



# Ameliorative Renal, Antioxidant and Antibacterial Activities of Zinc-nitazoxanide Complex with its Spectroscopic Characterization

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## ABSTRACT

**Background:** Nitazoxanide (NTZ) possesses a high potential activity against infections by various protozoa.

**Methods:** Nitazoxanide/zinc complex (NTZ/Zn) was synthesized and chemically characterized. Also, the antibacterial activity of NTZ/Zn was performed against *Staphylococcus aureus* strain. Then, twenty-one male mice were divided into three treatment groups as follows: Control, NTZ, NTZ/Zn complex; all animal treated for 30 successive days. The present study aimed to determine the physiological alterations, oxidative stress actions beside confirmatory part via the histopathological changes on the renal tissues of male mice and the possible ameliorative role of the recent complex (NTZ/Zn) to prevent the signs of renal toxicity.

**Result:** Electrolytic nature of (NTZ/Zn) complex was confirmed. The bidentate characteristic of NTZ through the nitrogen atom of the amide group and oxygen atom of the ester group toward Zn (II) metal ion was confirmed using IR, UV for NTZ and its complex (NTZ/Zn). SEM results showed that small particles agglomerated with different shapes. TEM showed that the zinc nitazoxanide complex has spherical black spots. Also, NTZ/Zn showed antibacterial activity against the *S. aureus* strain. The NTZ-treated group alone marked decline in renal antioxidant enzyme markers and elevated MDA). Meanwhile, The NTZ/Zn complex prevented the NTZ-induced renal injury as specified by ameliorating all the biochemical parameters with the appearance of glomerulus near o normal structure in NTZ/Zn complex. NTZ induced renal alterations that could be mitigated by NTZ/Zn complex via the physiological and biochemical mechanisms.

**Key words:** Antioxidant activities, Anti-parasitic drug, Nitazoxanide, Renal tissues.

## INTRODUCTION

Nitazoxanide (NTZ) is a common antiprotozoal White (2004), antibacterial Hong *et al.* (2021), antiviral Sharfalddin *et al.* (2023) and in some literature, it act as anticancer agent against a lot of cancer cell lines (Fan-Minogue *et al.*, 2013).

NTZ, is known as 2-(acetyloxy)-N-(5-nitro-2-thiazolyl) benzamide and was discovered by Rossignol (Di Santo and Ehrisman, 2014). Its effectiveness against the infections caused by parasites led to its approval in the U.S.A. for treatment of the diarrhea that may be caused by the common parasites like "*Giardia intestinalis*" and "*C. parvum*" (Amadi *et al.*, 2002).

The thiazolyl structure yielded a lot of capacities that are useful in treatment of a lot of viruses, such as hepatitis C virus and some strains of influenza Elazar *et al.* (2009). Additionally, *in vitro* efficacy against many viruses such as rotavirus, hepatitis B and C viruses (Lokhande and Devarajan, 2021).

NTZ has the great potency against several cancer cell lines either *in silico* and *in vitro* (Alonso and Farina, 2020). NTZ has unique structure, it has two moieties "Nitro thiazole and salicylamide moiety" in only one molecule (Fig 1), which is attributed to the multiple activities. The nitro thiazole exhibits inhibition as protozoal, anti-proliferative and anti-infective agents Colín-Lozano *et al.* (2017). Moreover, it is a good antioxidant that could disrupt pyruvate: Ferredoxin

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oxidoreductase by interfering with the electron transfer for anaerobic protozoa for their energy metabolism Hemphill *et al.* (2006).

Salicylamide moiety, plays a vital role as an anti-infective, antioxidant and analgesic agent and regulating the immune responses via its role in blocking of the viral entry and host cell membrane fusion (Lokhande and Devarajan, 2021). Introducing the metals into the novel and active bioactive molecules elevates the pharmacological properties (Sharfaldin *et al.*, 2021). Metals could elevate the lipophilicity of the drugs by donating free electrons from their free orbitals to those metals and thus delocalizing the free electrons over the metals' drug complexes (Tweedy, 1964).

Moreover, metal ions decline the toxicity of the drug molecules and elevate the compound stability in the approved biological systems. It is approved that some novel metal complexes exhibited antiviral properties against some known viruses, such as SARS-CoV-2 and influenza strains' viruses (El-Megharbel *et al.*, 2021 and Pal *et al.*, (2021). This potency includes the blockage of the viruses' entry into the host cells and even some blockage of RNA replication process (Pal *et al.*, 2021).

In this study, Zn (II) metal ion reacted with NTZ giving a stable metal complex and was characterized using IR, electronic spectra, magnetic measurements,  $^1\text{H}$  NMR, SEM and TEM and then its biological activity was evaluated on renal functions, antioxidant enzymes and lipid peroxidation marker in kidney tissue homogenates beside the histological alterations assessment.

## MATERIALS AND METHODS

### Chemicals

Nitazoxanide (NTZ) and  $\text{ZnCl}_2$  salt was purchased from national pharmaceutical company.

### Synthesis of zinc nitazoxanide complex

Zinc nitazoxanide chelates were synthesized as follow: mixing nitazoxanide (2 mmol, 0.614 g) in 30 mL of ethanol solvent with (1 mmol) zinc salt in 20 mL of ethanol, then make refluxing for mixture reaction for 4 h. The formed solid chelate was filtered, washed with ethanol and finally dried under a vacuum over  $\text{CaCl}_2$ .

### Chemical characterization methods

The C, H and N contents were analyzed using a perkin elmer CHN 2400 Elemental Analyzer (USA). The Jenway 4010 conductivity meter was used to test the electrolytic characteristics of zinc compound. A Bruker FT-IR spectrometer measured infrared spectra in the  $4000\text{--}400\text{ cm}^{-1}$  spectral region. UV-Vis spectrophotometer measure UV-Vis spectra in DMSO within the  $800\text{--}200\text{ nm}$ .  $^1\text{H}$ -NMR spectrum measured using Varian Mercury VX-300 NMR spectrometer, which runs at 300 MHz spectra, the six signals for nitazoxanide show six chemical environments of the H ions. Magnetic moments demonstrate how magnetized material might become. The magnetic susceptibility balance from Sherwood Scientific was used to compute magnetic moments. SEM pictures taken using the Quanta FEG 250 apparatus.

Through the imaging and diffraction of nanoscale materials, TEM is used to explain structural and chemical characteristics in the nanoscale range using JEOL 100s microscope was used for TEM examinations.

### Animal and experimental design

The present study was based on ethical approval of Zagazig University animal care committee (ZU-IACUC/1/F/103/

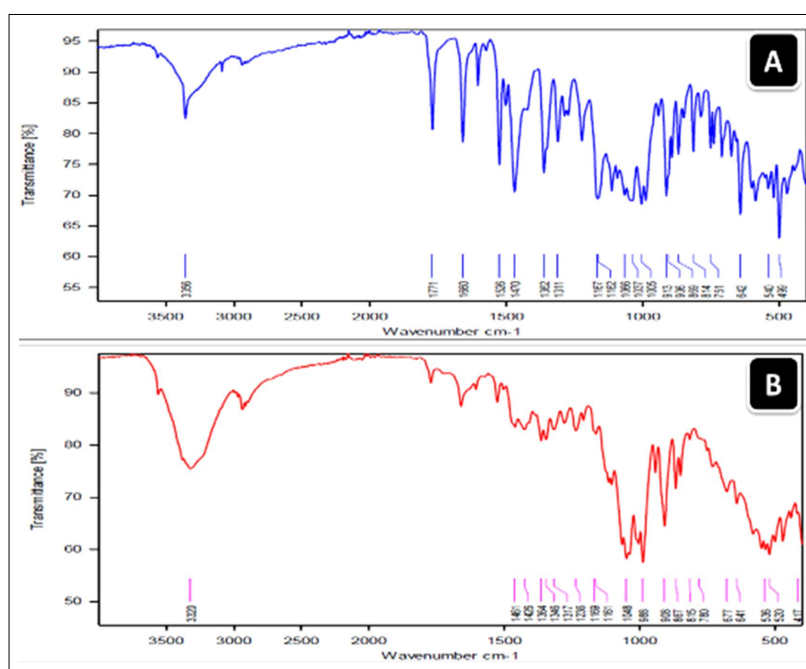


Fig 1: FT-IR of (A) Nitazoxanide and (B) Nitazoxanide/Zn.

2023). Animal experiments were performed according to the European Community Directive (86/609/EEC) and the 8<sup>th</sup> edition of NIH Guidelines for the Use of Animals. Experiments were all upon the ethical guidelines and international animal care.

### Experimental animals

21 male SWR mice, weighing between 25 and 30 g/2 months age. The male mice were kept in clean cages in the animal house with regular access of water and food, With very low dosage of both "Ketamine-xylazine" to induce light anaesthesia to prevent any pain, as ethically approved.

### Experimental design

21 male mice were divided equally into 3 groups, 7 male mice/each group, treated orally for 30 successive days as follows:

- (i) **Control group:** Male mice were fed a standard diet with water and balanced diets *ad libitum*, animals were given saline as a vehicle.
- (ii) **The NTZ-treated group:** Male mice were administered (NTZ 18 mg/kg) Albogami (2024).
- (iii) **The NTZ/Zn complex treated group:** Male mice were given NTZ/Zn complex at the same referenced dosage of (NTZ) as mentioned previously.

### Blood collection

The serum samples were harvested from the fasted animals via using the retro-orbital puncture method under light anesthesia. Serum was preserved at -80°C until biomarker estimation.

### Renal biomarkers evaluation

Creatinine and uric acid were evaluated according to Schirmeister *et al.* (1964) and (Fossati *et al.*, 1980). Urea levels were based on (Patton and Crouch, 1977).

### Renal homogenates preparation for estimation of the oxidative stress injury

Renal tissues were used for determination of the oxidative stress. Tissues were embedded with a 50 mM of sodium phosphate buffer (pH 7.4), 0.1 mM EDTA and 0.25 M sucrose. After centrifugation, the supernatants were modulated into Eppendorf and stored at -20°C until being used for further measurements. MDA was determined using manufacturer manual. Superoxide dismutase and catalase activity (SOD and CAT) were estimated (Marklund and Marklund, 1974) and Aebi (1984). The glutathione peroxidase (GPx) activity was evaluated by Beutler *et al.* (1963).

### Quantitative RT-PCR

RNA samples were purely isolated using by using the TRIzol and reverse transcription that was conducted by using the RT Master Mix Kits. qRT-PCR was performed using qPCR SYBR Green Master Mix. Primer sequences are listed in Table 1.

### JNK activity assay in kidney tissues

The JNK activity in kidney tissues was assayed by a cell JNK kinase activity kit (GENMED, China) according to the manual protocol. The absorbance was measured at ~340 nm using the digital Spectrophotometer (Thermo Scientific, USA).

### Histological evaluation of the renal tissues

The routine histological assessment to process the histological evaluation of the renal tissues. Using a light microscope (Olympus Co.), the renal tissue was investigated and evaluated.

### Statistical analysis

The data were expressed statistically as (Mean ± SE), One-way ANOVA is used. Value is considered as significant at (p<0.05).

## RESULTS AND DISCUSSION

### Elemental analysis and molar conductance

The electrolytic character for solid nitazoxanide zinc chelates was measured using molar conductance that measured value was  $\Lambda_m = 90 \text{ (}\Omega^{-1} \text{ mol}^{-1} \text{ cm}^{-1}\text{)}$ , due to presence of two Cl<sup>-</sup> ions outside the complex sphere. Elemental analysis C, H and N analyses and molar conductance values of nitazoxanide complex [Zn (NTZ)<sub>2</sub>]. Cl<sub>2</sub> with molecular formula (C<sub>24</sub>H<sub>18</sub>O<sub>10</sub>N<sub>6</sub>S<sub>2</sub>Cl<sub>2</sub>Zn) and White in colour with calculated elemental analysis of the sample as follows: C (38.40%), H (2.40%) and N (11.20%) and all confirmed the 1:2 zinc: nitazoxanide ratio. The mode of chelation of nitazoxanide with Zn (II) metal ion was characterized using IR, UV, <sup>1</sup>H-NMR spectroscopy.

### Infrared spectra (FT-IR)

The IR spectral bands for free NTZ and its Zn (II) complex are shown in (Fig 1). The mode of chelation of NTZ can be explained depending up on the main six donating sites that NTZ contains which can be coordinated with Zn (II), which gives more extraordinary compound properties: amide (NH) group, two oxygens of carbonyl (C=O), N atom in thiazole ring. By comparing IR spectrum of NTZ with its zinc complex showed that the stretching vibration of NH amide group disappeared upon chelation to Zn (II) complex and shifted to higher frequencies, confirming that the NH

**Table 1:** Primer sequence of JNK.

| Gene | Forward primer (5'-3') | Reverse primer (5'-3') | Size | Accession no. |
|------|------------------------|------------------------|------|---------------|
| JNK  | AGTGTAGAGTGGATGCATGA   | ATGTGCTTCCTGTGGTTTAC   | 182  | NM_053829.2   |

amide group is involved in the complex formation (Nakamoto, 1970). Furthermore, for this result, the stretching vibration for ester group appeared at  $1771\text{ cm}^{-1}$  and  $1167\text{ cm}^{-1}$  allocated to both "C=O and C-O", respectively.

A low-range change in the C=O carbonyl group indicated that it was not involved in the chelation process. The ester oxygen first emerged in NTZ at  $1167\text{ cm}^{-1}$ , to move to a higher frequency with low intensity. The NTZ molecule underwent chelation at these primary locations, as evidenced by the redshift and low intensity of the C=O amide and C=N modes of vibration in the thiazole ring following connection to the Zn(II) ion.

The zinc complex's infrared spectra show bands in the  $677\text{--}641\text{ cm}^{-1}$  and  $536\text{--}520\text{ cm}^{-1}$  range, which caused by  $\nu(\text{M-O})$ . The NTZ spectrum does not show these bands.  $\nu(\text{M-N})$  (from the NH amide group) may be responsible for the new band at  $417\text{ cm}^{-1}$  in the zinc complex spectra, which vanishes in ligands devoid of NTZ (Nakamoto, 1970 and Bellamy, 1975).

### H-NMR spectra

The main assignments of  $^1\text{H-NMR}$  spectra for NTZ and its  $[\text{Zn}(\text{NTZ})_2] \cdot \text{Cl}_2$  complex were carried out in DMSO- $d_6$ .

There are six signals appeared for NTZ: CH-Ar (7.81, 7.74, 7.34 and 7.31 ppm),  $\text{CH}_3$  (2.29 ppm) and NH (12.61). The singlet signal appeared at 12.61 ppm assigned to the amine-NH group in NTZ that are appeared at de-shielded range 13.31 for Zn (II) complex confirming binding of Zn (II) with NH amide group.

### UV-Vis spectra and magnetic measurements

Fig (2) show the uv-vis spectra and their assignments for the free ligand NTZ and its zinc complex in DMSO solvent. Two absorption maxima for NTZ may be found at 225 and 410 nm by comparing the spectra of the NTZ free ligand and its zinc complex. Because of the organic moiety, the

band that occurred at 225 nm is attributed to  $\pi \rightarrow \pi^*$  transitions, but the band that appeared at 240 nm is attributed to  $n \rightarrow \pi^*$  transitions. Weak bands at 265 and 410 nm are seen in the Zinc (II) complex, which might be attributed to  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  transitions. The square planner shape of the  $[\text{Zn}(\text{NTZ})_2] \cdot \text{Cl}_2$  complex was validated by the magnetic moment of 1.836 BM for the Zn (II) complex (Cotton *et al.*, 1962 and Figgis, 1967).

### SEM and TEM investigations

The physical and microscopic characteristics of solid surfaces are described by SEM pictures for NTZ and its  $[\text{Zn}(\text{NTZ})_2] \cdot \text{Cl}_2$  complex. A tiny particle size with nano feature products is depicted in Fig 3. Using SEM analysis, the surface morphology of NTZ and its  $[\text{Zn}(\text{NTZ})_2] \cdot \text{Cl}_2$  complex was investigated. All particles were shown to have a high capacity to form agglomerates with variety in shape. The compound NTZ and its  $[\text{Zn}(\text{NTZ})_2] \cdot \text{Cl}_2$  complex exhibits tiny kidney-shaped particles with tiny square fragments.

TEM pictures for NTZ and its  $[\text{Zn}(\text{NTZ})_2] \cdot \text{Cl}_2$  Fig 3, is described. A homogenous phase material is confirmed by the pictograph's ordered arrangement of the NTZ metal chelate matrix and its  $[\text{Zn}(\text{NTZ})_2] \cdot \text{Cl}_2$  complex. With particle sizes ranging from 4.90 to 16.80 nm. a spherical black spot shape appears  $[\text{Zn}(\text{NTZ})_2] \cdot \text{Cl}_2$  complex with particle size of 4.90-16.80 nm.

### Changes in kidney functions in different treated groups

Urea, uric acid and creatinine renal levels were used to estimate the renal functions as shown in (Table 2). The marked increment of all parameters in either NTZ or NTZ/Zn complex treated groups were all observed. The increment of urea levels were noticed by significant and noticed elevation to  $29.25\text{ mg dl}^{-1}$  in NTZ treated group only as compared to the normal control group. The uric acid level was elevated to  $21.52\text{ mg dl}^{-1}$  in NTZ treated group as

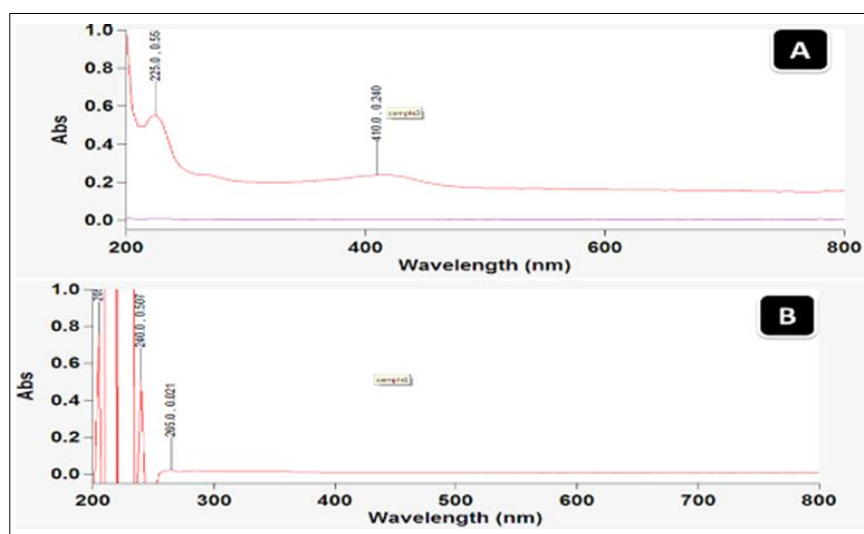


Fig 2: UV-vis for (A) NTZ and (B) NTZ/Zn complex.

compared to the normal control group, respectively. The male mice treated with NTZ only elevated the creatinine levels to  $1.87 \text{ mg dl}^{-1}$  comparison to the control group, meanwhile all these mentioned parameters were restored to about normal levels of control group in NTZ/Zn complex group. Urea, uric acid and creatinine levels declined in the group treated with the NTZ/Zn complex in comparison with NTZ only and confirmed the beneficial renal effect of the NTZ/Zn complex that outperformed the NTZ drug alone.

#### Changes in antioxidant enzymes (SOD, CAT and GPx) and non-enzymatic antioxidant marker of lipid peroxidation (MDA) in different treated groups

SOD, CAT and GPx antioxidant enzyme activities declined markedly in NTZ treated group when compared to the normal control group (Fig 4). Meanwhile, administration of NTZ/Zn complex ameliorated all the estimated antioxidant enzymes (SOD, CAT and GPx) incomparable to the normal control group. The results proved that MDA levels were elevated in the renal tissues of NTZ group (Fig 4). Meanwhile, NTZ/Zn complex administration mitigated the level of MDA stimulated by NTZ treatment only and thus, the used complex NTZ/Zn significantly exhausted the MDA levels in the renal tissues.

#### Oxidative stress regulates the expression of JNK axis

JNK axis was involved in oxidative stress series. The current results indicated that JNK significantly enhanced its

promoter activity indicating the mutation of endoplasmic reticulum site in response to oxidative stress (Fig 5). Notably, we observed a significant increment in JNK activity in NTZ treated group as compared with the control group and NTZ/Zn complex treated group.

#### Histological examination

Microscopic examination of the renal tissues (Fig 6A) of male mice that treated with either NTZ or NTZ/Zn complex (Fig 6 B,C). Control group of male mice showing a normal structure of the renal tissues (Fig 6A). Additionally, the section of male mice renal tissues treated with NTZ shows restoration of the normal renal tubules and glomeruli but with some dilated renal tubules' spaces and some dilation of the vascular glomerular space. Meanwhile, group treated with NTZ/Zn complex restoration of the normal tubules and normal renal glomeruli but with some dilated renal tubules' spaces and some dilation of the vascular glomerular space.

#### Antibacterial activity

The antibacterial properties of NTZ/Zn were studied using *Staphylococcus aureus*. This bacterium of *S. aureus* which is (gram+ve bacterium) was chosen as *Staphylococcus aureus* (*S.aureus*) can cause several serious renal infections and complications, primarily through hematogenous spread (bloodstream infection). Key renal manifestations include acute kidney injury (AKI), renal abscesses and *Staphylococcus*-induced glomerulonephritis

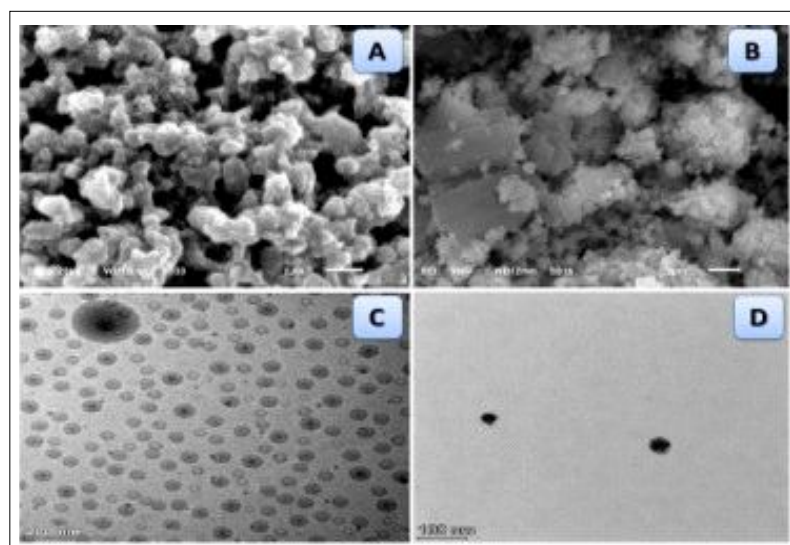


Fig 3: SEM and TEM of (SEM: A: Nita, B: Nita/Zn), (TEM: C: Nita, D: Nita/Zn).

Table 2: Effect on the kidney functions of either control SWR male mice or groups treated with either NTZ or NTZ/Zn complex.

| Parameters         | Control            | NTZ                | NTZ/Zn                |
|--------------------|--------------------|--------------------|-----------------------|
| Urea (mg/dl)       | $18.57 \pm 1.65^c$ | $29.25 \pm 2.41^a$ | $18.98 \pm 0.98^{bc}$ |
| Uric acid (mg/dl)  | $10.58 \pm 2.14^c$ | $21.52 \pm 1.74^a$ | $11.05 \pm 1.65^{bc}$ |
| Creatinine (mg/dl) | $0.74 \pm 0.05^c$  | $1.87 \pm 0.32^a$  | $0.79 \pm 0.098^{bc}$ |

Values are (Mean $\pm$ SE), n=7, NTZ: Nitazoxanide, NTZ/Zn: Nitazoxanide/Zn complex, Significance level ( $p < 0.05$ ).

(SAGN), which may result in rapid decline in renal functions. Measured zones of inhibition ranged from 8-100 mm (Table 3 and Fig 7), reporting that the NTZ/Zn complex generally more skilled performance than DMSO as control. NTZ-Zn (II) showed the highest antibacterial activity at the highest concentration 100 µg/ml. This high antibacterial activity of

NTZ/Zn against *S. aureus* could be attributed to the high electrophilicity of the complexes recoded from the DFT theoretical analysis as electrophilicity which is known primarily as the tendency of a molecule to accept electrons directly correlates with enhanced the antibacterial activity by promoting the bacterial membrane disruption and

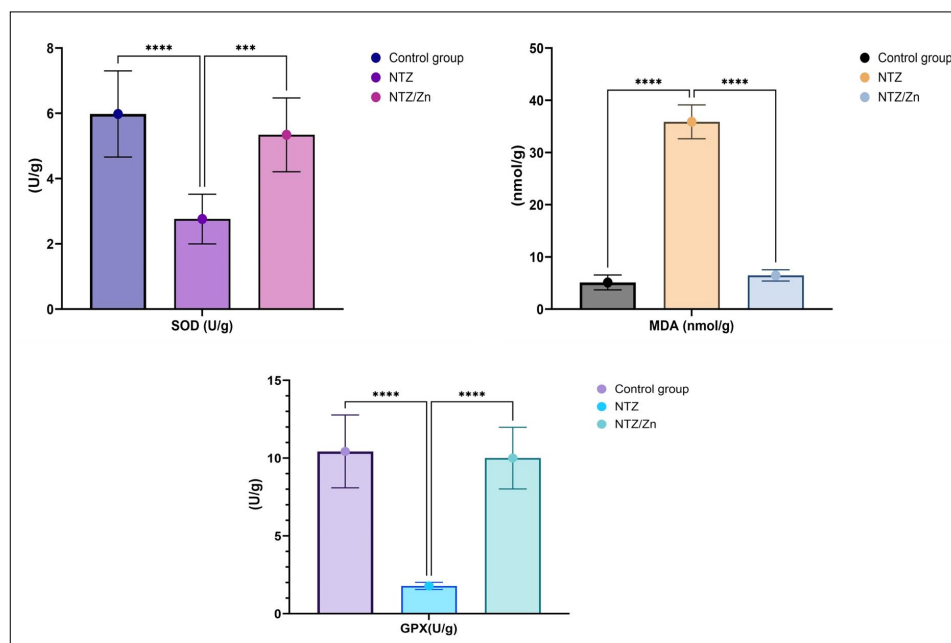


Fig 4: Changes in renal enzymatic antioxidants and non-enzymatic marker in different treated groups (Mean±SE )/n=7.

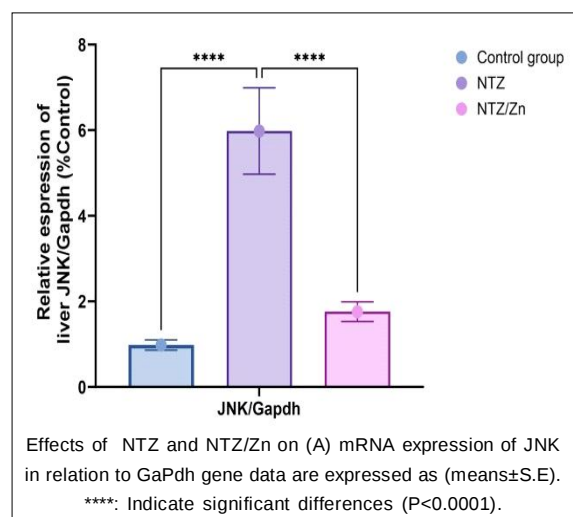


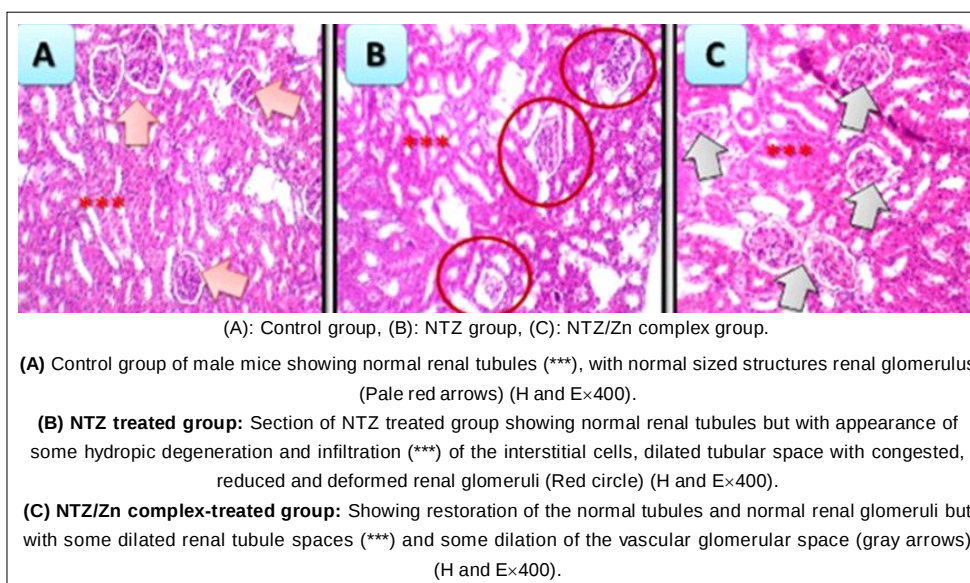
Fig 5: JNK expression in NTZ and NTZ/Zn complex treated groups.

increasing membrane permeability and enhancing excessive production of more reactive oxygen species (ROS) which is essential cause for induction of oxidative stress, DNA damage and can destroy proteins in bacteria, thus from DFT analysis, it is proved that NTZ/Zn possess

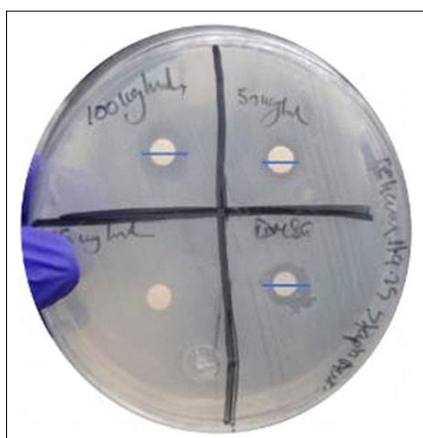
high electrophilic capacities and thus effective target key microbial proteins and genes, accelerating the destruction of bacteria, also electrophilicity is strongly linked to the disruption of bacterial adenosine triphosphate (ATP) synthesis and thus further compromising the cell viability.

NTZ, is a nitrothiazolebenzamide formula (Rossignol and Cavier, 1975). It is a widely known anti-parasitic drug that is commonly used against the protozoal infections Sisson *et al.* (2002). NTZ is known as an inhibitor of the "pyruvate ferredoxin/ flavodoxin oxidoreductases" of the parasites Hoffman *et al.* (2007).

NTZ is absorbed from the GIT, with about 1/3 and 2/3 excretion of the oral dose in both urine and faeces, respectively. Regarding the blood, NTZ is hydrolyzed speedily into desacetyl NTZ derivative, known as tizoxanide (TZX) Broekhuysen *et al.* (2000). TZX is the active metabolite of NTZ *in vivo* Stockis *et al.* (1996). TZX does not block cytochrome P450 enzymes and no interaction has been shown due to NTZ administration with other agents, which are cytochrome P450 enzyme blockers. This concept greatly reinforced the concept of binding of NTZ with Zn metal ion without any adverse effects of interactions, as NTZ did not affect the renal kinetics when two of the drugs were administered Romark (2006), respectively.



**Fig 6 A-C:** Kidney sections stained deeply with H and E.



**Fig 7:** Antibacterial activity of NTZ/Zn against *Staphylococcus aureus*, DMSO was use as a control.

**Table 3:** Diameters of inhibition zones (mm) of DMSO (control) and NTZ/ZN metal complex in three concentrations (25, 50 and 100 µg/ml) against *Staphylococcus aureus*.

| Compound | Concentration (µg/ml) | Zone of inhibition (mm) |
|----------|-----------------------|-------------------------|
| DMSO     | 100                   | 70                      |
| NTZ/Zn   | 25                    | 8                       |
| NTZ/Zn   | 50                    | 80                      |
| NTZ/Zn   | 100                   | 100                     |

NTZ has different adverse effects that included: vomiting, diarrhea, insomnia, abdominal pain, increased renal and hepatic enzymes, tachycardia and sometimes anemia Stockis *et al.* (2002), all these findings come in parallel with the current study findings and the current

findings regarding the biochemical and histological alterations in NTZ treated group, meanwhile restoration of the normal levels in the group treated with NTZ/Zn complex.

In this study, NTZ was used in a dosage of (18 mg/kg) for successive 30 days Albogami (2024). The current results suggested that NTZ treatment elicited a significant increment in the renal parameters (Urea, Uric acid and creatinine) with an increment in the marker of lipid peroxidation (MDA) with concurrent decline in the antioxidant enzymes SOD, CAT and GPx. The current results were in accordance with Shams *et al.* (2018), who found that the effect of NTZ included an increase in renal and hepatic markers with histological alterations.

NTZ significantly afforded a significant increment in renal markers (Urea, Uric acid and creatinine) and these results were in harmony with the results reported by Stockis *et al.* (2002) who found that NTZ afforded high increment in creatinine and urea levels after one day of treatment but contrary to this effect, the current study produced evidence for long term effect after successive 30 days of treatment.

Previous study of Shams *et al.* (2018) confirmed dilation of the congested vascular space between the renal tissues and heavy aggregation of the chronic inflammatory cells. The results of the current study were in accordance with Ferguson *et al.* (2008), who demonstrated that the change in glomerular dynamics, cellular renal tubules' toxicity and renal inflammation is among the general mechanisms that caused the renal functions' alterations, which are all in agreement with the present study findings.

The results of Shams *et al.* (2018) previously suggested that the administration of NTZ for 14 successive days resulted in a significant decrease in antioxidant enzyme activities, including CAT, SOD and GPx activity, after one day post administration. NTZ administration resulted

in a marked increment in MDA activity, which are in complete accordance with the findings of the current study.

Many pathological alterations happened due to the oxidative injury, which is a real marker of NTZ administration and results as imbalance between the oxidants and the antioxidants. Oxidative injury is a significant marker of NTZ significant toxicity. In the current study, MDA level in the NTZ-treated group was elevated significantly and joined by a significant decline in the other estimated antioxidant enzymes in the renal tissue homogenates as compared to the normal control group. SOD and CAT enzymes that were decreased in the NTZ-treated group are considered the primary defense antioxidant enzymes that have the potency to arrest the oxidative injury that could be induced by excessive production of the reactive oxygen species (Al-Eisa *et al.*, 2018).

CAT and GPx enzymes conserve SOD enzyme against  $H_2O_2$  inactivation. Alternatively, SOD conserves CAT and GPx against superoxide anion via the sudden dismutation of superoxide anion ( $O_2^-$ ) to  $O_2$  and  $H_2O_2$ . Meanwhile, the excessive production of the free radicals greatly disturbs these antioxidant mechanisms and regulations and this gives the real scientific explanation of the induction of oxidative stress induced by NTZ and the ameliorative antioxidant effect of NTZ/Zn complex in the amelioration of these antioxidant parameters as previously confirmed in a lot of previous studies (Hamza and Alsolami, 2024; El-Megharbel *et al.*, 2024; AlZahrani *et al.*, 2025; Al-Thubaiti *et al.*, 2025; Hamza and Alsolami, 2023).

It was confirmed that increment of the  $H_2O_2$  production could activate JNK series cells, resulting in immediate cell apoptosis (Weng *et al.*, 2016). In the current study, the JNK activity was markedly elevated in the  $H_2O_2$ -treated NTZ group. The current results showed that JNK is essentially involved in the regulation of the process of the cellular apoptosis mechanism; meanwhile, the expression of JNK was declined in NTZ/Zn complex-treated group, which confirmed the decline in oxidative stress series and thus confirmed all the biochemical analysis.

All the previous studies confirmed the concept of the current study which confirmed that administration of NTZ in the therapeutic dosage induced alteration in some renal functions with histological changes, meanwhile the used complex of NTZ/Zn greatly reduced any renal alteration either in biochemical or histological structures and produced noticeable ameliorative effect in renal functions beside amelioration of the antioxidant capacities by great notice of elevation of the antioxidant enzymes in the renal tissues and marked decline in the lipid peroxidation marker levels (MDA) such induced by treatment of NTZ alone.

Synthesized NTZ/Zn complexes were tested for their antibacterial efficacy via using the bacterial pathogen *S. aureus*. The antibacterial activity of the NTZ/Zn complex was evaluated via three concentrations and by measuring the diameter of the inhibition zone and comparing it with DMSO as a control standard, NTZ/Zn exhibited antibacterial activity via concentration gradients, recording the highest activity at

100 µg/ml concentration and based on the DFT analysis that showed high electrophilicity activity, this may be the main cause of the bacterial membrane damage and excessive production of reactive oxygen species (ROS) and thus it exhibited high antibacterial activity against serious infections caused by the *S. aureus* strain (Chai *et al.*, 2024).

Accordingly, in line with the previous research of Mansour (2016), especially with the appearance of methicillin-resistant strains of *S. aureus*, there is growing bacterial resistance towards the recent antibiotics and thus the recent scientific trend regarding the new effective antimicrobial agents is of real importance as a key that has a vital role. And as the finding of new drugs with new unstudied chemical structures is a very expensive way and also takes a lot of time, it is thus considered time-consuming. Thus, the modification of the molecular structure of the used drugs is a better way, especially with the improvement of the drug interactions. Thus, this is great confirmation for this scientific concept in our recent study, which confirmed the high susceptibility of the *S. aureus* strain towards the NTZ/Zn complex, which is considered promising against this serious bacterial strain.

## CONCLUSION

NTZ/Zn recent complex was synthesized and tested on the kidney with its comparison with NTZ treatment only to evaluate the signs of renal toxicity and enhancing the antioxidant activity. The bidentate characteristic of NTZ through the nitrogen atom of the amide group and oxygen atom of the ester group toward Zn (II) metal ion was confirmed using IR, UV for NTZ and its complex (NTZ/Zn). SEM results showed that small particles can be agglomerated with different shapes. TEM showed that NTZ/Zn complex has spherical black spots with particle sizes 4.90 nm to 93.87 nm. Also, NTZ/Zn showed antibacterial activity against *S. aureus* strain, the highest activity was recorded at conc. 100 µg/ml. Regarding the biological effect of the newly synthesized complex, NTZ treatment for successive 30 days caused renal damage and effect on the kidney functions. NTZ/Zn complex ameliorated these biochemical and histological changes. Renal tissues' treatment with NTZ/Zn complex showed an amelioration in renal glomerulus and renal tubules. Therefore, NTZ/Zn complex had the potent ability to scavenge the excessive production of free radical activities and it may produce potent beneficial effects against the potential renal injury produced by the drug NTZ alone. Additionally, NTZ intake must be administered in restriction or in complexation with Zn for safer anti-parasitic treatment.

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## Conflict of interest

All authors declare that there is no any conflict of interest.

## REFERENCES

- Aebi, H. (1984). Catalase *in vitro*. *Methods Enzymol.* **105**: 121-126.
- Albogami, B. (2024). Ellagic acid ameliorates hepatic functions against the hepatic alterations induced by nitazoxanide (antiprotozoal drug) in male rats. *Int. J. Pharmacol.* **20(7)**: 1198-1208.
- Al-Eisa, R.A., Hamza, R.Z., Mehana, A.E. and El-Shenawy, N.S. (2018). The influence of L-carnitine on aspartame toxicity in kidney of male rats. *Int. J. Pharmacol.* **14(8)**: 1118-1127.
- Alonso, D.F. and Farina, H.G. (2020). Repurposing of host-based therapeutic agents for the treatment of coronavirus disease 2019 (COVID-19): A link between antiviral and anticancer mechanisms? *International Journal of Antimicrobial Agents.* **56(3)**: 106125.
- Al-Thubaiti, E.H., Hamza, R.Z., Alaidaroos, B.A., Al-Gheffari, H.K., Albaqami, N.M. and El-Megharbel, S.M. (2025). Chemical characterization of pollen grains ethanolic extract (PGE) with evaluation of its potent antioxidant, antibacterial and anti-cancer activities against hepatocellular carcinoma (HepG2). *Indian Journal of Animal Research.* **59(10)**: 1653-1662. doi: 10.18805/IJAR.BF-1953.
- AlZahrani, S.S., El-Megharbel, S.M., Al-Thubaiti, E.H., Alghamdi, M.A., Hassoubah, S.A., Qattan, S.Y.A., Alaidaroos, B.A., Albaqami, N.M., AL-Harbi, M.S. and Hamza, R.Z. (2025). Chemical and spectroscopic characterization of novel dexamethasone/zinc complex as a potent antioxidant and antibacterial agent. *Indian Journal of Animal Research.* **59(8)**: 1329-1340. doi: 10.18805/IJAR.BF-1939.
- Amadi, B., Mwiya, M., Musuku, J., Watuka, A., Sianongo, S., Ayoub, A. and Kelly, P. (2002). Effect of nitazoxanide on morbidity and mortality in Zambian children with cryptosporidiosis: A randomized controlled trial. *The Lancet.* **360**: 1375-1380.
- Bellamy, L.J. (1975). *The Infrared Spectra of Complex Molecules*, Chapman and Hall, London, UK.
- Beutler, E., Duron, O. and Kelly, B.M. (1963). Improved method for the determination of blood glutathione. *J. Lab. Clin. Med.* **61**: 882-888.
- Broekhuysen, J., Stockis, A., Lins, R.L., Graeve, J. and Rossignol, J.F. (2000). Nitazoxanide: pharmacokinetics and metabolism in man. *Int. J. Clin Pharmacol. Ther.* **38(8)**: 387-394.
- Chai, L., An, H., Duan, Y. and Chai, Y. (2024). Antimicrobial activities and theoretical studies on the weak interactions formation in two different configuration complexes. *Journal of Molecular Structure.* **1295(1)**: 136727.
- Colín-Lozano, B., León-Rivera, I., Chan-Bacab, M.J., Ortega-Morales, B.O., MooPuc, R., López-Guerrero, V., Hernández-Núñez, E., Argüello-García, R., Scior, T. and Barbosa-Cabrera, E. (2017). Synthesis, *in vitro* and *in vivo* giardicidal activity of nitrothiazole-NSAID chimeras displaying broad antiprotozoal spectrum. *Bioorganic and Medicinal Chemistry Letters.* **27**: 3490-3494.
- Cotton, F.A., Goodgame, D.M.L. and Goodgame, M. (1962). Magnetic and spectral properties of the spin-free 3d6 systems iron(II) and cobalt(III) in cobalt(III) hexafluoride ion: Probable observation of dynamic jahn-teller effects. *J. Am. Chem. Soc.* **84**: 167.
- Di Santo, N. and Ehrisman, J. (2014). A functional perspective of nitazoxanide as a potential anticancer drug. *Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis.* **768**: 16-21.
- Elazar, M., Liu, M., McKenna, S.A., Liu, P., Gehrig, E.A., Puglisi, J.D., Rossignol, J.F. and Glenn, J.S. (2009). The anti-hepatitis C agent nitazoxanide induces phosphorylation of eukaryotic initiation factor 2alpha via protein kinase activated by double-stranded RNA activation. *Gastroenterology.* **137**: 1827-1835.
- El-Megharbel, S.M., Alsawat, M., Al-Salmi, F.A. and Hamza, R.Z. (2021). Utilizing of (Zinc oxide nano-spray) for disinfection against "SARS-CoV-2" and testing its biological effectiveness on some biochemical parameters during (COVID-19 pandemic)-"ZnO nanoparticles have antiviral activity against (SARS-CoV-2)". *Coatings.* **11(4)**: 388.
- El-Megharbel, S.M., Qahl, S.H. and Albogami, B. (2024). Chemical and spectroscopic characterization of (Artemisinin/ quercetin/zinc) novel mixed ligand complex with assessment of its potent high antiviral activity against SARS-CoV-2 and antioxidant capacity against toxicity induced by acrylamide in male rats. *Peer J.* **12**: e15638.
- Fan-Minogue, H., Bodapati, S., Solow-Cordero, D., Fan, A., Paulmurugan, R., Massoud, T.F., Felsher, D.W. and Gambhir, S.S. (2013). Ac-Myc activation sensor-based high throughput drug screening identifies an anti-neoplastic effect of nitazoxanide. *Molecular Cancer Therapeutics.* **12**: 1896-1905.
- Ferguson, M.A., Vaigya, V.S. and Bonventre, J.V. (2008). Biomarkers of nephrotoxic acute kidney injury. *Toxicology.* **245(3)**: 182-193.
- Figgis, B.N. (1967). "Introduction to Ligand Fields", Interscience Publishers, John Wiley and Sons, New York. pp. 285.
- Fossati, P., Prencipe, L. and Berti, G. (1980). Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. *Clin. Chem.* **26**: 227-231.
- Hamza, R.Z. and Alsolami, K. (2023). Ameliorative effects of Orlistat and metformin either alone or in combination on liver functions, structure, immunoreactivity and antioxidant enzymes in experimentally induced obesity in male rats. *Heliyon.* **9(8)**: e18724.
- Hamza, R.Z. and Alsolami, K. (2024). Orlistat and metformin combination ameliorates obesity-induced renal injury via suppressing renal oxidative stress in male rats. *Toxicology Research.* **13(4)**: tfae135.
- Hemphill, J., Mueller, M. and Esposito, M. (2006). Nitazoxanide, a broad-spectrum thiazolide anti-infective agent for the treatment of gastrointestinal infections. *Expert Opinion on Pharmacotherapy.* **7**: 953-964.
- Hoffman, P.S., Sisson, G., Croxen, M.A., Welch, K., Harman, W.D., Cremades, N. and Morash, M.G. (2007). Antiparasitic drug nitazoxanide inhibits the pyruvate oxidoreductases of *Helicobacter pylori*, selected anaerobic bacteria and parasites and *Campylobacter jejuni*. *Antimicrob Agents Chemother.* **51(3)**: 868-876.
- Hong, L., Lü, Z., Zhao, Z.D., Wang, X.Y., Leng, H.J., Niu, Y. and Wang, M.P. (2021). Nitazoxanide, an antiprotozoal drug, reduces bone loss in ovariectomized mice by inhibition of RANKL-induced osteoclastogenesis. *Front Pharmacol.* **12**: 781640.
- Lokhande, A.S. and Devarajan, P.V. (2021). A review on possible mechanistic insights of Nitazoxanide for repurposing in COVID-19. *European Journal of Pharmacology.* **891**: 173748.

- Mansour, A.M. (2016). Spectroscopic, DFT, magnetic and biological activity evaluation of Pd (II), Pt (II) and Ru (III) complexes of nitazoxanide. *Inorganica Chimica Acta*. **453**: 697-703.
- Marklund, S. and Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem.* **47**: 469-474.
- Nakamoto, K. (1970). *Infrared Spectra of Inorganic and Coordination Compounds*, 2<sup>nd</sup> edition Wiley Interscience, John Wiley and Sons, New York, NY, USA.
- Pal, M., Musib, D. and Roy, M. (2021). Transition metal complexes as potential tools against SARS-CoV-2: An *in silico* approach. *New Journal of Chemistry*. **45**: 1924-1933.
- Patton, C.J. and Crouch, S.R. (1977). Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. *Anal. Chem.* **49**: 464-469.
- Romark (2006). Alinia (Nitazoxanide) Tablets. (Nitazoxanide) for Oral Suspension. Romark Pharmaceuticals. Nov 24.
- Rossignol, J.F. and Cavier, R. (1975). New derivative of 2 benzamide 5 nitrothiazoles. *Chem. Abstr.* **83**: 28216.
- Schirmeister, J., Willmann, H. and Kiefer, H. (1964). Critical evaluation of plasma creatinine as a test of glomerulus filtrate. *Verhandlungen Deutschen Gesellschaft Innere Med.* **70**: 678-681.
- Shams, G., Fouad, A. and Naiem, N. (2018). Nitazoxanide adverse effects on biochemical markers of liver and kidney injury and antioxidant enzymes on rats. *International Journal of Pharmaceutical Research and Allied Sciences*. **7(4)**: 1-6.
- Sharfalddin, A.A., Al-Younis, I.M., Emwas, A.H. and Jaremko, M. (2023). Investigating the biological potency of nitazoxanide-based Cu(II), Ni(II) and Zn(II) complexes synthesis, characterization and anti-covid-19, antioxidant, antibacterial and anticancer activities. *Molecules*. **28**: 6126.
- Sharfalddin, A.A., Emwas, A.H., Jaremko, M. and Hussien, M.A. (2021). Complexation of uranyl (UO<sub>2</sub>)<sup>2+</sup> with bidentate ligands: XRD, spectroscopic, computational and biological studies. *PLoS One*. **16(8)**: 0256186.
- Sisson, G., Goodwin, A. and Raudonikiene, A. (2002). Enzymes associated with reductive activation and action of nitazoxanide, nitrofurans and metronidazole in *Helicobacter pylori*. *Antimicrob Agents Chemother.* **46(7)**: 2116-2123.
- Stockis, A., Allemon, A.M., DeBruyn, S., Gengler, C. and Rosillon, D. (2002). Nitazoxanide pharmacokinetics and tolerability in man using single ascending oral doses. *Int. J. Clin. Pharmacol. Ther.* **40(5)**: 213-220.
- Stockis, A., Deroubaix, X., Lins, R., Jeanbaptiste, B., Calderon, P. and Rossignol, J.F. (1996). Pharmacokinetics of nitazoxanide after single oral dose administration in 6 healthy volunteers. *Int. J. Clin. Pharmacol. Ther.* **34(8)**: 349-51.
- Tweedy, B.G. (1964). Plant extracts with metal ions as potential antimicrobial agents. *Phytopathology*. **55(8)**: 910-914.
- Weng, Q., Liu, Z., Li, B., Liu, K., Wu, W. and Liu, H. (2016). Oxidative stress induces mouse follicular granulosa cells apoptosis via JNK/FoxO1 pathway. *Plos One*. **11(12)**: e0167869.
- White, A.C. (2004). Nitazoxanide: A new broad spectrum antiparasitic agent. *Expert Review of Anti-Infective Therapy*. **2**: 43-49.