catalase, superoxide dismutase and lipid peroxidase activity (TBARS). The results of catalase and superoxide dismutase activities and formation of TBARS in rats of different groups are given in table 4. All the observed effects were compared to a control group of non-treated hepatocytes. The levels of antioxidant markers such as serum catalase activity, superoxide dismutase activity and TBARS in OXPA-treated rats indicated that the compound had antioxidant capacity against induced oxidative stress. Oxidative stress affects the hepatocytes which can be assessed by determining liver function markers. OXPA has shown promising antioxidant and hepato protective activity in rats at a dose of 100 mg/kg.

All such parameters were found to be significantly between the groups and within the groups ($p < 0.05$). Catalase activity of OXPA- and silymarin-treated rats was significantly higher than the toxic group (group-II). SOD activity of the compound was found to be comparable to that of the silymarin-treated and control (untreated)

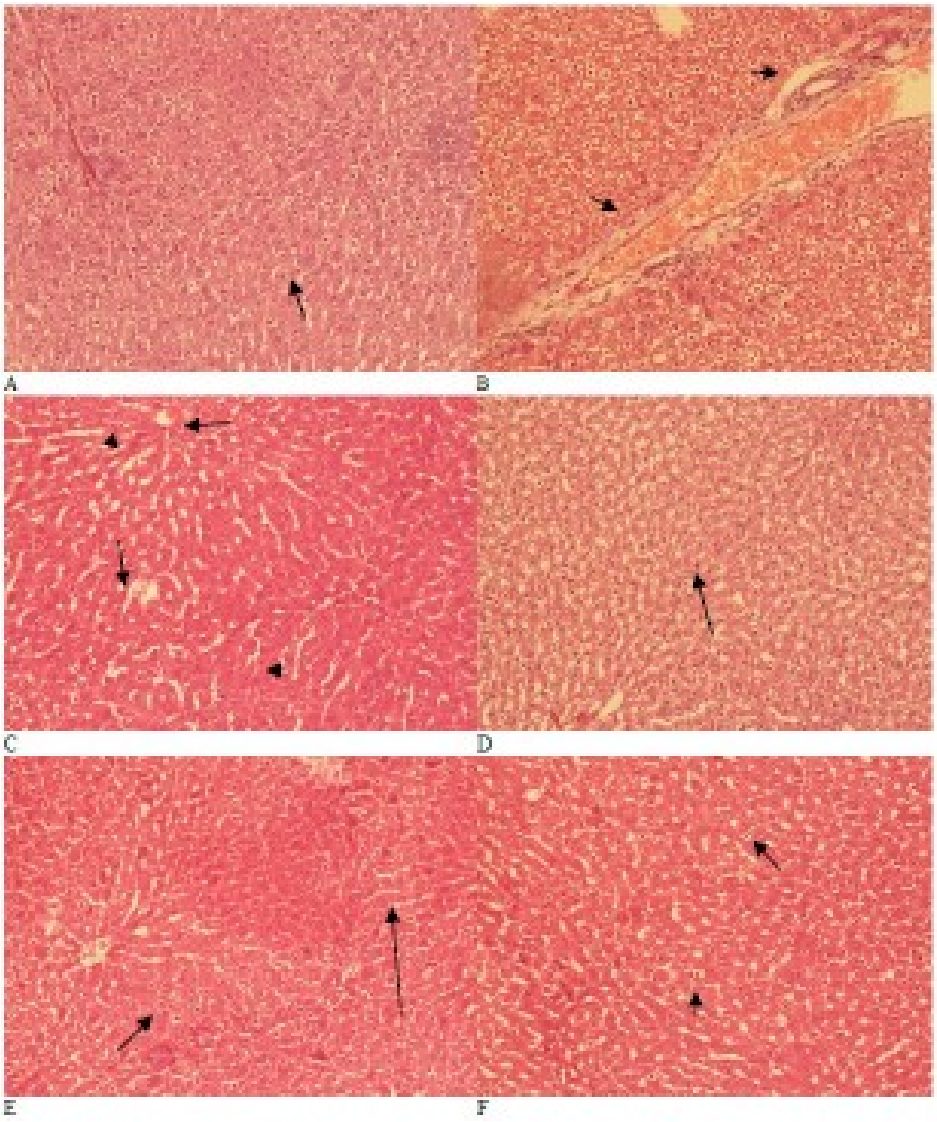

Fig. 3: Representative photomicrographs of liver samples in the INH-RIF model. A: Normal saline group, hepatocytes showing normal arrangement of cells (control, group I); B, C: Fatty liver showing portal inflammation, vacuolation, fatty changes and infiltration of mononuclear cells around the central port (INH-RIF, group II); D: treated cells having nuclei and sinusoids (Silymarin+ INH-RIF, group III); E, F: Amelioration effect of (OXPA+INH-RIF, group IV) on liver hepatocytes

groups. TBARS of OXPA-treated group were found to be comparable to that of the silymarin-treated group.

Histopathologic assessment

In contrary to the group I (control) shown in photo micrographs, the group II (hepatotoxicants) have shown abnormal architecture where fatty changes, vacuolation, moderate infilteration of neutrophils, inflammation can be seen in fig. 3 (B and C), and ballooning degeneration (60-65%) as shown in *non enzymetic* and *enzymetic* assays while the other intervention groups (III and IV) have about normal architecture as portal tract, liver parenchymal inflammation (mild), fatty changes, portal and central vein congestion and cirrhosis was nil. The ballooning degeneration in standard treated group (10-15%) while in OXPA treated group (10-20%) was observed as shown in fig. 3 (D,E,F).

Liver function markers

The results of liver function markers aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), bilirubin, total protein and albumin in different groups of rats are given in table 5. All such markers were found to significantly different within the groups and between the groups ($P<0.05$). ALT, AST and ALP levels of OXPA-treated (group-IV) and silymarin-treated (Group-III) groups were comparable with control (Group-I) whereas significantly lower than the toxic group (Group-II). Likewise, total protein and albumin levels of the toxic group were significantly lower

than that of control and treated groups ($P<0.05$). However, the level of both of the markers were comparable in silymarin-treated group (group-III) and OXPA-treated group (group-IV).

DISCUSSION

The compound under investigation was found to be in class-IV as per the Internationally Harmonized System of Classification of Labeling Chemicals. Toxicological studies classify the chemicals according to their intrinsic toxic effects produced in humans, animals, plants and environment (Arwa and Vladimir, 2016). It is performed to gain an insight of the behavior of a drug in the living system. Pharmaceutical agent anticipated for human use has to be necessarily checked for these studies. Such studies are useful for the selection of doses for repeat-dose studies, early identification of target-organ toxicity, overdosing and delayed toxicity. These may also help in Phase-I human trials for the selection of starting doses. OECD (2001c) and EEC (2009) have laid down guidelines which are being used in risk management, hazard identification and calculation of median lethal dose (LD_{50}). Classification of chemicals is based on LD_{50} value (Valerio, 2009). The obtained data are evaluated and used in drug development process by correlating different physicochemical and biological descriptors with toxicological predictions qualitatively such as genotoxicity, immune toxicity, hepatotoxicity and carcinogenicity while some tests are quantitative such as LD_{50}. Computational methods aim to complement the other *in vitro* and *in vivo* techniques. So in the above studies compound was found out to be non toxic to most of the toxicity enzymes and other toxicity considerations so some of the parameters were evaluated by using *in vivo* acute oral toxicity studies which declared it to be safe till experimental dose 1000mg/kg.

The antioxidant activity of the compound was predicted computationally by using Mcule's 1-Click Docking software, and the ligand-protein interactions were scored by using Auto Dock Vina which ease the drug discovery program by using default parameters to calculate the docking score which is being calculated by using evidence of molecular docking of a particular ligand to the selected target. The targets selected were of glutathione reductase and glutathione S-transferase, when hepatocytes deplete GSH, this act is considered as indicator of mitochondria-associated apoptosis and oxidative stress (Mari *et al*., 2009). Glutathione reductase prevent oxidative stress by providing reduced glutathione, which catalyze reduction of glutathione di-sulphide to sulphydryl glutathione, so ultimately play an important role in redox balance of the body. It helps in detoxification of reactive oxygen species by giving reducing equivalents to glutathione peroxidase and detoxifies electrophilic xenobiotics with glutathione S-transferase which ultimately catalyze reduced form of GSH to substrate-compound complex for detoxification. Molecular interaction studies of OXPA with the above enzymes have shown good score.

After the *in vitro* antioxidant evaluation (Qamar *et al*., 2019), response of the compound was confirmed by *in vivo* studies, in which level of biomarkers/antioxidant enzymes in serum and liver tissue was evaluated after inducing two clinically used anti-tuberculosis drugs isoniazid and rifampicin (hepatotoxic) in combination.to induct oxidative stress (Tostmann *et al*., 2008). As a known fact oxidative stress has a very crucial role in liver diseases and this can be assessed by the increase production of malondialdehyde (MDA), an aldehyde produced in biological system from the lipid peroxidation process due to the overproduction of ROS. The catalase enzymes secreted from liver tissues scavenge free radicals (H_2O_2) (Thumvijit *et al*., 2013), Superoxide dismutase (SOD) preserved tissue oxidation due to superoxide anion radical and provided defense by catalyzing the radical into peroxide and oxygen. Thus, H_2O_2 scavenging is very important for protecting living organisms. Results have shown significant protective effect of OXPA on these three enzymes by maintaining the membrane structural integrity towards normal by regenerating damaged cells as seen in photomicrographs, it may be due to significant H_2O_2 scavenging activity as described earlier (Qamar *et al*., 2019). According to (Güngör et al., 2011; Ulrich and Jakob, 2019), thiol containing compounds protect biological systems from oxidative injury by scavenging H_2O_2 (The pi bonds of the phenyl ring of the compound may also be involved in the scavenging activity of H_2O_2, while decrease in MDA level by TBARS activity was due to preservation of glutathione peroxidase in the tissues according to Arivazhagan *et al*., 2000 , this decrease may indicate that the compound had reactivated level of GSH ,which is a non enzymetic antioxidant responsible for protection of cellular proteins against ROS. The lipid soluble character of compound also plays a key role in its penetration to the membrane and reducing character of thiol contributes in redox reactions taken place in the body while free radical and H_2O_2 scavenging capacity of the compound is already proven. These findings may indicate that OXPA had capability to preserve the antioxidant enzymes against induced oxidative stress.

Liver diseases always remain a matter of mortality and morbidity worldwide, and especially the toxicity induced by the drugs, are the major cause of dysfunction and ultimately damage to the liver (Abbound and Kaplowitz., 2007). The occurrence and extent of damage depends upon the drug used, so it is an intense matter of concern to lead forward for a compound having such properties to protect the liver from the oxidative stress. Glutathione and thioredoxin (Onoja *et al*., 2014) release provide safety to liver, therefore, exogenous thiol compounds have been

successfully used to control antioxidant level of animals and humans in oxidative stress (Deneke, 2001). The severity in hepatotoxicity is also assessed by the total protein contents (Taniyama and Griendling, 2003) and the other biomarkers considered as liver profile indexing enzymes. In the present study the compound has shown a profound effect in restoring total protein and albumin, it may be due the stabilization of endoplasmic reticulum (Mahmud *et al*., 2012). Effect of the OXPA on liver biomarkers clearly indicate its hepato protective effects against free radicals generated after inducing hepatotoxicant, however these results were contrary to those predicted computationally where the compound was predicted to be at the borderline of hepatotoxicant. It is worth mentioning that the results of *in vitro* and *in vivo* antioxidant activity of OXPA confirmed the findings of each other and can be correlated with the previous report (Poole, 2015) that thiol containing compounds due to hydrogen-donating ability can attribute reducing potential which is proved by results of *in silico* and *in vivo* studies although exact mechanism is not known. The tendency of the liver enzymes towards normality after OXPA treatment shows the clear manifestation of its hepato protective effect.

CONCLUSION

In conclusion, this study demonstrates that presence of functional groups in OXPA present at 2nd and 5th position of active oxadiazole ring have the capability to interact with the toxicity and liver enzymes analyzed by *in silico* and *in vivo* methods and this can be attributed due to the presence of –H atom donating ability of -benzyl and -thiol group which make this compound a “superb antioxidant”. by preventing lipid peroxidation and ultimately retard formation of toxic oxidation products. Further studies are needed to explore the *in vitro* effect of OXPA on glutathione reductase and glutathione transferase enzymes.

The coherence of both *in silico* and *in vivo* study of 5-benzyl 1, 3, 4-oxadiazole 2-thiol (OXPA) indicate that it possesses good safety profile and promising antioxidant and hepato protective activity on the *enzymetic* and *non enzymetic* behavior of serum and liver, hence may be developed in to a drug.

ACKNOWLEDGEMENT

Authors are thankful to the Quality operation lab, University of Veterinary and Animal Sciences, for the histopathological study of processed liver, Pathological lab, University of Health Sciences for the study of biochemical parameters in serum and Government college University, Lahore, Pakistan for their gift of 5-Benzyl-1,3,4-oxadiazole -2-thiol.

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