Spectrophotometric and Toxicological Investigation of Thioridazine Hydrochloride in Tablets, Biological Fluids, and Albino Rats

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ABSTRACT

Two simple, sensitive, and selective spectrophotometric methods have been described for the determination of the psychoactive drug, thioridazine HCl (THZ) in dosage forms and in biological fluids. The methods are based on the reaction of THZ with measured excess of $KMnO_4$ under acidic conditions followed by the determination of unreacted oxidant using indigo carmine and methyl orange. Optimization of the different experimental conditions is described for both methods. The methods have been successfully applied to the assay antipsychotic drug in pharmaceutical formulations and biological fluids. For toxicological studies, Twenty-eight mature albino rats of both sexes were used for toxicological studies. The rats were divided equally into four group's seven rats from each sex intraperitoneally administered THZ (5 mg/kg B. wt) once daily for one month, seven rats from each sex served as control. Results revealed significant increase in the level of the enzyme activities of both superoxide dismutase (SOD) and catalase and significant decrease in gonadotrophins hormones luteinizing hormone (LH) and follicular stimulating hormone (FSH) in serum samples of treated groups. Histopathological findings of liver, brain and the reproductive organs proved the previously mentioned results.

INTRODUCTION

Thioridazine hydrochloride (THZ), the hydrochloride of 10-[2-(lmethyl-2-piperidyl)ethyl]-2-methylthiophenothiazine, is a phenothiazine neuroleptic drug used for the treatment of schizophrenia and other psychiatric disorders *(1)*. Phenothiazines also possess antiemetic, sedative, antipruritic, antidyskinetic, analgesic and antihistaminic properties *(2)*. Thioridazine has been known as one of the most potent phenothiazines, which inhibit trypanothione reductase irreversibly *(3)*. Additionally, thioridazine was effective in the treatment of sweating *(4)*, as an antidepressant and sedative drug. The ranking of phenothiazines, in terms of cytotoxic activity corresponds with that reported in the literature for calmodulin-inhibiting potency. The principle pharmacological function of phenothiazine is related to their ability to inhibit dopaminic receptors of CNS. Because of this, are able to create an indifferent psychomotive being very effective in stress situations. For this reason, they are suspected to be administered in animals, in order to decrease their motive activity and aggressiveness *(5)*. It is conjectured that the cytotoxic effect of phenothiazines could be wholly or partially explained based on their interaction with calmodulin. The most toxic drug tested was thioridazine with SCH_3 at position 2 and the amino group incorporated into a piperazine ring. Thiorizadine also showed the greatest degree of specificity for hypoxic cells *(6)*.

The phenothiazine – derived antipsychotics, namely chlorpromazine and thioridazine, have been associated with very rare but severe incidences of hepatotoxicity in patients. While the mechanism of idiosyncratic hepatotoxicity remains unknown, it is possible that metabolic activation and subsequent covalently bending of reactive metabolites to cellular proteins play a causative role *(7)*.

Due to the clinical importance of thioridazine hydrochloride, it is significant to establish a simple and sensitive method for its determination in biological fluids. Different pharmacopoeias recommend the determination of thioridazine base or the hydrochloride by titrating the drug in glacial acetic acid and acetic anhydride against perchloric acid. The BP

recommends the titration of the drug in acetone solution containing about 7% mercury (II) acetate solution and using methyl orange as indicator *(8)*. Several analytical methods have been described for determination of thioridazine hydrochloride. Among the methods, UV spectrophotometry and conventional high performance liquid chromatography *(9)*, are most often used. Voltammetry *(10)*, spectrophotometry *(11)*, and chemiluminescence method *(12)*, have been reported for the determination of thioridazine hydrochloride. Supercritical fluid chromatography *(13)*, and LC combined with other techniques *(14)*.

Experimental

Part I: Spectrophotometric studies

Equipment

An spectroscan 80 D double-beam UV/visible spectrophotometer (Biotech Engineering Ltd., UK), with wavelength range 190 nm-1100 nm, spectral bandwidth 2.0 nm with matched 10 mm quartz cells were used for all the absorbance spectral measurements.

Chemicals and materials

All chemicals used were of an analytical reagent grade and solutions were made in distilled water.

Indigo carmine (IC)

A 5×10^{-3} M of indigo carmine (Aldrich) was prepared by dissolving of an accurate weight dye (99% purity) in water and diluting to 100 mL in a calibrated flask.

Methyl orange (MO)

A 5×10^{-4} M of methyl orange was prepared by dissolving an accurate weight of dye in least amount of water and completed to the mark in a 100 mL calibrated flask.

Potassium permanganate

A stock solution of 1.0×10^{-3} M $KMnO_4$ (Aldrich) was prepared by dissolving an accurate weight in 10 mL of warm distilled water, then completed to the mark in a 100 mL calibrated flask and standardized using sodium oxalate. A 2.0 M H_2SO_4 was prepared.

Thioridazine hydrochloride (THZ)

Pharmaceutical grade thioridazine hydrochloride was received as a gift from Delta Pharm Company, 10th of Ramadan, Egypt; it was reported to be 99.8% pure and was used as received. A stock standard solution equivalent to 10 mg of THZ was prepared by dissolving an accurately weighed amount of pure drug in distilled water. The solution of THZ was diluted stepwise to obtain working concentration of 100 $\mu g\ mL^{-1}$. The standard solutions were kept in amber colored bottles and stored in a refrigerator when not in use.

Recommended procedures and calibration curves

Oxidation reaction using $KMnO_4$

Varying aliquots (0.1 – 0.7 mL) of the standard 100 $\mu g\ mL^{-1}$ THZ solution were transferred into a series of 25 mL calibrated flasks by means of a micro burette. To each flask, 2.0 mL of 2.0 M H_2SO_4 and 1.2 mL of 1.0×10^{-3} M $KMnO_4$ solution were added. The content was mixed well and the flasks were kept aside for 20 min, at room temperature with intermittent shaking. Finally, 1.1 mL of 5×10^{-3} M of IC and 1.4 mL of 5.0×10^{-4} M of MO solution were added to each flask and the volume was diluted to the mark with water and mixed well. The absorbance was measured against a reagent blank at 610 nm and 508 nm, respectively. In both methods, a standard graph was prepared by plotting the absorbance *vs.* the concentration of THZ. The unknown concentration was read from the calibration graph or computed from the regression equation derived using Beer's law data.

Procedure for pharmaceutical preparations

At least twenty of THZ tablets (Thiozine tablets, Delta Pharm Company, Cairo Egypt, are labeled to contain 25 mg of thioridazine HCl per tablet) were weighed to obtain the mean tablet weight and then ground to a homogenized powder. A quantity of the powdered tablets equivalent to 30 mg was transferred into a 100 mL calibrated flask and dissolved in distilled water and filtered. A suitable amount of filtrate was taken and analyzed as described under

recommended procedures. For the proposed methods, the content of a tablet was calculated using the corresponding regression equation of the appropriate calibration graph.

Procedure for spiked urine and serum

The proposed methods were applied to the determination of THZ in spiked urine and serum provided from several healthy volunteers. Spiked urine was 50-fold diluted with distilled water. A 10 mL of serum sample was deproteinzed by adding 5 mL of acetonitrile in a centrifuge for 5 min at 1000 rpm. The supernatant was used to investigate recovery. Add an aliquot of standard aqueous solution of THZ to 1.0 mL of diluted urine or serum. The analysis was completed as in the recommended procedures. A blank value was determined by treating THZ-free urine and THZ-free serum in the same way. The absolute recovery was determined for THZ by comparing the representative absorbance of the treated urine or serum samples with the absorbance of the standard drug at the same concentration.

Part II: Toxicological studies

Dose of THZ: 5 mg/kg B. wt according to *(15)*. Intraperitoneally administered once daily for one month after dissolving in the suitable vehicle (distilled water).

Animals

Twenty eight mature albino rats of both sex (14 males and 14 females) were obtained from laboratory animal breeding unit (Faculty of Veterinary Medicine, Zagazig University) weighting 140 to 170 g. Animals were classified and treated as depicted in Table 1. Rats were kept in metal cages during the whole experimental period under hygienic conditions, fed on well balanced ration and provided with water *ad-libtum*, through the experiment. The experiment was terminated after one month of first administration, where the animals were sacrificed.

Sampling and analysis

Hormonal estimation

Blood samples were collected at the end of the experiment for separation of the serum which kept at -20 °C till used. Serum LH, FSH were estimated using electrochemiluminescence immunoassay according to *(16)*.

Biochemical analysis

Serum levels of antioxidant enzymes as superoxide dismutase (SOD) was assayed by *(17)*, and catalase was determined according to *(18)*.

Histopathological examination

Specimens were collected from liver, brain, ovary and testis and fixed in 10% neutral buffer formalin then processed for histopathological investigation according to *(19)*.

Statistical analysis

The obtained data were analyzed statistically using ANOVA test. The level of significance was accepted at $p \leq 0.05$.

Table 1.Classification of the experimental groups.

Groups	Sex	No	Treatment (dose, duration)
1st group G1	Male	7	5mg/kg B.wt. I/P administered once daily for one month
2nd group G2 (control)		7	----------
3rd group G3	Female	7	5mg/kg B.wt. I/P, administered once daily for one month
4th group G4 (control)		7	----------

RESULTS AND DISCUSSION

Optimization of variables

The optimum conditions for the assay procedures have been established by studying the reactions as a function of reaction time, concentration of reagents, and stability of the colored species. Such variables were changed individually while the others were kept constant.

Oxidation methods

In the present method, two dyes indigo carmine and methyl orange have been used for the determination of THZ. The determinations of THZ are indirect and are based on the determination of surplus $KMnO_4$ after the oxidation reaction of THZ by $KMnO_4$, (Fig. 1) according to the reaction scheme given in Scheme 1 ***(20)***.

Effects of temperature and reaction time

Keeping other conditions constant, the effect of temperature on the oxidation product was studied. The reaction between THZ and $KMnO_4$ was found to be instantaneous. However, the reaction is complete within 15 min at room temperature (25±2 °C), but 20 min was sufficient to get maximum intensity. Therefore, 20 min at room temperature have been selected for further experiments.

Effect of acid concentration

Preliminary investigation showed that sulphuric acid was the medium of choice for the oxidation of THZ by $KMnO_4$. Two milliliters of 2.0 M H_2SO_4 was ideal for the oxidation step in both methods and the same quantity of acid was employed for the estimation of the dye. It was found the maximum color was developed within 20 min and remained almost stable for about 24 h.

Effect of oxidant concentration

The effect of oxidant concentration was studied by adding different volumes of 1×10^{-3} M of $KMnO_4$ solution to a constant amount of THZ (2.4 μg mL^{-1}). It was observed that the maximum color intensity was obtained with 1.2 mL of $KMnO_4$, after which further increase in volume resulted in a decrease of absorbance. Thus, 1.2 mL of $KMnO_4$ was sufficient to reach with the maximum drug concentration in the Beer's range.

Effect of dye concentration

The effect of the dye-concentration on the intensity of the color developed at the selected wavelengths was studied separately by measuring the absorbance's of final solutions resulting from reaction mixtures containing a fixed concentration of THZ (2.4 μg mL^{-1}) and various amounts of dyes (0.5–5 mL). The use of 1.1 mL of 5×10^{-3} M IC and 2.8 mL 5×10^{-4} M of MO, were found to be necessary to produce constant absorbance values. The use of excess of reagent produced no further increase in absorbance.

Analytical data

The calibration graphs were constructed for the two methods under the optimum conditions described above. The molar absorptivity, concentration range, regression equation, and correlation coefficient are tabulated in Table 2. A linear relationship was found between the absorbance at λ_{max} and the concentration of the drug in the range 0.4 – 3.2 μg mL^{-1}. Regression analysis of the Beer's law plotted at λ_{max} reveals a good correlation (r^2 = 0.9980 – 0.9993). The graphs showed a negligible intercept, which was calculated by the least squares method's regression equation. The relative sensitivities of the reagents can be determined by comparing the molar absorptivities of the complexes.

Accuracy and precision

The inter-day repeatability of the proposed methods was studied by performing five independent analyses of THZ in pure form at three different concentration levels on six consecutive days (Table 3). The intraday reproducibility of the proposed method was determined by measuring the drug at three concentration levels within one day five times (Table 3). The reagent solutions were prepared freshly and analyzed as described under recommended procedures and calibration graphs. Data of Table 3 show that within day the relative standard deviations are less than 1.8%

and inter-day relative standard deviations are not exceeding 1.88%, which indicates that the proposed methods are highly reproducible.

Analytical applications

a- Analysis of tablets

The proposed methods were successfully applied to the determination of THZ in representative tablets and the results are summarized in Table 4. For the brands/doses examined, the methods gave results, which were in agreement with the declared content. The performance of the proposed methods was judged by calculating the student *t*- and *F*-values. At 95% confidence level, the calculated *t*- and *F*-values did not exceed the theoretical values as evident from Table 4. Hence, it was concluded that there is no significant difference between the proposed and official methods *(8)* with respect to accuracy and precision. Moreover, to check the validity of the proposed methods, we applied the standard addition method by adding THZ to the previously analyzed tablets. The recovery of THZ was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug and the recoveries of added quantity were found to be more than 98.74%. This indicates that there is no interference from any excipients, which are present in tablets. These results are given in Table 5.

b- Analysis of human samples

The quantitative assessment of added THZ in serum and urine of healthy human beings is also determined as shown in Table 6. High accuracy and good recovery are obtained, which indicates that the proposed methods can be successfully applied to recover thioridazine in human samples.

Toxicological and biochemical results

Concerning the effect of THZ on the activity of antioxidant enzymes, Table 7 is showing significant increase in the levels of both serum and brain SOD in male and female treated rats comparing with their control groups. While, there was no significant difference when comparing results of treated male groups with those of treated female ones in both levels of SOD either in serum or in brain samples.

The results of catalase enzyme activity were demonstrated in Table 7 which showed significant increase in the level of its activity in serum samples of both male and female treated groups compared to the control one. Also, level of catalase enzyme activity in brain samples were shown in Table 7, which revealed significant increase in the treated female only but male groups showed non significant increase compared to the control one.

Aforementioned results came in harmony with findings recorded by *(21)*, who found in the plasma of schizophrenics, that neuroleptics increase the amounts of Mn-SOD in the brain of psychotics. In addition, our results coincide with those obtained by *(22)*, who reported that the in vivo administration of thioridazine in fed of mice revealed a significant increase of liver residual catalase activity RCA.

In contrary to our results *(23)*, reported that neuroleptics as chlorpromazine equivalents (thioridazine and others) did not affect level of antioxidant enzyme activities especially SOD and GSH-Px giving reason that the dose and the duration of treatment with drugs have no influence on the results, it can be interpreted that the findings are more likely to be related mainly to the underlying disease rather than to the drugs. The changes in the activities of these antioxidant and oxidant enzymes studied in schizophrenic patients seem to be independent from antipsychotic treatment and may reflect the pathophysiological process of the disease. The increase in the activity of antioxidant enzymes demonstrated in the present work may be also, confirmed by the histopthological findings of the brain of both sexes of albino rats.

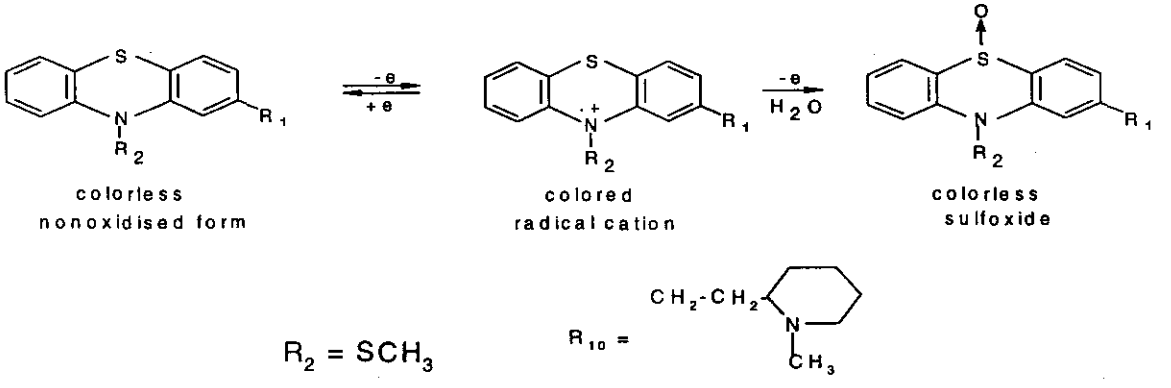
The current study proved that their was a significant decrease in both gonadotrophine hormones LH and FSH estimated in male and female treated groups compared to their control ones, as shown in Table 8. In this direction our results are nearly similar with those obtained *(24)*, used single subcutaneous doses of predominantly antiadrenergic neuroleptics (thioridazine, propericiazine) significantly depressed the preovulatory serum LH surge. FSH titers were significantly altered by thioridazine. The results, suggest a stimulatory

role of norepinephrine/epinephrine in LH-RH secretion and an inhibitory action of dopamine on the same process. The level of LH and FSH investigated in the current work could be confirmed by the histopathological changes observed in the examined testis and ovary.

Regarding the histopathological findings. Treatment of both male and female rats with THZ revealed similar lesions of the examined liver and brain samples. Meanwhile, the reproductive organs; testis and ovary showed various histological lesions (Figs. 2-9). Liver revealed proliferation duct epithelium forming numerous bile ductules and congested portal veins were observed in (Fig. 3). The hepatic cells had hydropic degeneration hyperplastic kupffers cells were seen. These results are nearly on the same context with those observed by *(25)*, who showed rigid lamellae in Kupffer cell lysosomes of rats treated with thioridazine for two weeks in liver cells. In addition, the same results obtained showed that irregularly spaced lamellae in liver parenchymal cells of a patient with pseudo-neonatal adrenoleukodystrophy. On the other aspect, our results are nearly in an agreement with that investigated by *(26)*, reported that there are late preventive effects of anticalmodulin drug (Thioridazine) on galactosamine-induced liver necrosis.

Brain tissue showed of a few psychotic neurons with per cellular edema was common in cerebellum (Fig. 5). Congested and memingeal and cerebral blood vessels with focal areas of demyelization were seen. Perivascular lymphomas aggregations in Virchow robin space and focal gliosis could be cleared. Our results were in partial agreement with *(27)*, who reached to that thioridazine administration causes undernourishment which is known to influence myelination in developing rat brain. However, known consequences of undernourishment, such as decreased myelin concentration in whole brain, decreased percentage of myelinated fibers, and decreased granule-to-Purkinje cell ratio are not present.

The histopathological findings of the testis by investigated sever thickening of tunica albugenia by fibrous tissue with disintegrations of some spermatogonial cells and spermatid of seminiferous tubules (Fig. 7). Interstitial edema, congested blood vessels and mild spermatogenesis in other tubules could be seen. Ovaries showed partial destruction of surface germinal epithelium absence of ovum and degenerated theca cells together with mild fibrous strands in the stroma as appearin (Fig. 9). The effects of THZ on the reproductive organs (testis and ovaries) like other phenothiazines such as chlorpromazine where our results were consistent to those recorded by *(28)*, who observed that chlorpromazine treated rats revealed various testicular lesions included moderate to sever degeneration of seminiferous tubular epithelium. Also our ovarian findings approximately similar to that obtained by *(29)*, who found that chlorpromazine administration in female rats resulted in ovarian toxicity; increase atretic follicles with various atrophic findings.



Scheme 1. The probable mechanism of the oxidation of THZ.

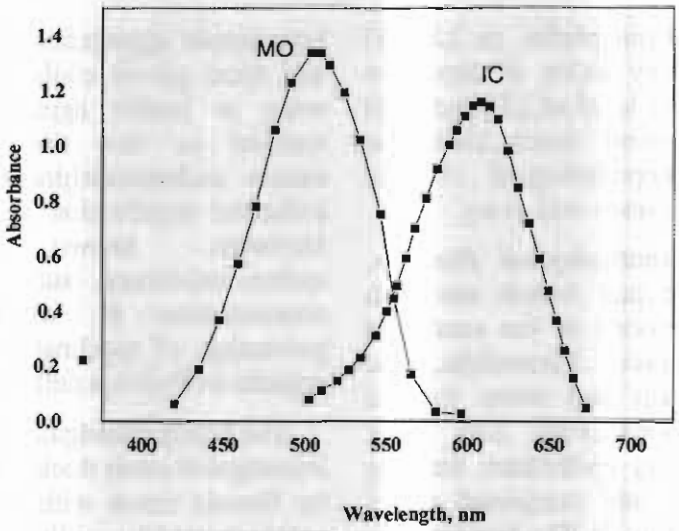


Fig. 1: Absorption spectra of the reaction products.

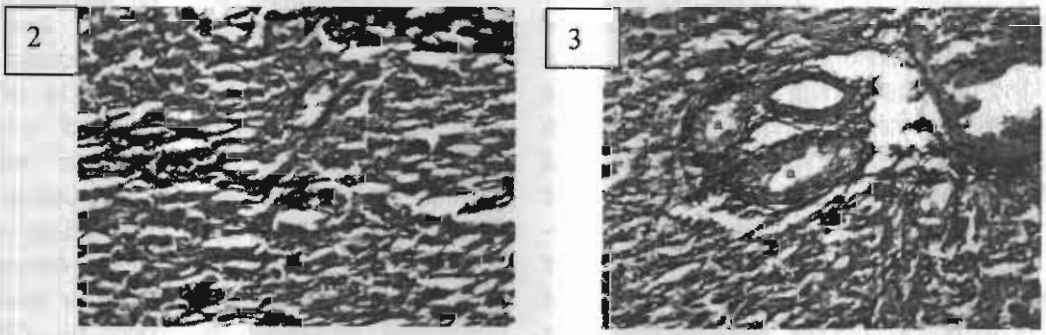


Fig. 2: Section in liver of control male albino rat showing normal histological structure, H & E. x 300.

Fig. 3: Section in liver of male albino rat I/P administered with thioridazine (5mg/kg B.wt) once daily for one month showing (a) numerous bile ductules and (b) congested portal vein, H & E. x 300.

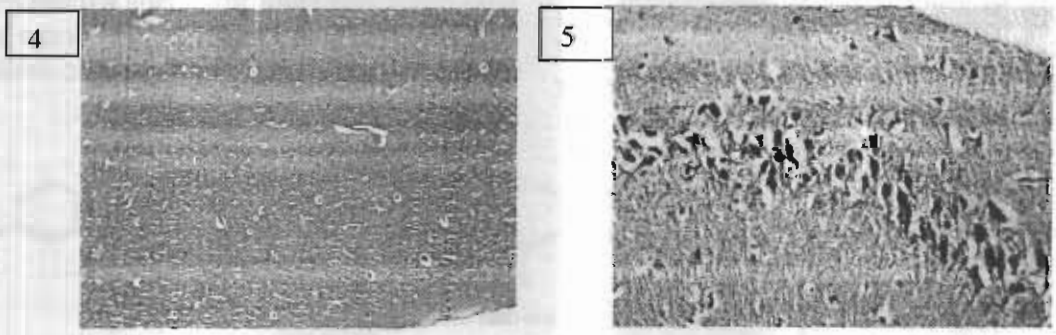


Fig. 4: Section in brain of control female albino rat showing normal histological structure, H & E. x 300.

Fig. 5: Section in brain of female albino rat I/P administered with thioridazine (5mg/kg B.wt) once daily for one month showing (a) degenerated purkinje cells and (b) perneuronal edema, H & E. x 300.

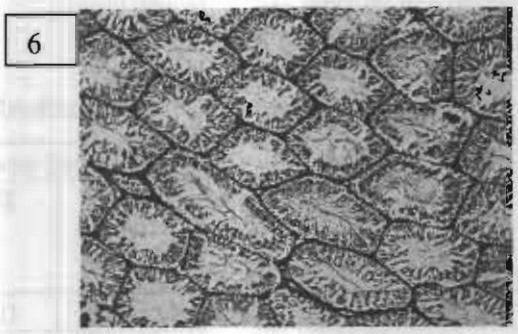
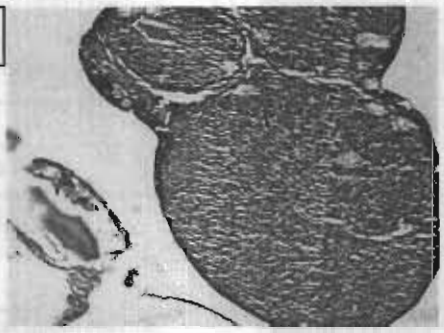


Fig. 6: Section in testis of control male albino rat showing normal histological structure, H & E. x 300.

Fig. 7: Section in testes of male albino rat I/P administered with thioridazine (5mg/kg B.wt) once daily for one month showing (a) severe thickening tunica albugenia and (b) disintegrated spermatogonialcells and spermatocytes, H & E. x 300.



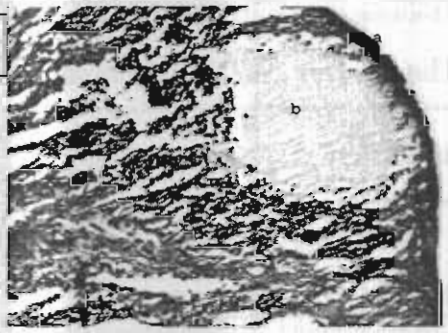


Fig. 8: Section in ovary of control female albino rat showing normal histological structure, H & E. x 300.

Fig. 9: Section in ovary of female albino rat I/P administered with THZ(5mg/kg B.wt) once daily showing (a) partial destruction of germinal epithelium and (b) absence of ovum from some mature follicles, H & E. x 300.

Table 2. Optical characteristics and statistical data for the proposed methods.

Parameters	THZ+$KMnO_4$	
	IC	MO
λ_{max} (nm)	610	508
Beer's law limit (μg mL^{-1})	0.4 - 3.2	0.4 - 2.4
Molar absorptivity (L mol^{-1} cm^{-1})	3.82×10^{4}	5.28×10^{4}
Sandell's sensitivity (μg cm^{-2})	0.0106	7.71×10^{-3}
Correlation coefficient (r)	0.9980	0.9993
Linear regression equation[a]		
$S_{y/x}$	0.0105	0.0104
Slope (b)	0.24	0.2455
Intercept (a)	0.4912	0.635
S.D. of slope (S_b)	8.29×10^{-3}	8.25×10^{-3}
S.D. of intercept (S_a)	0.0223	0.0222
LOD (μg mL^{-1})	0.0583	0.0641
LOQ (μg mL^{-1})	0.1943	0.2133

[a] A = a + bC, where A is the absorbance and C is the concentration of THZ in μg mL^{-1}.

Table 3. Evaluation of intra-day and inter-day accuracy and precision of the methods.

Method	THZ Taken µg mL^{-1}	Intra-day accuracy and precision			Inter-day accuracy and precision		
		THZ found[a], µg mL^{-1}	RE[b], %	RSD, %	THZ found, µg mL^{-1}	RE, %	RSD, %
$KMnO_4$ + IC	0.8	0.801	0.026	1.236	0.799	-4×10^{-3}	0.929
	1.6	1.599	-4×10^{-3}	1.052	1.599	-2×10^{-3}	0.974
	2.4	2.399	-2×10^{-3}	0.717	2.399	-2×10^{-3}	0.424
$KMnO_4$ + MO	0.4	0.399	-4×10^{-3}	1.648	3.999	-4×10^{-3}	1.355
	0.8	0.799	-2×10^{-3}	1.807	0.799	-2×10^{-3}	1.889
	1.6	1.599	-2×10^{-3}	1.660	1.599	-4×10^{-3}	0.803

[a]Mean value of five determinations. [b]RE: Relative error.

Table 4. The recovery of THZ in tablets formulation using the proposed methods.

Formulation	Found[a] (% of nominal amount ± RSD)		
	$KMnO_4$+IC	$KMnO_4$+MO	Reference method
Thiozine 25 mg/tablet	98.92±1.51	99.92±0.78	99.88±0.901
	t = 2.046	t = 1.876	
	F = 2.808	F = 1.334	

[a]Mean value of five determinations. Tabulated *t*-value at the 95% confidence level is 2.77. Tabulated *F*-value at the 95% confidence level is 6.39.

Table 5. Results of the recovery study by the standard addition method.

Formulation	Method	Amount of drug in formulation, µg	Amount of pure drug added, µg	Total found, µg	Recovery of pure drug, %	RSD, %
Thiozine, 25 mg/tablet	$KMnO_4$ + IC	1.2	0.4	1.610	100.68	0.9703
			0.8	1.974	98.74	1.6282
			1.0	2.174	98.81	1.4965
	$KMnO_4$ + MO	0.8	0.4	1.189	99.16	1.1380
			0.8	1.581	98.85	1.4635
			1.2	1.974	98.77	1.5673

Table 6. The recovery of THZ in serum and urine (n = 5, t = 2.78).

Method	Added, µg mL^{-1}	Serum			Urine		
		Found, µg mL^{-1}	Recovery, %	RSD, %	Found, µg mL^{-1}	Recovery, %	RSD, %
$KMnO_4$ + IC	0.4	0.3999	99.996	1.633	0.80	100.00	1.839
	0.8	0.7999	99.996	2.418	1.1751	97.93	2.612
	1.6	1.5999	99.996	1.779	1.5645	97.78	2.849
$KMnO_4$ + MO	0.8	0.7999	99.998	0.8563	0.4059	101.49	2.109
	1.2	1.1999	99.996	1.927	0.80	100.00	1.025
	1.6	1.5999	99.996	0.846	1.1999	99.99	0.7039

Table 7. Effect of THZ (5mg/kg B.wt) intraperitoneally administered daily for one month on serum level of SOD and catalase of mature male and female albino rats (means±S.E.) n=7.

Parameters	Sample	Groups			
		Male		Female	
		Treated male (G1)	Control male (G2)	Treated female (G3)	Control female (G4)
SOD (μM/mg tissue)	Serum	61.61^{ab}± 0.53	41.76^{c}± 4.35	60.58^{a}± 4.11	52.48^{c}± 6.77
	Brain	72.45^{a}± 0.60	54.33^{bc}± 3.65	65.49^{a}± 0.09	51.92^{c}± 3.13
Catalase (μM/mg tissue)	Serum	71.16^{c}± 6.58	57.70^{e}± 2.17	82.87^{bc}± 1.89	46.3^{de}± 2.00
	Brain	51.32^{b}± 3.21	40.07^{bcd}± 1.67	83.67^{a}± 0.51	48.63^{cde}± 5.27

Means within the same row carrying different superscripts are significantly different at ($p < 0.05$)

Table 8: Effect of THZ (5mg/kg B.wt) intraperitoneally administered daily for one month on serum level of LH and FSH of mature male and female albino rats (means±S.E.) n=7.

Parameters	Group and treated			
	Male		Female	
	Treated male (G1)	Control male (G2)	Treated female (G3)	Control female (G4)
LH (mL U/mL)	0.250^{a}±0.039	0.343^{b}±0.012	-	-
FSH (mL U/mL)	-	-	0.360^{a} ±0. 026	0.493^{b} ± 0. 008

Means within the same row carrying different superscripts are significantly different at ($P < 0.05$)

Conclusion

Oxidation reaction and ion-pair complex formation of THZ have been investigated. The obtained complexes were studied by UV–vis spectrophotometry techniques. The obtained colored complexes were utilized in the development of simple, rapid and accurate spectrophotometric methods for the analysis of THZ in tablets and biological fluids. From the results of experimental study, it could be concluded that THZ elicited an obvious ruinous effect on both male and female rats *via* investigating its effects on the antioxidant enzyme activities, reproductive hormones and the histopathological findings as expected outcome.

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الملخص العربى

دراسات طيفيـــة وسميـــة على عقارالثيوريدازين هيدروكلوريد فى الأقراص الدوائية والسوائل الحيوية والفئران البيضاء

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يقوم هذا البحث على دراسة التقدير الكمي لعقارالثيوريدازين باستخدام التحليل الطيفي على المواد الخام وتطبيقها على الأقراص الدوائية والسوائل الحيوية (البول ومصل الدم). يعتمد التقدير الكمي لعقارالثيوريدازين على أكسدته بإضافة كمية زائدة من برمنجنات البوتاسيوم في وجود حامض الكبريتيك ثم تقدير الكمية الزيادة من العامل المؤكسد مع صبغة (Methyl orange) وقياس قيم الامتصاص عند طول موجى 505 نانومتر او باستخدام (Indigo carmine) وقياس قيم الامتصاص عند طول موجي 608 نانومتر. وقد اوضحت النتائج أنه أمكن تطبيق الطرق المقترحة فى مدى من التركيز 0.4-2.4 و 0.4-3.2 ميكروجرام/مللي ثيوريدازين مع صبغة (Methyl orange) و (Indigo carmine) على الترتيب. أمكن تطبيق الطرق المقترحة في تقدير العقاقير الأربعة قيد الدراسة في الأقراص الدوائية أوفى السوائل الحيوية (البول ومصل الدم). وكانت النتائج المعملية متفقة إلى حد كبير مع النتائج القياسية والمرجعية. امتازت هذه

الطرق بانخفاض نسبة الانحراف المعياري ونسبة الخطأ في جميع التجارب، مما يسمح بتطبيق هذه الطرق بدقة وكفاءة فى مختلف المعامل العلمية على المستحضرات الدوائية والسوائل الحيوية. أثبتت هذه الدراسة أن تجريع الفئران البيضاء يوميا لمدة شهر في الغشاء البريتونى بعقار الثيوريدازين (5مجم/كجم) يؤدى الى زيادة معنوية فى مستوى إنزيم السوبر اكسيد ديسميوتاز (SOD) والكتالاز(catalase) بكل من مصل ومخ ذكور وإناث الفئران البيضاء مقارنة بمجموعات الفئران القياسية فيما عدا لم يظهر زيادة معنوية فى مستوى انزيم الكتالاز(catalase) فى مخ ذكور الفئران البيضاء. وقد أثبتت هذه الدراسة أن عقارالثيوريدازين يؤدى إلى نقص معنوي في مستوى هرمونات الخصوبة (هرمون ال اتش (LH) وهرمون اف اس اتش(FSH)) عند ذكور وإناث الفئران البيضاء مقارنة بمجموعات الفئران القياسية. أظهر الفحص الميكرسكوبى أن ذكور وإناث الفئران تظهر تأثيرا مرضيا متشابها بالكبد والمخ ومختلفا بالأعضاء التناسلية (المبيض والخصية) وأن جميع التأثيرات المرضية الناتجة عن العقار تتوافق مع نتائج تأثيره على الإنزيمات المضادة للأكسدة وهرمونات الخصوبة.