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Full Length Research Paper

Synthesis and characterization of Cu(II) and Fe(II) metal complexes of oxazepine derivative via Schiff base [Fe(HPOHBOT)Cl_2] and [Cu(HPOHBOT)Cl_2]

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A Schiff base and its derivative (oxazepine) have been synthesized by the reaction between thio-semicarbazide and aromatic aldehyde 4-hydroxybenzaldehyde in ethanol in the presence of acetic acids to yield the Schiff base. This Schiff base on treatment with phthalic anhydride to give seven-member heterocyclic ring called oxazepine. Oxazepineas di-dentate ligand treated with hydrated metal chlorides $CuCl_2$ and $FeCl_2$ in the presence of ethanol as solvent to yield tetrahedral complexes. The structures of synthesized ligand and complexes have been established on the basis of their spectral Fourier transform infrared (FTIR), mass, 1H-NMR, elemental analysis C, H, N as well as molar conductance. The purity of the compounds was confirmed by thin layer chromatography (TLC).

Key words: Characterization, complexes, oxazepine, Schiff bases.

INTRODUCTION

1,3-Oxazepine is unsaturated seven-member heterocyclic ring containing oxygen atom in position1 and nitrogen atom in position 3 in addition to the five carbon atoms (Zeid, 2013).

It is synthesized by (2+5) → 7 cycloaddition reaction of imine group (Schiff bases) as two-member component to five-member component such as maleic or phthalic, nitrophthalic and succinic anhydrides to give a seven-membered heterocyclic ring (Rahman, 2011).

Oxazepine derivatives showed a vast variety of biological activities like cancer diseases, psychotic depression (Khalid et al., 2014), mental depression associated with schizophrenia, affecting the nervous centre (CNS) (Khuluod and Hamid, 2013) used for the control of anxiety and tension states, the relief of muscle spasm and for the management of acute agitation during with drawls from alcohol (Saoud, 2011). Oxazepine derivatives showed biological activities against different types of bacteria (Abood, 2009).

The aim of this study was to synthesize new metals complexes of oxazepine derivative via Schiff base which are expected to have enhanced biological activity

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Table 1. The experimental result and physical data of ligand and its complexes.

Code No.	Compounds M.F.	M. W. (g/mole)	Colour	M.P. (°C)	Yield (%)	Elemental analysis of ligand		
						C	H	N
Ligand	$C_{16}H_{13}N_3O_4S$	343	Yellow	153	94	-	-	-
						% Theoretical data		
Ligand +Fe	$C_{32}H_{26}Cl_3CoN_6O_8S_2$	470	Green Brownish	266	62	55.97	3.82	12.24
						% Practical data		
Ligand +Cu	$C_{16}H_{13}Cl_2CuN_3O_4S$	477	Deep Green	272	64	56.12	3.71	12.15

compared to free oxazepine (Nagham et al., 2014).

EXPERIMENTAL

Melting points of ligand and metal complexes were taken in melting points apparatus U.k. 1H NMR spectra was recorded on a Bruker avance Mercury-300BB NMR 300 spectrometer. FT-IR spectra was obtained in KBr pallet in the 4000 to 200 cm^{-1} region on a Fourier transform infrared spectrophotometer Shimandzu. Mass spectra were recorded in the range of 0 to 900 m/z on a 5973 network mass selective detector. Elemental analysis C, H, and N were carried out on a Thermo finigan flash analyser, molar conductance, and molar conductance measurements were made in anhydrous DMSO at 25°C using Inolabcond 720 professional bench top meter.

Step I: Synthesis of 2-(4-hydroxybenzylidene) hydrazine carbothioamide (Schiff bases)

An equimolar amount of thiosemicarbazide (0.01 mole) and 4-hydroxybenzaldehyde (0.01 mole) was dissolved in 60 ml ethanol. The resulting mixture was refluxed for 6 h in the presence of few drops of catalytic amount of glacial acetic acid. After completion of the reaction, the mixture was poured into crushed ice; thereafter, the separated product was filtered and dried at room temperature. The product was purified by re-crystallization from ethanol, and was followed by TLC giving a yellow colour, yield percent of 81 and a melting point (m.p) of between 141 to 143°C (Rakesh et al., 2011).

Step II: Synthesis of 1-[3-(4-hydroxyphenyl)-1,5-dioxo-1,5-dihydro-2,4-benzoxazepin-4(3*H*)-yl]thiourea (Ligand) (HPOHBOT) .

The resulting mixture of an equimolar amount (0.02 mole) of Schiff's bases and 0.02 mole phthalic anhydride in 25 ml of dry toluene was refluxed for 7 h. After completion of the mixture, it was allowed to cool down at room temperature. The separated product was filtered and dried at room temperature. The product was purified by re-crystallization from dioxin. The purity of the compound was followed by TLC. The physical appearance yield and melting point are shown in Table 1 (Zainab and Hasan, 2011).

Step III: Synthesis of Cu(II) and Fe (II) metal complexes of oxazepine derivative

Ligand HPOHBOT was obtained by refluxing the mixture of hydrated metal chlorides $CuCl_2$ and $FeCl_2$ (0.001) and (0.001) of the ligand (HPOHBOT) in 70 ml ethanol until the complexes precipitated out. The colours of the complexes were filtered, washed with water, ethanol and dried under vacuum. The purity of the compound was followed by TLC. The physical appearance yield and melting point are shown in Table 1 (Matheel et al., 2009).

RESULTS AND DISCUSSION

The HPOHBOT ligand and their metal complexes were subjected to elemental analyses. The results of elemental analyses (C, H, N) with molecular formula and melting points are presented in Table 1. The results obtained are in good agreement with those calculated for the suggested formula. The structures of the ligand and metal complexes are also confirmed by IR, MASS, 1H NMR spectra and molar electrical conductivity which are discussed subsequently.

Infra-red spectroscopy

FTIR (KBr, cm^{-1}) of ligand HPOHBOT showed 3483(O-H), 3409 (N-H), 3062(C-H)A, 2940(C-H)Ali, and 1639 (C=O)1482 (Abood, 2009). The band (N-H) of the complexes was shifted to a lower frequency, indicating its involvement in coordination with metal ion. These findings were further supported by the appearance of new bands at 694 to 696 and 700 to 703 cm^{-1} which belong to both v(M–N) vibrations, respectively. All data tabulated in Table 1 are as shown in Figures 1 and 2 (Nagham, 2013).

^{1}H NMR spectra data of ligand

The 1H NMR spectra of the HPOHBOT (L) in DMSO solutions with assignments are collected in Table 2 and 3. The 1H NMR spectra of the free ligand (Figure 3) showed the aromatic proton signals appearing at 7 to 8 ppm and also showed C-H proton at 8.2 ppm, secondary amine proton at 9.8 and 9.2 ppm of primary amine proton. The phenol OH proton has a signal at 10.5 ppm (Dhanya et al., 2014).

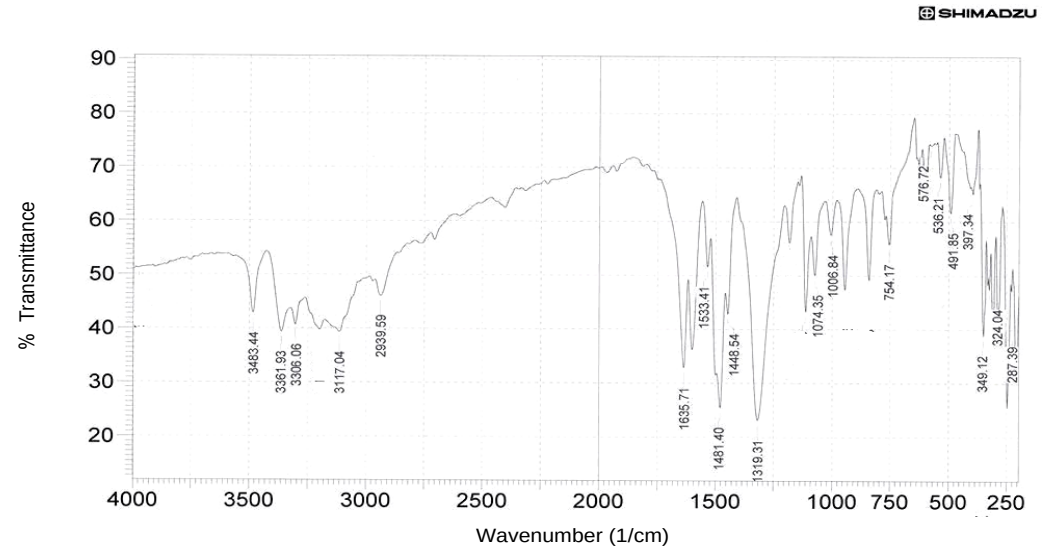


Figure 1. IR spectra of ligand.

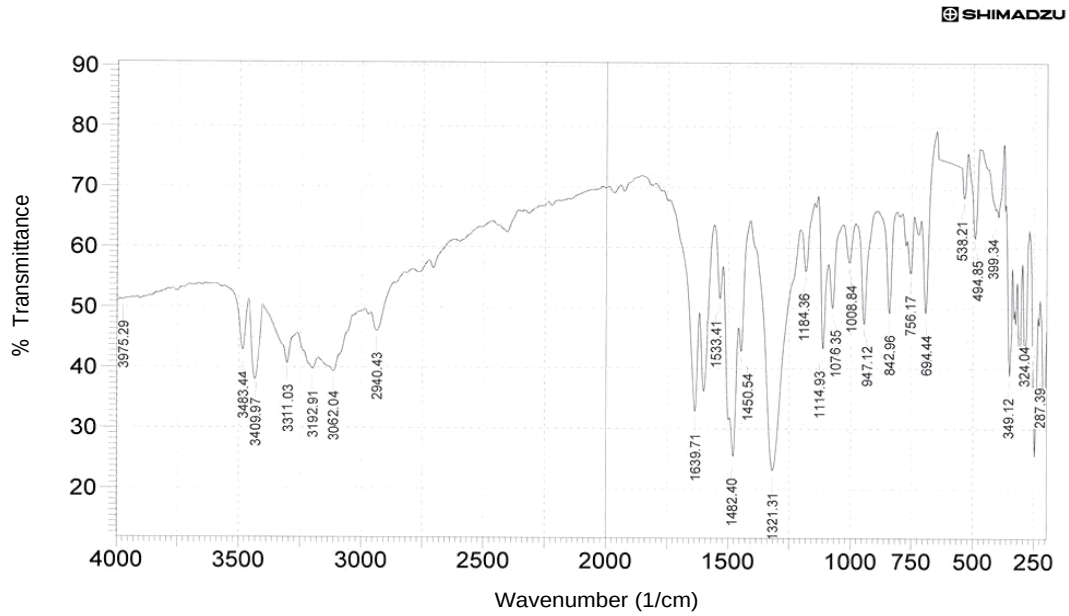


Figure 2. IR spectra of Cu(II) complexes.

Table 2. IR spectral data (cm-1) of the ligand and their metal complex in KBr pellets.

Vibration mode	Ligand	Ligand+Fe	Ligand+Cu
ν(O-H)	3483	3483	3488
νN-H)	3361	3409	3416
ν(C-H)Aro	3061	3062	3067
ν(C-H)Ali	2939	2940	2945
ν(C=O)	1635	1639	1644
ν(C=S)	1481	1482	1487
ν(M-N)	576	669	694

Table 3. 1H NMR spectra data of ligand.

Signal No.	Signal position (ppm)	Relative No. of Protons	Inference
1	7-8	2H	Ar-H
2	7-8	2H	Ar-H
3	7-8	2H	Ar-H
4	7-8	1H	Ar-H
5	7-8	1H	Ar-H
6	8.2	1H	-CH
7	9.2	2H	-NH
8	9.8	1H	-NH
9	10.5	1H	-OH

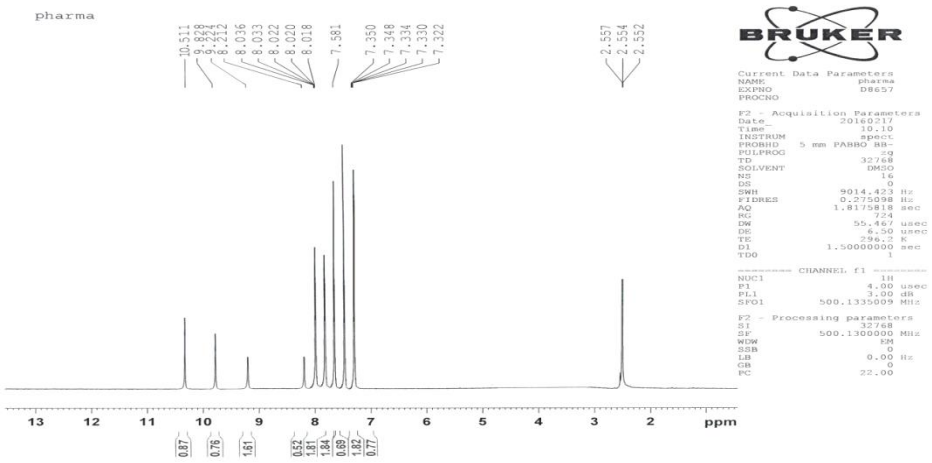
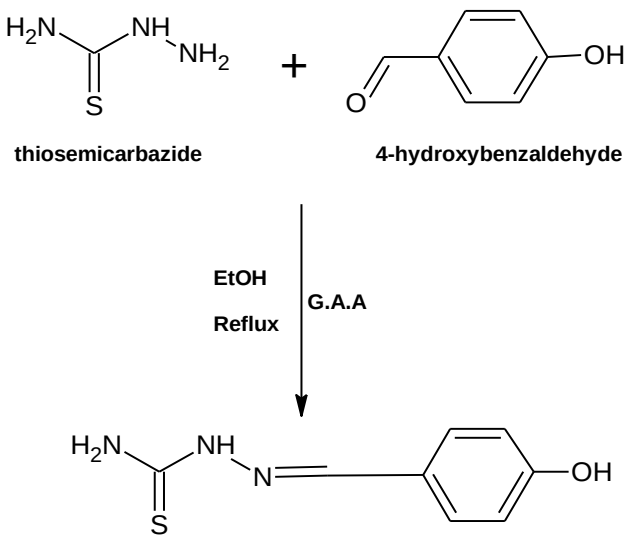
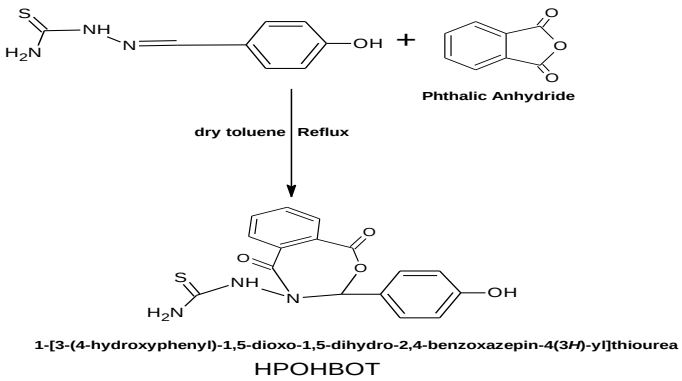


Figure 3. 1H NMR spectra of ligand.



Scheme 1. Step I: Synthesis of 2-(4-hydroxybenzylidene) hydrazinecarbothioamide.

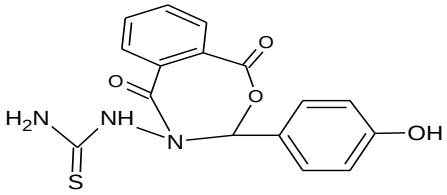
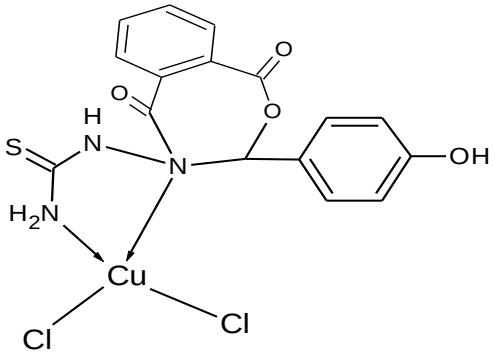
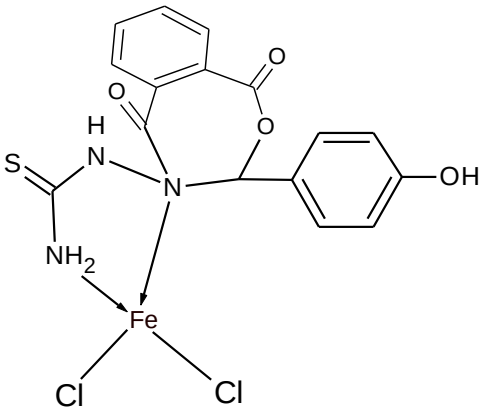


Scheme 2. Step II: Synthesis of 1-[3-(4-hydroxyphenyl)-1,5-dioxo-1,5-dihydro-2,4-benzoxazepin-4(3*H*)-yl]thiourea.

Mass spectra

Mass spectral data confirm the structure of the ligand and their Cu(II) and Fe (II) complexes as indicated by the molecular ion peaks corresponding to their molecular weight. All data are as shown in Scheme 1 and 2,

Table 4. Mass spectral data of the ligand and their Cu (II) and Fe (II) complexes.

Ion	The structure	Molecular ion
Ligand		343
$C_{15}H_{13}N_3O_3S$	-	315
$C_{10}H_7N_2O_3S$	-	250
$C_{10}H_6N_2O_3S$	-	234
$C_9H_7N_2O_3$	-	191
$C_9H_6NO_3$	-	176
C_5H_5	-	65
$[Cu(L)Cl_2]^{+\cdot}$		477
$[Cu\ (L)Cl]^{+\cdot}$	-	442
$[Cu\ (L)]^{+\cdot}$	-	406
$[Fe\ (L)Cl_2]^{+\cdot}$		470
$[Fe\ (L)Cl]^{+\cdot}$	-	435
$[Fe\ (L)]^{+\cdot}$	-	399

tabulated in Table 4 (Figures 4, 5 and 6) (Mukhlus et al., 2012).

Molar conductance measurements

The molar conductance data of the prepared complexes solution tabulated in the Table 6 were measured at room temperature in 10^{-3} M DMSO solvent. All exhibited low value of molar conductivity (0 to 20) which indicates that complexes under study is non-electrolyte Table 5. The obtained value suggested that no anions (Counter Ions) present outside the coordination sphere and showed

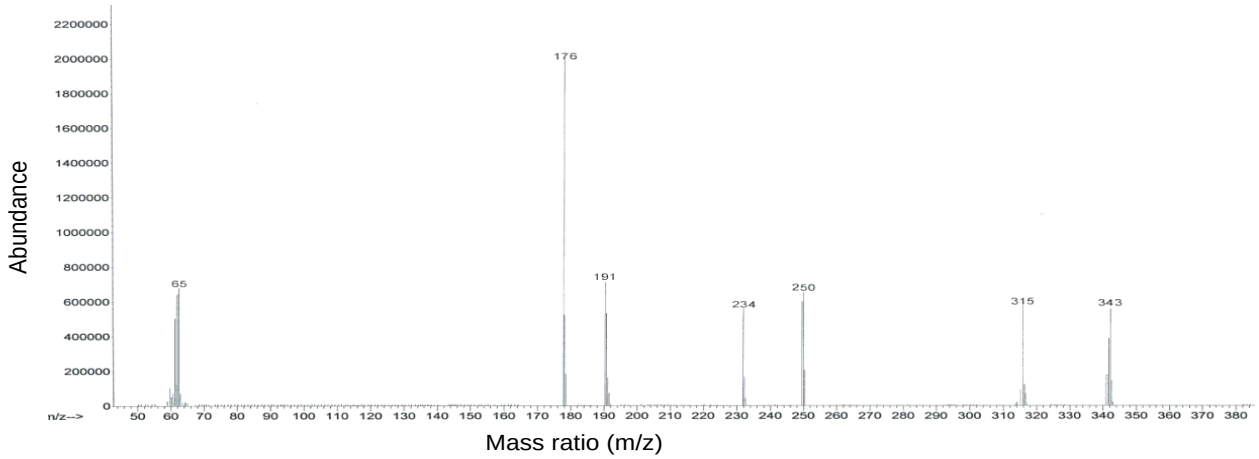


Figure 4. Mass spectral data of the ligand.

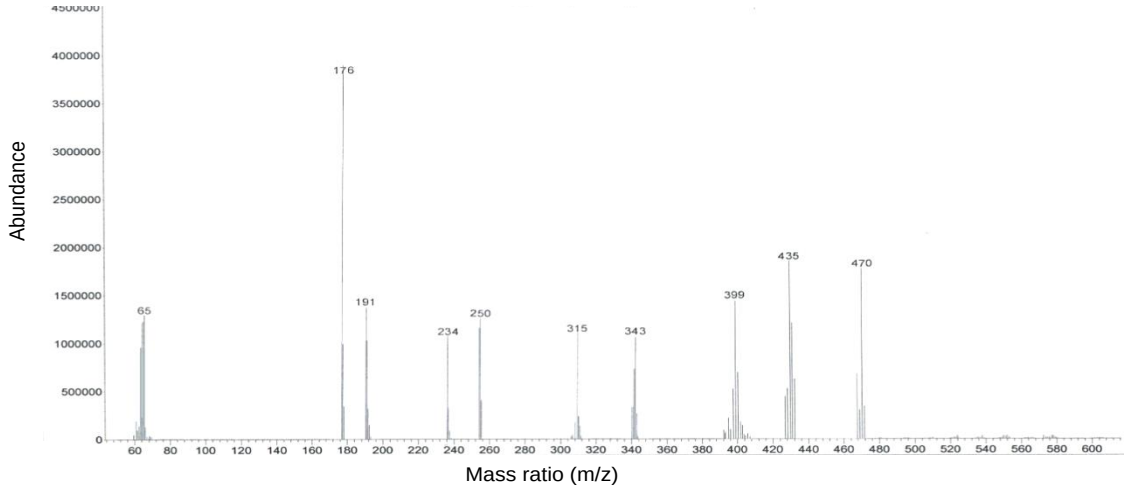


Figure 5. Mass spectral data of the Fe(II) complexes.

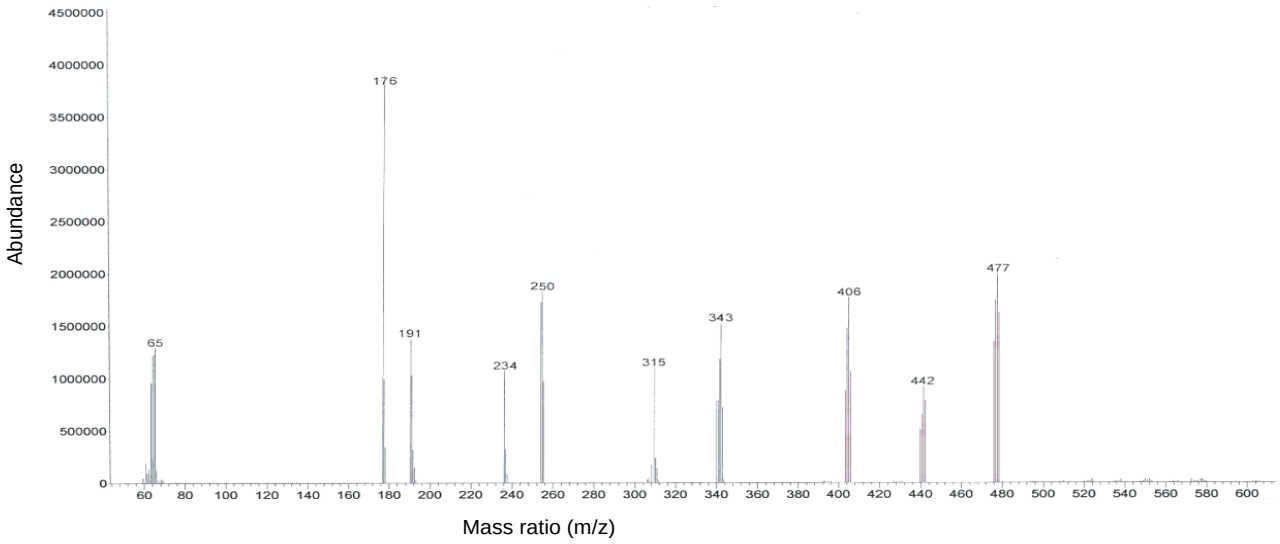


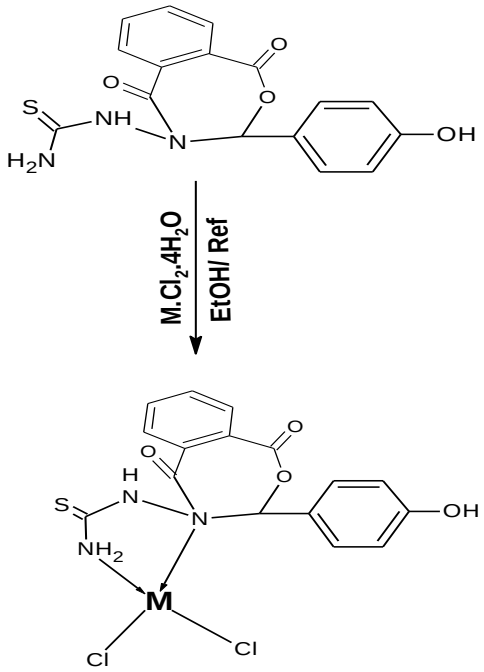
Figure 6. Mass spectral data of the Cu(II) complexes.

Table 5. Standard value of molar conductance data.

Solvent	Electrolyte type Λ_M(S .cm^2 .mole^{-1})		
	Non electrolyte	1:1	1:2
Dimethyl sulfoxide	0-20	30-40	70-80

Table 6. Molar conductance data of all complexes measurements were made in anhydrous DMSO at 25°C, concentration 10^{-3} at 298 K.

N	Formula	Solvent	Standard ΛM	Practical ΛM (S.cm^2.mol^{-1})	Electrolyte type
1	[Fe(L) Cl_2]	Dimethyl sulfoxide	0-20	13.3	Non electrolyte
2	[Cu(L)Cl_2]	Dimethyl sulfoxide	0-20	12.6	Non electrolyte



M= Cu,Fe

Scheme 3. Step III: Synthesis of Fe (II) and Cu (II) metal complexes of Oxazepine derivative. [Fe(HPOHBOT)Cl_2] and [Cu(HPOHBOT)Cl_2].

good agreement with that reported in the literature (Alya, 2015).

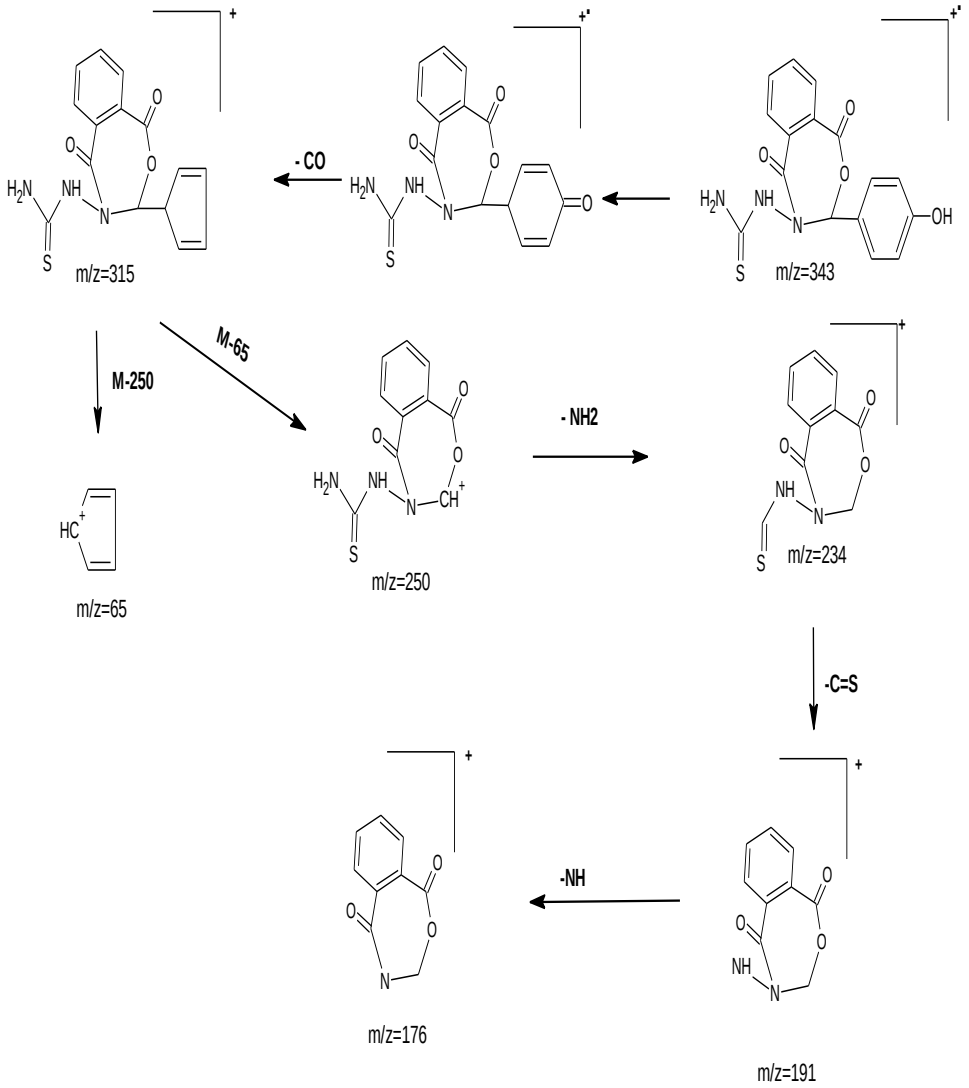
Conclusion

In the present study, Fe(II) and Cu(II) complexes with ligand HPOHBOT (L) have been synthesized and identified by IR, ^{1}HNMR, mass spectra, elemental analyses C, H, N, and molar conductance. v(M-N) band of ligand appeared in the prepared complexes at 694 cm^{-1} in Cu complex and 669 cm^{-1} in Fe complex and also (N-H) band of NH_2 for the synthesized ligand shifted from 3361 to 3416 cm^{-1} in Cu complex and 3409 cm^{-1} in Fe complex due to the coordination with the matal. This view further support that the coordinate appeared through the nitrogen of (C-N) and N of NH_2.

In all the physical and chemical measurements, it was suggested that the chemical configuration of the prepared complexes as tetrahedral geometry complexes is as shown in Scheme 3 and 4 (Selvana, 2012).

Conflict of Interests

The authors have not declared any conflict of interest.



Scheme 4. fragmentation mass spectral of the prepared ligand.

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Full Length Research Paper

Long term treatment of fluexotine and buspirone on gastric mucosal integrity in rats

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The highly selective serotonin reuptake inhibitors are successful in the treatment of depression mood and anxiety disorders. The objective of this study is to investigate the effect of fluexotine and buspirone when given orally in three dose levels (5, 10 and 15 mg/ kg) not only for eight weeks on gastric lesions and oxidative markers in normal rats' mucosal integrity, but also for four weeks on gastric mucosal lesions in rats treated with indomethacin- induced ulcers. The results of this study showed that in normal rats, the administration of fluoxetine induced gastric lesions while buspirone caused no lesions. The indomethacin administration resulted in the development of gastric mucosal lesions. Furthermore, co-administration of fluoxetine and indomethacin enhanced the development of gastric mucosal lesions that were coupled with disturbance in antioxidant status. Buspirone, in contrast, significantly decreased the development of gastric mucosal lesions in rats treated with indomethacin. In conclusion, fluoxetine caused the development of gastric mucosal lesions and aggravate the effect of indomethacin to induce ulcer, however, buspirone had a protective effect that may be attributed to its antioxidant properties.

Key words: Fluoxetine, buspirone, peptic ulcer, indomethacin-induced ulcer, oxidative stress.

INTRODUCTION

Serotonin is a neurotransmitter that plays an integral role in mediating a number of physiological processes; a wide distribution in the brain and gut (van Praag, 1980; Hanson and Hurley, .2014). Several studies (Kuhn et al., 1980; Rickels and Schweizer, 1990; Ahmed and Simmons, 2013) said that selective serotonin reuptake inhibitors (SSRIs) are very successful in the treatment of psychological depression (Dworkin et al., 2007). Interestingly, although administration of an SSRI causes an immediate increase in synaptic release of 5-HT, antidepressant effects are not experienced until approximately 3 to 4 weeks of chronic administration in humans (Rickels and Schweizer, 1990; Stahl, 1988) and 2 weeks in rat models of affective disorder (Gambarana et al., 2001).

Most serotonin is found in the gut being produced by

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the enterochromaffin cells in the gastric and intestinal mucosa (Ener et al., 2003). Gut serotonin is involved in the control of smooth muscle tone and motility (Mazda et al., 2004; Fink et al., 2006). Studies in humans have suggested an association between the intake of SSRIs and increased episodes of gastric bleeding in patients with depressive disorders (De Abajo et al., 2006). As serotonin promotes platelet aggregation, and it is thought that SSRIs limit the uptake of blood serotonin by platelets consequently to serotonin promoting platelet aggregation (Meijer et al., 2004). The present work aimed to investigate the effect of long term treatment with drugs influencing serotonergic neurotransmission on gastric mucosal integrity in rats.

In this experimental study, the effect of fluoxetine and buspirone were investigated in normal rats' stomach. In addition, the effect of both drugs on oxidant and antioxidant parameters in rats' stomach tissue was evaluated, as a first approach to investigate the mechanism behind their effect on the stomach.

MATERIALS AND METHODS

Animals

Adult albino wistar rats, weighting 120 to 130 g of body weight, were obtained from the animal house colony in National Research Center (Giza, Egypt). The animals were housed in a conditioned room at 25 ± 2ºC, standard diet and tap water source were supplied *ad libitum*. Approval for this study was obtained from the Ethics Committee of The National Research Center-Egypt and in accordance with the recommendations of the proper care and use of laboratory animals.

Drugs

Fluexotine was purchased from lilly (England), buspirone from Squibb (Egypt) and indomethacin from Khahira Pharma-ceutical and Chemical IND Company (Egypt). The doses of the drugs were selected from published literature according to Abdel-Salam et al. (2003), Chial et al. (2003) and Ilahi et al. **(**2006), respectively.

Studies in the intact rat

Rats were randomly allocated into 7 groups, each consisting of 16 rats. Daily administration orally of the test drugs in three dose levels was carried out for eight weeks. The animals were treated according to the following scheme: Group 1: Received saline and served as a negative control. Groups 2, 3 and 4 received fluexotine 5, 10 and 15 mg/kg, respectively. Finally groups 5, 6 and 7 received buspirone 5, 10 and 15mg/kg, respectively.

Studies in combination with indomethacin on normal rats

Eleven groups, each consisting of 8 rats, daily administration orally of the test drugs in three dose levels was carried out orally for four weeks, along with indomethacin 1 mg / Kg / 24 h (Ilahi et al., 2006). Animals were treated according to the following scheme: Group 1: Received saline and served as the control. Group 2: Received indomethacin and served as the control +ve. Groups 3, 4 and 5 received fluexotine 5, 10 and 15 mg/kg, respectively and indomethacin. Groups 6, 7 and 8 received buspirone 5, 10 and 15 mg/kg, respectively and indomethacin.

At the end of the first experiment, eight animals of each group were sacrificed by decapitation. After which the brains were quickly isolated, the brains were frozen stored at -80°C, until analysis of serotonin brain levels.

At the end of two experiments, animals were sacrificed by cervical dislocation then the abdominal cavity was opened and the stomach was removed. The stomach was opened along the greater curvature and pinned on a plastic board. The mucosa was examined for mucosal necrotic lesions, red streaks and red erosions (Mózsik et al., 1982). Immediately after gross lesion examination, the stomach was placed over an ice-cold surface. The glandular mucosa was cut, weighed then homogenized in ice-cold saline to obtain a 10 % (W/V) homogenate by using glass homogenizer. This was performed to determine the level of glutathione, lipid peroxides and nitric oxide.

The stomach mucosa was examined for mucosal necrotic lesions, red streaks and red erosions (Mózsik et al., 1982). Total lesion number was counted and the lesion severity was determined based on the following scores:

0 = no ulcer
1 = lesion size ≤ than 1 mm.
2 = lesion of size 1-2 mm.
3 = lesion of size 2-3 mm.
4 = lesion of size 3-4 mm.
5 = lesion of size > 4 mm.

Determination of oxidative stress

Lipid peroxides were determined according to the method described by Mihara and Uchiyama (1978**)** and expressed as nmol/g wet tissue. Lipid peroxidation products were estimated by the determination of the level of TBARS that were measured as malondialdehyde (MDA). The latter is the decomposition product of the process of lipid peroxidation and is used as an indicator of this process. The principle of the assay depends on the colorimetric determination of a pink pigment product, resulting from the reaction of TBARS with thiobarbituric acid (TBA) in an acidic medium, at high temperature. Reduced glutathione (GSH) content was determined in the stomach homogenates according to the method of Beutler et al. (1963) and expressed as mg/g wet tissue. The method depends on the fact that both protein and non-protein thiol (SH-) groups (mainly GSH) react with Ellman's reagent [5,5' -dithiobis (2- nitrobenzoic acid)] to form a stable yellow color of 5-mercapto -2- nitrobenzoic acid, which can be measured colorimetrically at 412 nm. Stomach NO metabolites were determined according to the method described by Miranda et al. (2001) and expressed as µM/g wet tissue. The assay determines the total NOx content based on the reduction of any nitrate to nitrite by vanadium, followed by the detection of total nitrite (intrinsic + nitrite obtained from reduction of nitrate) by Griess reagent. The Griess reaction leads to the formation of a chromophore from the diazotization of sulfanilamide by acidic nitrite, followed by coupling with bicyclic amines such as N-(1-naphthyl) ethylenediamine. The chromophoric azo derivative can be measured colorimetrically at 540 nm.

Estimation of serotonin

Estimation of serotonin in the midbrain region using rapid and precise liquid chromatography with mass spectrometric detection (LC/MS) method for the identification and quantification of serotonin from rat brain tissue without any pre-analysis adjustment of the

Table 1. Effect of fluoxetine and buspirone on gastric mucosal integrity.

Groups	Number of ulcers (mean)	Ulcer severity (score)	Incidence of lesions
Normal	No lesion	Zero	Zero
Fluoxetine 5 mg/kg	2.3 ± 0.4	3 ± 0.7	3/6
Fluoxetine 10 mg/kg	2 ± 0.6	2 ± 0.6	4/6
Fluoxetine 15 mg/kg	4 ± 0.6	6 ± 1	4/6
Buspirone 5 mg/kg	No lesion	Zero	Zero
Buspirone 10 mg/kg	No lesion	Zero	Zero
Buspirone 15 mg/kg	No lesion	Zero	Zero

Values are means ± SE of 6 rats.

Table 2. Effect of concurrent administration of fluoxetine and buspirone with indomethacin on gastric mucosal integrity.

Groups	Number of lesions (mean)	Severity of lesions (score)
Indomethacin 1 mg/kg	3.2 ± 0.03	5 ± 0.09
Fluoxetine 5 mg/kg	4.3 ± 0.04	5.3 ± 0.07
Fluoxetine 10 mg/kg	5 ± 0.05	6.3 ± 0.08
Fluoxetine 15 mg/kg	6.8 ± 0.07[a]	11.7 ± 0.14[a]
Buspirone 5 mg/kg	Zero	Zero
Buspirone 10 mg/kg	Zero	Zero
Buspirone 15 mg/kg	Zero	Zero

Values are means ± SE of 6 rats. [a]P<0.05 vs. control group.

sample such as pre-concentration or derivatization has been developed (Cao et al., 2006).

Histopathological examination

The dissected stomachs of different groups were washed with saline and fixed in 10% formalin for histopathological assessment. The prepared sections were stained with Haematoxylin and Eosin for assessing histopathological changes.

Statistical analysis

Values were expressed as means ± S.E.; the results of the ulcer number and severity were analyzed using Kruskal-Wallis non-parametric one way analysis of variance (ANOVA), followed by Mann Whitney multiple comparison test. The results of the remaining experiments were analyzed using one way ANOVA followed by least significant difference (LSD) multiple comparison test. P<0.05 was accepted as being significant in all types of statistical tests. Statistical analysis of results, were done using software SPSS 17.

RESULTS

Effect on ulcer index

In normal rats, after eight weeks of drugs administration, fluoxetine caused lesions compared with buspirone (Table 1). The concurrent administration of fluoxetine and indomethacin for four weeks enhanced the development of gastric mucosal lesions (Table 2).

After eight weeks the administration of fluoxetine reduced gastric NOx content while buspirone elevated gastric NOx (Figure 1). The concurrent administration of fluoxetine and buspirone with indomethacin for four weeks results in gastrointestinal mucosal damage decreasing NO cause by indomethacin. Moreover, gastric lesions caused by NSAIDs can be aggravated by vascular ischemia, which can explain why fluoxetine enhanced lesions with indomethacin co-administration through their decrease in nitric oxide.in the same manner gastric NOx content was reduced by fluoxetine (Figure 2).

Effect on gastric NOx content

Effect of fluoxetine and buspirone on gastric NOx in normal rats

The effect of fluoxetine on NO concentration, in normal rats that received fluoxetine at 5 mg/kg, showed a significant decrease by 35% compared to the control (saline treated) group. The other two doses of fluoxetine 10 and 15 mg/kg resulted in an insignificant reduction in NO concentration compared to the normal group.

The rats, that received buspirone at 5 mg/kg showed

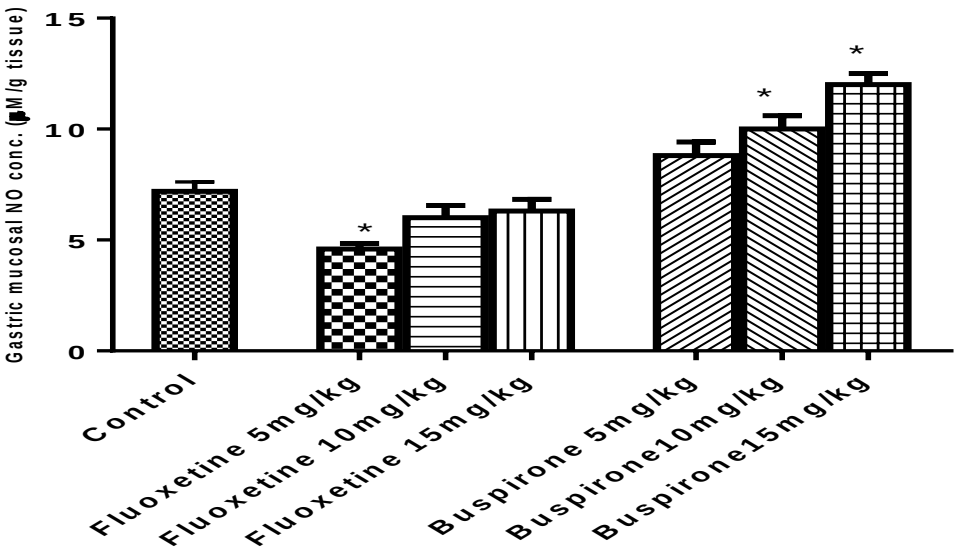

Figure 1. Effect of fluoxetine and buspirone at 5, 10 and 15 mg/kg on rat stomach nitric oxide (NO) concentration.

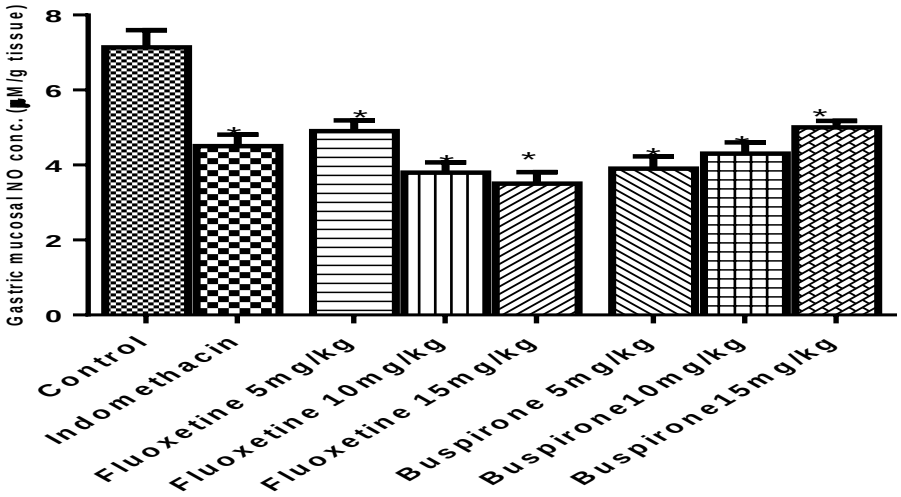

Figure 2. Effect of fluoxetine and buspirone NO concentration in indomethacin- induced gastric ulcer in rats.

an insignificant increase in NO concentration by 23% compared to the normal group. In rats that were administered buspirone at 10 and 15 mg/kg, NO concentration was significantly increased compared to normal group by 41 and 55%, respectively (Figure 1).

Drugs were suspended in 1% Tween 80 and orally administered daily for 8 weeks. The normal and control group received 1% Tween 80 daily. In all groups, animals were sacrificed by cervical dislocation under ether anesthesia. Stomachs were dissected and part of each stomach was homogenized and the homogenate was used for the determination of gastric total nitrate/nitrite (NO_x) content. Each value represents the mean of 6 rats ± S.E. Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by least significant difference (LSD) multiple comparison test.

Effect of fluoxetine and buspirone NO concentration in indomethacin induced gastric ulcer in rats

Indomethacin significantly decreased NO content by 36% compared to the control group. Fluoxetine at 5, 10 and 15 mg/kg was given to indomethacin treated rats resulting in significant decrease in NO concentration compared to the

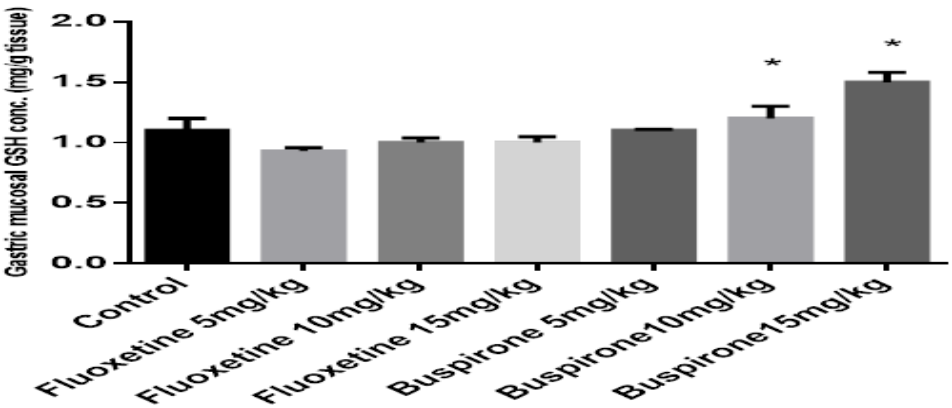

Figure 3. Effect of fluoxetine and buspirone at 5, 10 and 15 mg/kg bodyweight on rat gastric reduced glutathione (GSH) content.

control group by 31, 46 and 50% respectively. In the same manner, NO concentration showed insignificant decreased compared to the indomethacin treated group. Buspirone was given at 5, 10 and 15 mg/kg to indomethacin treated rats resulting in a significant decrease in NO concentration compared to the control group by 45, 40 and 30%, respectively. However, no significant effects were seen after treating with buspirone in NO concentration compared to indomethacin treated group.

Drugs were suspended in 1% Tween 80 and orally administered daily for four weeks with 1% indomethacin. The normal received 1% Tween 80 daily and the control group received 1% indomethacin. In all groups, animals were sacrificed by cervical dislocation under ether anesthesia. Stomachs were dissected and part of each stomach was homogenized and the homogenate was used for the determination of gastric total nitrate/nitrite (NO_x) content. Each value represents the mean of 6 rats ± S.E. Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by least significant difference (LSD) multiple comparison test.

Effect on gastric GSH content

Effect of fluoxetine and buspirone gastric on glutathione (GSH) content in normal rats

Normal rats were given fluoxetine; GSH content showed an insignificant change compared to the control group. Rats that received buspirone at 5 mg/kg showed an insignificant increase in GSH content compared to the control group. In rats treated with buspirone at 10 and 15 mg/kg, GSH content was a significantly increased compared to the control group by 27 and 36% respectively (Figure 3).

Drugs were suspended in 1% Tween 80 and orally administered daily for 8 weeks. The normal and control group received 1% Tween 80 daily. In all groups, animals were sacrificed by cervical dislocation under ether anesthesia. Stomachs were dissected and part of each stomach was homogenized and the homogenate was used for the determination of gastric total reduced glutathione content. Each value represents the mean of 6 rat's ± S.E. Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by least significant difference (LSD) multiple comparison test.

Effect of fluoxetine and buspirone GSH content in indomethacin- induced gastric ulcer in rats

Indomethacin was given at 1 mg/kg for four weeks, resulting in an insignificant increase in GSH content compared to the control group. Fluoxetine administration to indomethacin treated rats at 5 mg/kg caused an insignificant decrease in GSH content compared to both the control and the indomethacin treated group. Fluoxetine at 10 and15 mg/kg administrations to indomethacin treated rats showed a significant increase in GSH content compared to the control group by 38 and 84%, respectively. Fluoxetine at 15 mg/kg caused a significant increase in GSH content by 72% compared to the indomethacin treated group.

Buspirone at 5, 10 and 15 mg/kg was given to indomethacin treated rats resulted in a significant increase in GSH content not only compared to the control group by 29, 57and 84%, but also to the indomethacin treated group by 27, 54 and 72%, respectively (Figure 4).

Drugs were suspended in 1% Tween 80 and orally administered daily for four weeks with 1% indomethacin. The normal group received 1% Tween 80 daily and the control group received 1% indomethacin. In all groups animals were sacrificed by cervical dislocation under ether anesthesia. Stomachs were dissected and part of each stomach was homogenized and the homogenate was used for the determination of gastric reduced glutathione content. Each value represents the mean of 6

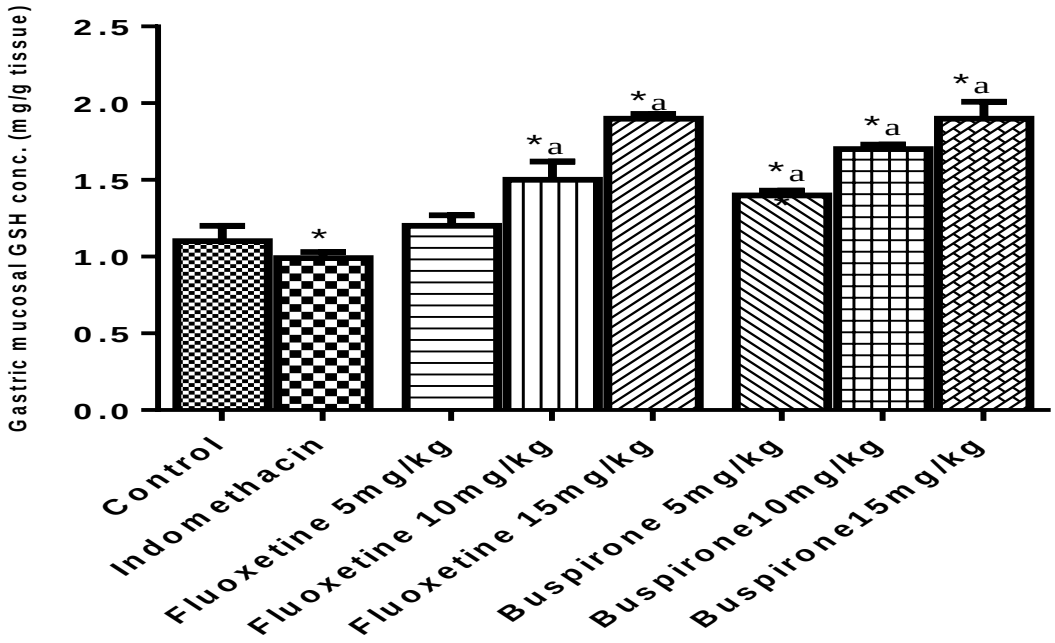

Figure 4. Effect of fluoxetine and buspirone GSH content in indomethacin- induced gastric ulcer in rats. *Significantly different from normal group at $p<0.05$.

rats ± S.E. Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by least significant difference (LSD) multiple comparison test.

Effect on gastric MDA content

Effect of fluoxetine and buspirone on normal rat stomach malondialdehyde (MDA) content

The fluoxetine group resulted in a significant increase in MDA content compared to the control group. Buspirone at 5, 10 and 15 mg/kg, MDA content showed an insignificant change compared to the control group (Figure 5).

Effect of fluoxetine and buspirone MDA content in indomethacin induced gastric ulcer in rats

Indomethacin given at 1 mg/kg for four weeks resulted in significant increase in MDA content compared to the control group. Fluoxetine at 5 and 10 mg/kg given to indomethacin treated rats, resulted in a significant increase in the MDA content compared to the control group. This elevation was insignificant compared to the indomethacin treated group. However, fluoxetine at 15 mg/kg given to indomethacin treated rats, resulted in a significant increase in the MDA content compared to both the control and the indomethacin treated groups.

Buspirone was given to indomethacin treated rats, resulted in a significant decrease in gastric mucosal the MDA content compared to the indomethacin treated group (Figure 6).

Effect on serotonin level

Effect of fluoxetine and buspirone on rat gastric mucosal serotonin

In normal rats, that were given fluoxetine at 5 and 10 mg/kg, the gastric mucosal serotonin level was decreased compared to the control group by 50 and 12%, respectively. Fluoxetine at 15 mg/kg increased gastric mucosal serotonin level by 32% compared to the control group. Buspirone at 5 and 10 mg/kg decreased the gastric mucosal serotonin level compared to the control group. Buspirone at 15 mg/kg showed no effect on the gastric mucosal serotonin level compared to the control group (Figure 7).

Effect of fluoxetine and buspirone on rat midbrain serotonin level

Fluoxetine at 5 and 10 mg/kg, decreased the brain serotonin level compared to the control group by 82 and 52% respectively. Fluoxetine at 15 mg/kg increased the brain serotonin level by 42% compared to the control group. Buspirone at 5, 10 and 15 mg/kg, increased the brain serotonin level compared to the control group (Figure 8).

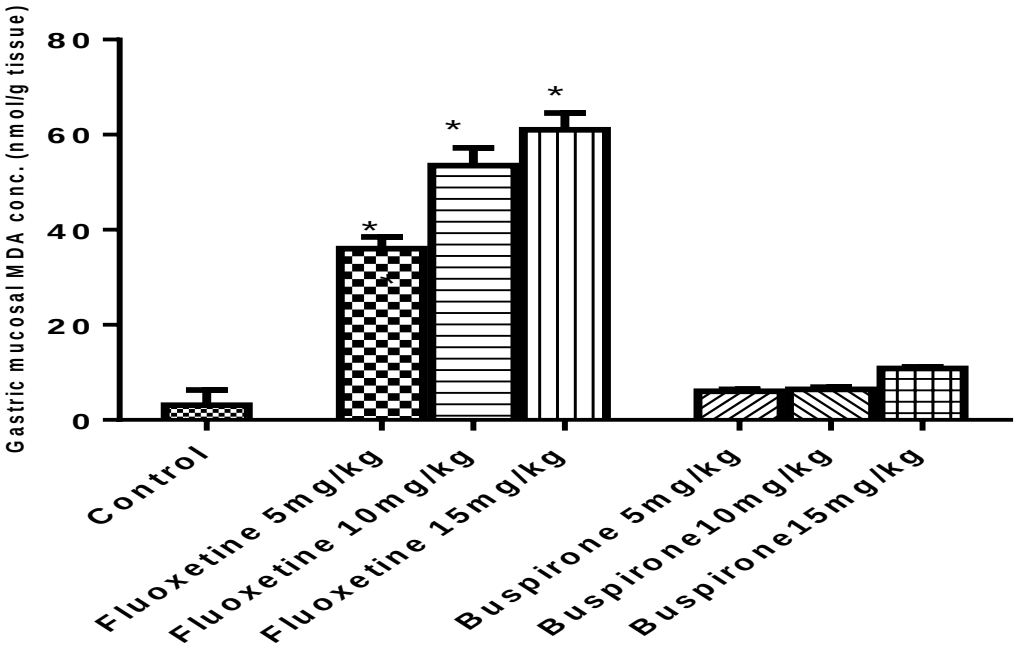

Figure 5. Effect of fluoxetine and buspirone at 5, 10 and 15 mg/kg bodyweight on rat stomach malondialdehyde (MDA) content. Drugs were suspended in 1 % Tween 80 and orally administered daily for 8 weeks. The normal and control group received 1% Tween 80 daily. In all groups animals were sacrificed by cervical dislocation under ether anesthesia. Stomachs were dissected and part of each stomach was homogenized and the homogenate was used for the determination of gastric total reduced glutathione content. Each value represents the mean of 6 rats ± S.E. Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by least significant difference (LSD) multiple comparison test. *Significantly different from normal group at $p<0.05$.

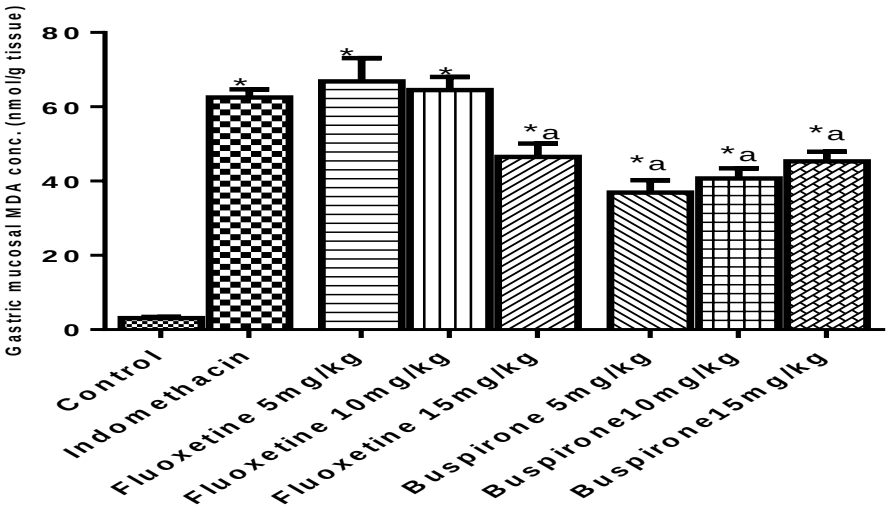

Figure 6. Effect of fluoxetine and buspirone MDA content in indomethacin-induced gastric ulcer in rats. Drugs were suspended in 1% Tween 80 and orally administered daily for four weeks with 1% indomethacin. The normal received 1% Tween 80 daily and the control group received 1% indomethacin. In all groups animals were sacrificed by cervical dislocation under ether anesthesia. Stomachs were dissected and part of each stomach was homogenized and the homogenate was used for the determination of gastric mucosal thiobarbituric acid reactive substances (MDA) content. Each value represents the mean of 6 rats ± S.E. Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by least significant difference (LSD) multiple comparison test. *Significantly different from normal group at $p<0.05$; [a]Significantly different from indomethacin group at $p<0.05$.

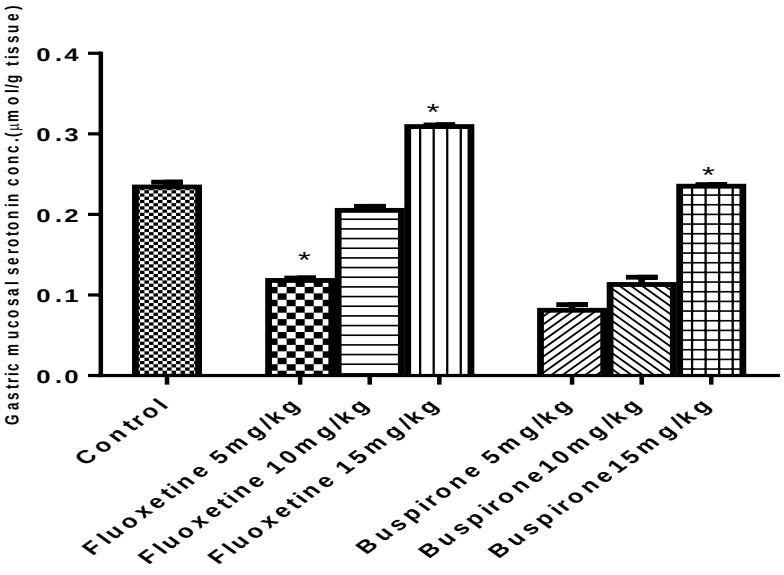

Figure 7. Effect of fluoxetine and buspirone at 5, 10 and 15 mg/kg on rat gastric mucosal serotonin. Drugs were suspended in 1% Tween 80 and orally administered daily for 8 weeks. The normal and control group received 1% Tween 80 daily. In all groups animals were sacrificed by cervical dislocation under ether anesthesia. Stomachs were dissected and part of each stomach was homogenized and the homogenate was used for the determination of gastric mucosal serotonin level. Each value represents the mean of 6 rat's ± S.E. Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by least significant difference (LSD) multiple comparisons test. *Significantly different from normal group at $p < 0.05$ using one way ANOVA followed by LSD for multiple comparisons.

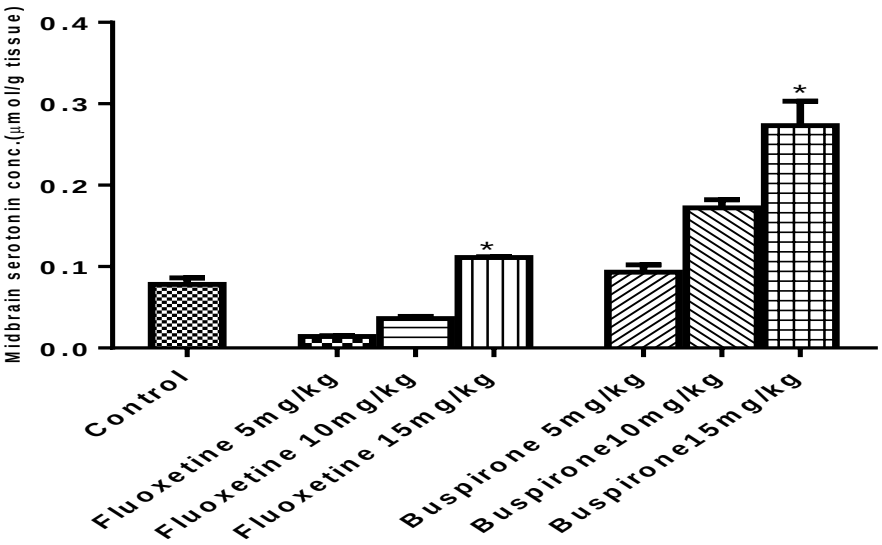

Figure 8. Effect of fluoxetine and buspirone at 5, 10 and 15 mg/kg on rat midbrain serotonin level. Drugs were suspended in 1 % Tween 80 and orally administered daily for 8 weeks. The normal and control group received 1% Tween 80 daily. In all groups animals were sacrificed by cervical dislocation under ether anesthesia. Brain was dissected and midbrain rejoin was homogenized and the homogenate was used for the determination of midbrain serotonin level. Each value represents the mean of 6 rats ± S.E. Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by least significant difference (LSD) multiple comparison test. *Significantly different from normal group at $p < 0.05$ using one way ANOVA followed by LSD for multiple comparisons.

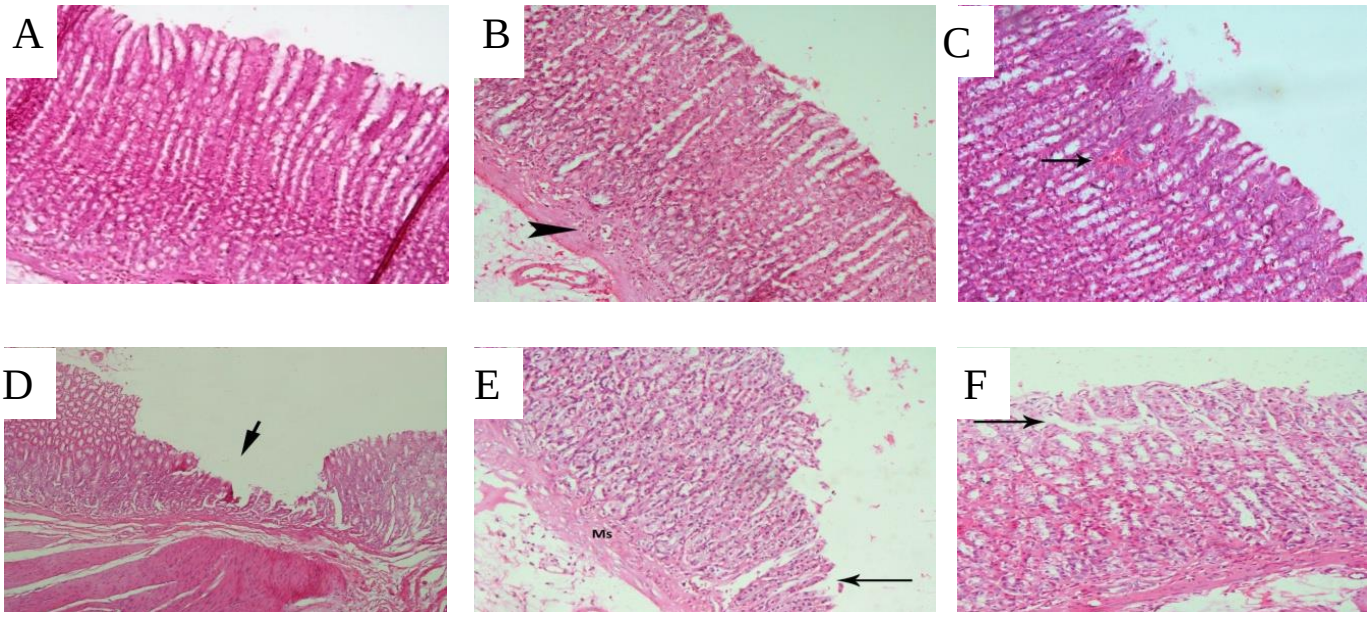

Figure 9. Photomicrograph of sections of gastric mucosa of normal rats (A), fluoxetine 15 mg/kg (B), buspirone 15 mg/kg (C), Indomethacin 1 mg/kg (D), fluoxetine 15 mg/kg + Indomethacin 1 mg/kg (E) and buspirone 15 mg/kg + Indomethacin 1 mg/kg (F) (Hx. & E. X 100).

Histopathological changes

Examination of the gastric mucosa of normal rats showed normal stomach structure (Figure 9A). Gastric mucosa of normal rats treated with fluoxetine at 15 mg/kg showed slight cellular infiltration in the muscularis mucosa layer. The rest of the tissue appears normal (Figure 9B). Gastric mucosa of normal rats treated with buspirone at 10 mg/kg showed quite normal gastric mucosal structure (Figure 9C).

Gastric mucosa of rats subjected to indomethacin showed a wide area of damage in the upper 2/3 of the gastric mucosa. Only the bases of the fundic glands are still present, while numerous sorts of cells were separated. (Figure 9D). Gastric mucosa of rats subjected to indomethacin and fluoxetine at 15 mg/kg showing marked thickening of muscularis mucosa (Ms). The gastric mucosa shows partial detachment of the upper third or half of the gastric glands (Figure 9E). Gastric mucosa of rats subjected to indomethacin and buspirone at 15 mg/kg showed mild gaps in between the gastric glands occupied by connective (fibrous) tissue, with a mild deformity of the normal structure of gastric mucosa (Figure 9F).

DISCUSSION

The gastric side effects of SSRI drugs have been reported (Lewis et al., 2008). However, numerous serotonin 1A receptor (5HT1-A) agonists, as buspirone, developed as anxiolytics, appeared with antisecretory and gastroprotective effects in rats (Glavin et al., 1995), by decreasing stomach and intestinal distension (Tack, 1999). Fluoxetine was found to cause a few number of ulcers with moderate severity in a dose-dependent manner. On the other hand, buspirone was found to be safe on the gastric mucosal. This study investigated fluoxetine and buspirone effect on GSH, NO and MDA activities in normal stomach tissue of rats, as a first approach to investigate the mechanism behind their effect on the stomach.

In the current study, all doses of fluoxetine and buspirone increased gastric NO levels significantly when compared to the normal group.

The present investigation revealed that eight weeks administrations of fluoxetine resulted in a decreased production of gastric NO content of the gastric mucosa in a dose dependent-manner with a marked decrease in low doses. By contrast, administrations of buspirone resulted in an increased gastric NO content in a dose dependent-manner; however, both markedly increased gastric mucosal lipid peroxides.

The increase in gastric GSH content was reported in the current results. Buspirone showed marked increase in the gastric GSH content; in the same way fluoxetine showed a slight increase in the gastric GSH content. This lesser increase in reduced glutathione was observed at the higher dose of fluexotine which markedly enhanced lipid peroxidation in gastric content.

The findings that lipid peroxidation was markedly increased by fluoxetine, was correlated with its ability to

cause gastric lesions. Alternatively, buspirone insignificantly increased gastric lipid peroxidation was visually previewed compared to control group in all experiments. Conversely, the increase of GSH by buspirone, may explain the effect of buspirone on gastric mucosa compared to fluoxetine.

SSRIs increase the 5-HT concentration in tissue, as a result of the inhibition of 5-HT reuptake at nerve endings and platelets. An increased serotonin level in the stomach plays a role in the aggravation of gastric lesions due to the vasoconstrictor effect of serotonin as shown by Cho et al. (1989); that the blood flow was decreased by 5-HT in a dose dependent-manner. On other hand, Takeuchi et al. (2011) found that aggravating effect of SSRIs to induce lesions were mimicked by exogenous 5-HT, symptomatic of the effect of endogenous 5-HT in this action. Ohta et al. (1997) confirmed the current results by serum serotonin concentration, an index of mast cell degranulation, increased with the formation of gastric mucosal lesions, and this increased serotonin level was attenuated with lesion progression and recovery.

A conseguential increase in the serotonin level in the midbrain may be due to the enhancement of serotonin synthesis secondary to an increased hydroxylation of tryptophan by tryptophan hydroxylase; the rate-limiting enzyme of the serotonin biosynthetic pathway. Serotonin additionally represents the aggressive ulcerogenic amines, which stimulate ulcer formation by raising total acidity and decreasing the volume of gastric secretion (Ibrahim et al., 1996).

Conclusion

The administration of fluoxetine caused the development of gastric mucosal lesions. This effect may be attributed to oxidative stress and an imbalance in gastric acid, peptic activity, mucin, GSH and NO, as well as increase in MDA contents. Buspirone appeared to be devoid of a deleterious effect on the gastric mucosa through antioxidant properties and by improving of the immunity in the gastric mucosa.

Conflict of Interests

The authors have not declared any conflict of interests.

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Ocular effects of oral acetazolamide on visual functions of normotensive individuals

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Acetazolamide is a carbonic anhydrase inhibitor which is on World Health Organization's list of essential medicines, a list of most important medications needed in a basic health system. The ocular effects of acetazolamide was studied in 30 normotensive volunteers. Each volunteer received a loading dose of 500 mg at 0700 h followed by a maintenance dose of 250 mg at 1300 and 1800 h, respectively on day one. From day two to day five, the volunteers received 250 mg acetazolamide thrice daily at the established time intervals. Each volunteer served as his or her own control. Ocular effects of the drug were assessed on the following visual functions; pupil diameter, near point of convergence, visual acuity, amplitude of accommodation, accommodation/convergence accommodation ratio and the phoria status. Results showed that the pupil diameter and near point of convergence increased while the amplitude of accommodation and the accommodation/convergence accommodation ratio decreased. The phoria tended towards exophoria. The aggregate effect on the volunteers was convergence insufficiency with the accompanying asthenopic symptoms, transient myopia and photophobia.

Key words: Acetazolamide, asthenopic, symptoms, convergence, insufficiency.

INTRODUCTION

The use of high potent drugs by the public and clinically in the treatment or management of ocular or systemic ailment could affect the visual functions. Among such drug is acetazolamide, a carbonic anhydrase inhibitor (Dutta and Goodsell, 2004), unsubstituted sulphonamide derivative (Rossi, 2013; Tripathi, 2013), and a bicarbonate diuretic (Reiss and Oles, 1996). The drug is used for medical treatment of glaucoma (Ives, 2013), epileptic

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seizures (Reiss and Oles 1996; Porter and Meldrum, 2013); idiopathic intracranial hypertension (Brayfield, 2014), altitude sickness (Leaf and Goldfarb, 2007), cystinuria (Wikipedia, the free encyclopedia), periodic peristalsis, central sleep apnoea (Aurora et al., 2012), dural ectasia (Brayfield, 2014; SRS, 2006). It has also been used to prevent methotrexate induced kidney damage by alkalinizing the urine, hence facilitating the excretion of methotrexate by increasing its solubility in urine (Shamash et al., 1991; Brayfield, 2014). Acetazolamide is not an immediate cure for acute mountain sickness rather, it facilitates the acclimatization process which in turn helps to relieve symptoms (Muzza et al., 2004; Leaf and Goldfarb the study: 2007; Low et al., 2012).

Acetazolamide has been extremely effective in inhibiting the production of aqueous humor by the ciliary body (Dutta and Goodsell, 2004) and most valuable as preoperative adjunct in the treatment of acute glaucoma as well as secondary glaucoma (PID, 2005; Rossi, 2013). It is on the WHO list of essential medicines that is, a list of the most important medications needed in a basic health system (WHO, 2013). The drug produces its primary pharmacologic effects through reversible, non-competitive binding with the enzyme carbonic anhydrase in the red blood cells. The enzyme catalyses the cellular production of carbonic acid (H_2C0_3) and the formation of hydrogen and bicarbonate ions (H^+ and $HC0_3$, respectively). Hence the inhibition of carbonic anhydrase activity in the ciliary body is the mechanism responsible for the decreased aqueous formation produced by acetazolamide (Ives, 2013). In the management of raised intraocular pressure, glaucoma or any condition requiring the reduction of intraocular pressure or as a diuretic or in the treatment of epilepsy, etc (Brayfield, 2004) where acetazolamide is used as a sole drug or in combination with other drugs, no attempt has been made to evaluate the effect of this drug on visual functions such as, pupil size (diameter), near point of convergence, amplitude of accommodation, distant and near visual acuity, accommodative-convergence/accommodation ratio, near and distant phoria, etc. The purpose of this study is to bridge the gap and provide the needed information to clinicians and practitioners since the literature is devoid of such information. It will further serve as an advocate in creating such therapeutic awareness.

MATERIALS AND METHODS

Volunteers who are mainly undergraduate students of Abia State University, Uturu, of both sexes, whose ages ranged from 21 to 30 years (mean 23.6 ± 1.6 years) and body weight 58 to 62 kg were used for the study. Informed verbal consent was obtained from these volunteers. They were further subjected to ocular examination by the optometrist and subjects who from history or examination with any of the following conditions were excluded from the study those with ocular diseases requiring treatment, amblyopic subjects or those with refractive errors of any type, subjects on any types of systemic medication, which might obscure the results, pregnant or lactating women, etc. After the pre-study examination (screening) thirty normal volunteers of either sexes, male: female ratio (50:50) were selected. Each volunteer had an average intraocular pressure, measured with schiotz tonometer of 20.5 ± 0.1 mmHg, and the following visual functions were measured to establish their initial values: pupil diameter, near point of convergence, amplitude of accommodation, visual acuity, etc. Furthermore, each volunteer acted as his or her own control. This study was approved by the University Ethical Committee on such studies.

Measurements

a. The pupil size (pupil diameter, PD) was measured in millimeters using the pupil distance rulers or pupilliometer. Readings were taken at three different positions and the mean calculated.
b. The near point of convergence (NPC) was measured with the subject fixating at the tip of a pencil positioned initially at 10 cm, then adjusted towards the subject until the subject reports diplopia. The distance between the position of doubling and the central plane of the subject was measured with a meter rule in centimeter to give the NPC.
c. The visual acuity (VA) was measured for near and far, respectively using the standard illuminated Snellen optotypes at appropriate measuring distance (0.4 m for near and 6m for far, respectively).
d. The phoria was measured using the phoropter, and for the distant phoria, the subject was asked to fixate at the far Snellen chart placed at 6 m, then a 15 prism diopters base-in was introduced on the right eye while 6 prism diopters base-out was introduced on the left eye. The chart will appear double. The 15 prism diopters base-in was gradually reduced until there was vertical alignment of the chart. The amount of prism diopters obtained was recorded as the phoria value. For near phoria, the procedure was repeated at a distance of 0.4 m.
e. The amplitude of accommodation (AA) was accomplished using the minus lens to the blur method. The amount of lens added to the blur point plus + 2.50 to compensate for the reduced target gave the AA in diopter.

Drug administration

Day 1, 500 mg acetazolamide tablets as loading dose, at 0700 h, thereafter maintenance dose of 250 mg at 1300 h and 1800 h, respectively. From day 2 to day 5, 250 mg acetazolamide was administered to the volunteers thrice daily at established time intervals. There was no restriction to fluid intake. Each volunteer agreed to comply with the research protocol. The general illumination was maximum, which was about 100 foot candles bearing in mind the volunteers comfort. The volunteers were allowed sufficient time for their eyes to adapt to this level of illumination before the following visual functions were measured: Pupil size (diameter) PD, near point of convergence, (NPC), visual acuity, (VA), amplitude of accommodation, (AA), accommodative convergence accommodation ratio, (AC/A) ratio and habitual phoria.

Materials

Acetazolamide tablets (Diamox^R) 250 mg, Lederle Laboratories

Table 1. Change in Pupil size in mm following oral administration of acetazolamide.

S/N	Initial pupil Size (mm)	Pupil size (mm) After first week	Pupil size (mm) After second week
1	2.8	3.0	2.8
2	2.8	3.0	2.8
3	2.8	2.8	2.8
4	3.0	3.2	3.0
5	3.0	3.4	3.0
6	3.2	3.4	3.1
7	3.2	3.3	3.1
8	3.2	3.2	3.2
9	3.4	3.5	3.4
10	3.4	3.6	3.4
11	3.4	3.6	3.4
12	3.4	3.5	3.4
13	3.5	3.6	3.5
14	3.5	3.5	3.5
15	3.5	3.7	3.6
16	3.6	3.8	3.6
17	3.6	3.7	3.6
18	3.6	3.7	3.6
19	3.7	3.8	3.7
20	3.7	3.8	3.7
21	3.7	3.9	3.8
22	3.8	3.9	3.8
23	3.8	4.0	3.9
24	3.8	3.9	3.8
25	3.8	3.8	3.8
26	3.9	3.9	3.9
27	3.9	4.0	3.9
28	4.0	4.2	4.1
29	4.0	4.1	4.0
30	4.0	4.0	4.0
Total	106	108	103
Mean	3.5 ± 0.2	3.6 ± 0.3	3.4 ± 0.2

Division, Cyanamide (Pakistan) Ltd, Karachi. Potable water, Pupillometer for the measurement of the pupil size (diameter) standard illuminated Snellen Optotypes, distant chart and reduced Snellen chart for the measurement of visual acuity, phoropter and its accessories for the measurement of other visual parameters involved in the study, Schiotz tonometer and 0.4% xylocaine.

Statistical analysis

Data were presented in tabular form. Each subject served as his or her own control. Differences between the initial value of each visual function prior to drug administration and the value obtained after administering acetazolamide was regarded as the change in that particular visual function and expressed as mean ± SEM for six readings, or as percentage.

RESULTS

The results of this study are presented in Tables 1 to 6. Table 1 shows the changes in pupil diameter following oral administration of acetazolamide and is indicative of transient mydriasis during the first week and reversal by the second week. Table 2 shows the changes observed in the values of NPC, an increase in the first week (43.9%), a fall in the second week (23.6%) which was still above normal value while Table 3 represents changes in

Table 2. Changes in the near point of convergence (NPC) in cm following oral administration of acetazolamide.

S/N	Initial NPC (cm) before drug	NPC after first week in (cm)	NPC after second week in (cm)
1	9.0	15.0	13.0
2	9.0	15.5	13.0
3	9.0	15.5	12.0
4	9.5	15.0	13.0
5	9.5	15.0	13.5
6	9.5	16.0	13.5
7	10.0	16.0	12.0
8	10.0	16.5	12.5
9	11.0	16.5	13.5
10	11.0	16.5	14.0
11	12.0	16.5	14.0
12	12.0	16.5	14.5
13	12.5	16.0	13.0
14	12.5	16.0	12.0
15	12.5	17.0	15.0
16	13.0	17.5	15.0
17	13.0	17.5	15.5
18	13.0	17.0	16.0
19	13.0	17.0	15.0
20	13.0	17.0	15.0
21	14.0	17.0	14.2
22	14.0	18.0	15.0
23	14.0	18.5	16.0
24	14.0	18.0	17.0
25	14.0	20.0	18.0
26	15.0	20.0	18.0
27	15.0	22.0	18.5
28	15.0	22.0	19.0
29	15.0	25.0	22.0
30	15.0	25.0	23.0
Total	369.0	531.0	465.7

Mean NPC before drug administration = (12.3 ± 0.7) cm. Mean NPC after first week = (17.7 ± 0.6) cm. Mean NPC after second week = 15.2 cm. (15.2 ± 0.5) cm. The table shows an increase of about 43.9% in the 1st week and 23.6% in the 2nd week following drug administration.

the VA. At near, there was 80% increase in the first week and 23% in the second week and there was no significant change in the distant VA throughout the study. Table 4 shows changes observed in the AA which declined by 11.3% in the first week and 8.2% in the second week while Table 5 shows a similar trend in the AC/A ratio in which the value decreased during the first week and increased slightly by the second week. Table 6 shows that before acetazolamide administration, 73.3, 16.7 and 10% of the study group were esophoric, exophoric and orthophoric, respectively. During the first and second week, the esophoric subjects demonstrated a decline, 50 and 36.7%, respectively while the exophoric and orthophoric subjects increased in the number of subjects (40 and 40%) and (10 and 23.3%), respectively. However, the near phoria was not affected by the acetazolamide administration hence all the subjects remained exophoric at near throughout the study.

Table 3. Changes in the visual acuity and effect on distant visual acuity following oral administration of acetazolamide during the two weeks.

S/N	V.A drug	Before administration	V.A first	After week	V.A second	After week
	At far	At near	At far	At near	At far	At near
1	6/4	N.5	6/4	N.5	6/4	N.5
2	6/4	N.5	6/4	N.5	6/4	N.5
3	$6/4^{+2}$	N.5	6/4	N.5	$6/4^{+2}$	N.5
4	$6/4^{+2}$	N.5	6/4	N.6	$6/4^{+2}$	N.5
5	$6/4^{+2}$	N.5	$6/4^{+1}$	N.5	$6/4^{+2}$	N.5
6	6/5	N.5	6/5	N.6	6/5	N.5
7	6/5	N.5	6/5	N.8	6/5	N.6
8	6/5	N.5	6/5	N.5	6/5	N.5
9	6/5	N.5	6/5	N.6	6/5	N.5
10	$6/5^{-1}$	N.5	6/5	N.6	6/5	N.5
11	$6/5^{-1}$	N.5	6/5	N.6	$6/5^{-1}$	N.5
12	$6/5^{-1}$	N.5	$6/5^{-1}$	N.6	$6/5^{-1}$	N.5
13	$6/5^{-2}$	N.5	$6/5^{-1}$	N.8	$6/5^{-1}$	N.6
14	$6/5^{-2}$	N.5	$6/5^{-1}$	N.6	$6/5^{-2}$	N.5
15	$6/5^{-2}$	N.5	$6/5^{-2}$	N.6	$6/5^{-2}$	N.5
16	$6/5^{-2}$	N.5	$6/5^{-1}$	N.5	$6/5^{-3}$	N.5
17	6/6	N.5	6/5	N.5	$6/5^{-2}$	N.5
18	6/6	N.5	$6/5^{-2}$	N.6	$6/5^{-3}$	N.5
19	6/6	N.5	6/6	N.8	$6/5^{-3}$	N.6
20	6/6	N.5	6/6	N.8	6/6	N.6
21	6/6	N.5	6/6	N.8	6/6	N.6
22	6/6	N.5	6/6	N.6	6/6	N.6
23	6/6	N.5	6/6	N.6	6/6	N.6
24	6/6	N.5	6/6	N.6	6/6	N.5
25	6/6	N.5	6/6	N.6	6/6	N.5
26	6/6	N.5	6/6	N.6	6/6	N.5
27	6/6	N.5	6/6	N.6	6/6	N.5
28	6/6	N.5	6/6	N.6	6/6	N.5
29	6/6	N.5	6/6	N.6	6/6	N.5
30	6/6	N.5	6/6	N.6	6/6	N.5

Percentage change in near VA is 80% in the first week and about 23% in the second week following drug administration, while no appreciable effect in distant VA was observed.

DISCUSSION

Acetazolamide a diuretic, is a carbonic anhydrase inhibitor causing the accumulation of carbonic acid, catalyzing the reaction that ultimately leads to reduction in aqueous humour (PID, 2005) and fall in intraocular pressure. There is paucity of information on ocular manifestation of the drug on other visual functions when used therapeutically. However, during the period of this study which lasted three weeks, some of the subjects complained of mild dizziness (a central effect), and slight discomfort in vision. The PD increased by 3.1% during the first week of drug administration and decreased to 1.1% during the second week, still showing mydriatic effect with a tendency to returning to normal by the end of the study. The transient mydriasis observed in the subjects led to double vision (Table 1). The state of the pupil at any moment is determined by a variety of synergistic and antagonistic nervous influences over the ocular muscles. Generally, external factors such as light and proximity of the fixation point tend to cause constriction while internal factors of sensation and psychic activity cause dilatation (Grosvenor, 1989), and in the present study oral acetazolamide aids the second process of dilatation, hence the transient mydriasis.

The NPC increased following oral administration of

Table 4. Changes in amplitude of accommodation in diopters after oral administration of acetazolamide during the two weeks.

S/N	Initial AA before Drug Administration (D)	AA After First Week (D)	AA After Second Week (D)
1	7.0	6.3	6.5
2	7.0	6.3	6.5
3	7.0	6.0	6.0
4	7.5	6.0	6.3
5	7.5	6.5	6.5
6	8.0	7.0	6.8
7	8.0	7.3	7.0
8	8.0	7.0	7.3
9	8.0	7.0	7.5
10	9.0	7.8	8.0
11	9.0	8.0	8.0
12	9.0	8.5	8.3
13	9.5	8.0	8.5
14	9.5	8.5	8.5
15	9.5	8.5	9.0
16	9.8	9.0	9.3
17	9.8	9.3	9.8
18	10.0	9.3	10.0
19	10.0	9.0	10.0
20	10.5	9.8	10.3
21	10.5	10.0	10.5
22	10.5	10.0	10.3
23	11.0	10.3	10.5
24	11.0	10.3	10.8
25	11.0	10.0	11.0
26	11.0	10.5	11.0
27	11.0	10.5	11.0
28	11.0	10.0	11.0
29	11.0	10.8	11.0
30	11.0	10.8	11.0
Total	291.1	258.0	268.3

Mean (AA) before drug administration = (9.7 ± 0.4)D. Mean (AA) after first week of drug administration = (8.6 ± 0.3)D. Mean (AA) after second week of drug administration = (8.9 ± 0.6)D. The table shows a decrease in (AA) of about 11.3% in the first week and about 8.2% in the second week following oral administration of acetazolamide.

acetazolamide during the first and second week of the study, the normal NPC is between 8 and 10 cm as measured from the spectacle plane. If the NPC exceeds 10 cm, there will be problem of convergence insufficiency and in the study the NPC exceeded the range during the first and second week that is, (17.7 ± 0.6) cm and (15.2 ± 0.5) cm, respectively and the subjects would be predisposed to asthenopic symptoms during near work. Asthenopia is the weakness of the eye due to fatigue of the ciliary muscle or the extraocular muscle which is manifested in painful vision. This asthenopia is further aggravated by the mydriatic effect of the drug. During the course of the study, more than 60% of the population complained of tearing, dizziness, ocular discomfort and mild headache which are symptoms of asthenopia. Tearing which was experienced by all the subjects was indicative of diuretic action of acetazolamide.

It is known that when the NPC recedes beyond 10 cm, the individual will have convergence insufficiency. Since there was pupillary dilatation (mydriasis), the depth of

Table 5. Changes in accommodation-convergence accommodation ratio (AC/A) following oral administration of acetazolamide.

S/N	AC/A ratio before drug administration	AC/A ratio after first week	AC/A ratio after second week
1	8/1	7/1	7.5/1
2	8/1	7/1	7.5/1
3	8/1	7/1	7.0/1
4	7/1	6/1	6.5/1
5	7/1	6/1	6.5/1
6	7/1	6/1	6.4/1
7	7/1	6/1	6.4/1
8	7/1	6/1	6.4/1
9	6/1	6/1	5.2/1
10	6/1	5.2/1	5.5/1
11	6/1	5.2/1	5.5/1
12	6/1	5.3/1	5.6/1
13	6/1	5/1	5.6
14	6/1	5/1	5.6
15	6/1	5/1	6/1
16	6/1	5/1	6/1
17	5/1	5/1	4.5/1
18	5/1	4.0/1	4.5/1
19	5/1	4.0/1	4.5/1
20	5/1	4.0/1	4.5/1
21	5/1	4.0/1	4.8/1
22	5/1	4/1	5/1
23	4/1	4/1	3.4/1
24	4/1	3/1	3.5/1
25	4/1	3/1	3.5/1
26	4/1	3/1	3/1
27	4/1	2/1	3/1
28	4/1	2/1	3/1
29	4/1	2/1	2.5/1
30	4/1	2/1	3/1

Mean (AC/A) ratio before drug administration = 6.56/1. Mean (AC/A) ratio after first week following drug administration = 4.72/1. Mean (AC/A) ratio after second week following drug administration =5.29/1. The result shows a decrease in (AC/A) ratio in the first week and a slight increase in the second week.

focus has been compromised, the image tends to blur faster than normal hence the asthenopia. However, by the second week of the study, the NPC had started to improve, because the pupil had started to constrict.

The changes in near VA occasioned by the mydriatic effect of acetazolamide and the receding NPC shifted the refractive error to transient myopia, the myopia subsided upon discontinuing the acetazolamide therapy (Leaf and Goldfarb, 2007). Furthermore, it is noted that the myopia worsens with low NPC and convergence insufficiency as near work is attempted with the accompanying asthenopic symptoms.

The amplitude of accommodation (AA) represents the maximum amount of accommodation which the eye can exert and it is expressed in diopters. The ability of the eye to effect this is influenced by various factors such as age, previous refractive status of the patient, ciliary muscle balance, drug therapy etc and in the present study and in the first week, the AA dropped by 11.3% following oral acetazolamide administration in concert with the mydriatic effect. This further explains the transient myopia, receded NPC and poor near vision during the first week of the study and tended towards reversal by the second week (8.2%). The study also showed that a change towards

Table 6. Changes in habitual phoria values at far and near following oral administration of acetazolamide .

S/N	Habitual Phoria before drug administration		Habitual Phoria after first week		Habitual Phoria after second week	
	Far	Near	Far	Near	Far	Near
1	Ortho	6exo	0.5exo	8exo	0.5exo	7exo
2	0.5exo	6exo	1exo	8exo	1exo	7exo
3	0.5exo	6exo	1exo	7.5exo	1exo	6.5exo
4	Ortho	6.5exo	1exo	7.5exo	1exo	6.5exo
5	Ortho	6exo	1exo	7exo	1exo	6.5exo
6	1exo	6.5exo	1exo	7exo	1exo	6exo
7	1exo	6exo	1exo	7exo	1exo	6exo
8	1exo	6exo	1.5exo	6.5exo	1exo	6exo
9	0.5eso	5.5exo	Ortho	6.5exo	Ortho	5exo
10	0.5eso	5.5exo	Ortho	6exo	Ortho	5exo
11	0.5eso	5.4exo	Ortho	6exo	Ortho	5exo
12	1exo	5exo	0.5exo	5.5exo	0.5exo	5exo
13	1exo	5exo	0.5exo	5.5exo	0.5exo	5exo
14	1exo	5exo	0.5exo	5.5exo	0.5exo	5exo
15	1exo	5exo	0.5exo	5exo	0.5exo	4exo
16	2eso	4.5exo	1eso	5exo	Ortho	4exo
17	2eso	4.5exo	1eso	4.5exo	Ortho	4exo
18	2eso	4exo	1eso	4.5exo	1eso	4exo
19	2eso	4exo	1.5eso	4exo	1eso	4exo
20	2eso	3.5exo	1eso	4exo	1eso	3exo
21	3eso	3.5exo	2eso	4exo	1eso	3exo
22	3eso	3.5exo	2eso	4exo	1.5eso	3exo
23	3eso	3exo	2eso	3.5exo	1.5eso	3exo
24	3eso	3exo	2eso	3.5exo	1eso	3exo
25	3eso	3exo	2eso	3exo	Ortho	3exo
26	3.5eso	2.5exo	2.5eso	3exo	Ortho	2exo
27	3.5eso	2.5exo	2eso	3exo	1eso	2exo
28	4eso	2exo	2eso	3exo	1eso	2exo
29	4eso	2exo	2eso	3exo	1eso	2exo
30	4eso	2exo	3eso	2exo	1eso	2exo

The table shows an increase in exophoria in first week of drug administration which improved in the second week.

exophoria existed from 16.7 to 40% during the first and second week of the study. In a similar manner, the esophoric volunteers declined from 73.3 to 50% and 36.7% in the first and second week, res-pectively. The number of volunteers in the orthoposition increased from 10 to 23.3% in the second week of the study. However, all these subjects demonstrated exophoria at near which exacerbated the asthenopic symptoms if the fusional reserves are not enough (Grosvenor, 1989).

The accommodative-convergence accommodation (AC/A) ratio represents the change in convergence that accompanies a change in accommodation when fusion is interrupted per unit change in accommodation (Bruce et al., 1995; Mutti et al., 2000). It is used for clinical analysis of visual problems and it was found in this study that following oral administration of acetazolamide the AC/A ratio decreased (Table 5) thereby causing convergence insufficiency (Igwe et al., 2015) and the associated visual problems. Table 5 shows that the changes in AC/A ratio were transient as the AC/A ratio increased following cessation of therapy.

It is noteworthy that drug-induced (acetazolamide) myopia and convergence insufficiency contributed to the visual problems of the volunteers, visual discomfort,

asthenopic sysmptoms, etc and the unique benefit of this study is that it would enable clinicians gain more knowledge of the ocular pharmacology of such routine essential drug.

Conflict of Interests

The authors have not declared any conflict of interests.

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