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Effects of the New Antifungal Thio Halo-Benzene Derivative 5-Bromo-2-Iodo-1,3-Bis(Phenylthio)Benzene Molecule on Rat Liver and Kidney Tissue Oxidative Events

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Abstract

Nowadays, fluconazole is a widely used antifungal drug. Besides its similarities with the carbon structure of fluconazole, the newly synthesized 5-bromo-2-iodo-1,3-bis(phenylthio)benzene is also more active than low concentrated of fluconazole. Oxidative events occur in the organism during the drug metabolism. In this study, effects of previously proved as an antibacterial and antifungal molecule 5-bromo-2-iodo-1,3-bis(phenylthio)benzene investigated ($C_{18}H_{12}S_2IBr$) on mammal liver and kidney tissue using biochemical methods. 80 male and female Wistar albino rats between the ranges of 150-200 gr have been used for this study. Kidney and liver tissues malondialdehyde (MDA), glutathione (GSH), nitric oxide (NO) levels, and myeloperoxidase activities (MPO) studied using spectrophotometric methods. We found that this new compound as a potential alternative to fluconazole did not cause any lipid peroxidation in the liver tissue of female rats and in kidney tissue of male rat. This could be because of hormone (gonadal hormone) or metabolism differences of the sexes. It may be remarkable to note that 5-bromo-2-iodo-1,3-bis(phenylthio) benzene molecule does not cause nitrosative species formation at both doses and sexes. Also 5-bromo-2-iodo-1,3-bis(phenylthio) benzene may increase tissue antioxidant capacity in rats. Both doses of the newly synthesized 5-bromo-2-iodo-1,3-bis(phenylthio) benzene molecule may not cause respiratory burst in the liver and kidney tissues in both sexes.

1. INTRODUCTION

Nowadays fungal infections, tuberculosis, cancer and AIDS have become the main cause and the most important complexities of death and/or morbidity for the people who had an organ transplant and their immune system depressed [1,2]. Triazole antifungal compounds like fluconazole and voriconazole function inhibiting the lanosterol cytochrome P450 14α-demethylase (CYP51) enzyme. However, clinical values, relatively high toxicity, the drug resistance and pharmacokinetic shortcomings of these compounds are limited because of their lack of antifungal activities. Broad spectrum antifungal agents with low toxicities are still needed in despite of the recent developments [3-6]. In this sense the antifungal and antibacterial effects of the synthesized 5-bromo-2-iodo-1,3-bis(phenylthio)benzene have been revealed in previous studies [7-10]. This compound has similar carbon structure with commonly used antifungal drug fluconazole, not to mention that it is more active than low concentrations of fluconazole. Fluconazole has common side effects such as vomiting, nausea, headache and toxicity through interactions with drugs that are metabolized by CYP3A4 (statins and cyclosporine) [8].

Oxidative processes in the organism takes place during and after the use of drugs. There is little research into oxidative stress during drug uses. At the same time, there is not enough information about the oxidative events that occur during the use of this new drug. Therefore, We plan to investigate the parameters mentioned below: Myeloperoxidase (MPO) is an enzyme activated by macrophages, neutrophils and monocytes, found in phagocytes, involved in the mechanism of inflammatory regulation [11]. Nitric oxide (NO) is an important signaling molecule in mammals to assess various pathological states. NO can be produced in damaged cells in various cells such as platelets, keratinocytes, fibroblasts and macrophages

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[12]. Glutathione (GSH) is a tripeptide involved in the prevention of tissue damage caused by radicals and peroxides released by free ROS. MDA is a frequently used parameter to observe increased lipid peroxidation [13].

In this study, it is aimed to investigate the effects of 5-bromo-2-iodo-1,3-bis(phenylthio)benzene molecule ($C_{18}H_{12}S_2IBr$) as a new raw drug material on mammal liver and kidney tissue oxidative events in both gender.

2. MATERIAL and METHOD

In this study, 80 male and female Wistar albino rats between the range of 150-200 g have been used. Total 16 rats have been used as subjects in this experiment and each group consisted of 8 males and 8 females. Before and during the experiments, the rats were housed in individual cages with unrestricted standard rat chow and water. They were maintained in a 12 h light/12 h dark cycle at room temperature (25 ± 3 °C). Fluconazole and $C_{18}H_{12}S_2IBr$ were dissolved in ethanol (absolute alcohol: 0.145 mg kg^{-1} of bw) and applicated by oral gavage once a week for four weeks. The control group was taking no treatment; the vehicle group was receiving 0.145 mg kg^{-1} bw of ethanol, which served as the vehicle for active compounds, once a week; the fluconazole group was receiving 19.6 mg kg^{-1} bw of fluconazole a week; the lowdose $C_{18}H_{12}S_2IBr$ group was receiving 3.92 mg kg^{-1} bw of $C_{18}H_{12}S_2IBr$ a week; and the high-dose $C_{18}H_{12}S_2IBr$ group 7.84 mg kg^{-1} bw of $C_{18}H_{12}S_2IBr$ a week. We used fluconazole dose of 200 mg in the face of a 70-kg human receives every day during a 28-day treatment [14]. The tested concentrations of $C_{18}H_{12}S_2IBr$ were two and a half times and five times lower than that of fluconazole, based on earlier in vitro efficiency findings [15], as no in vivo efficiency has been studied yet.

Rats have been given fluconazole as well as 5-bromo-2-iodo-1,3-bis(phenylthio)benzene orally, each dissolved in alcohol, once in a week for 4 weeks. All subjects grouped as;

1. Control group,
2. Alcohol control,
3. Fluconazole group (0.28 mg/100 g),
4. 5-bromo-2-iodo-1,3-bis(phenylthio)benzene (0.112 mg/100 g),
5. 5-bromo-2-iodo-1,3-bis(phenylthio)benzene (0.056 mg/100 g).

Kidney and liver tissues obtained from subjects using cervical dislocation and preserved at -30 °C until examination. Kidney and liver malondialdehyde (MDA) [16], glutathione (GSH) [17], nitric oxide (NO) [18,19] and myeloperoxidase activity (MPO) [20] studied using spectrophotometric methods.

3. STATISTICAL ANALYSIS

Mean differences were compared by Anova Variance Analysis (ANOVA) followed by Tukey' s test for post-hoc analysis. Values of $P< 0.05$ were considered to be statistically significant. The results were expressed as the mean ± standard deviation.

4. RESULTS and DISCUSSION

Over the last two decades, invading fungal infection rates and its difficulties are highly increasing due to the host's deficiency of the immune system and its suppression. Curative, prior or prophylactic antifungal therapies are being developed to overcome Candida infections.

The resistance of fungal infections to antifungal agents is another big problem. For these reasons, wide spectrum antifungal agents with low toxicity are still needed [3-6].

A B

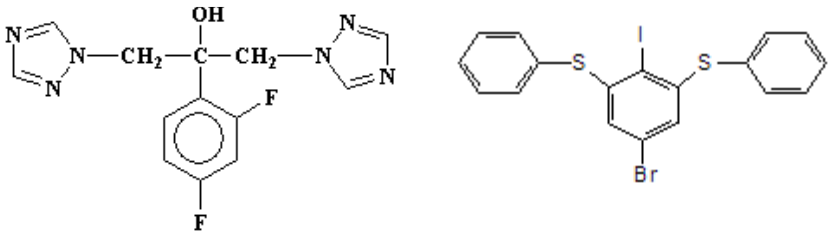

Figure 1. *Chemical structure of (A) Fluconazole, (B) 5-bromo-2-iodo-1,3-bis(phenylthio)benzene ($C_{18}H_{12}S_2IBr$)*

The molecular structures of fluconazole and newly synthesized 5-bromo-2-iodo-1,3-bis(phenylthio)benzene are shown in Figure 1. The effects of these molecules on mammal tissues examined on both male and female rat liver and kidney tissues.

In this study, the effects of newly synthesized 5-bromo-2-iodo-1,3-bis(phenylthio)benzene chemical on liver and kidney of both male and female subjects have been examined in the terms of oxidative damage, separately.

This effect on mammal tissues examined as liver and kidney MDA, GSH, NOx levels and MPO activity. Liver MDA, GSH, NOx levels and MPO activity of female rats have been given in Table 1, also results for all parameters of male rats have been given in Table 2. Kidney MDA, GSH, NOx levels and MPO activity of female rats have been given in Table 3, also results for all parameters of male rats have been given in Table 4.

4.1. Biochemical Parameters

4.1.1. MDA levels of liver and kidney tissues in rats

MDA levels of the liver tissues in the female rats statistically increased in group 3 when compared to group 4 and group 5 ($p< 0.05$). There wasn't any statistically a difference between group 4 and group 5 ($p> 0.05$) in terms of MDA levels of the liver tissues in the female rats. When 5-bromo-2-iodo-1,3-bis(phenylthio)benzene molecule and fluconazole compared, it is shown that 5-bromo-2-iodo-1,3-bis(phenylthio)benzene does not cause any oxidative damage on liver tissue particularly on female rats, in fact it is even acts like an antioxidant because of its lower MDA levels than control group (Table 1).

However, the MDA levels of the liver tissues in the male rats were not change among all experimental groups except group 2. 5-bromo-2-iodo-1,3-bis(phenylthio)benzene does not cause lipid peroxidation at liver tissues of male rats (Table 2).

There wasn't any difference between group 3 and group 4 in terms of MDA levels of the kidney tissues in the female rats ($p> 0.05$). MDA levels in kidney tissue were determined in the female rats that were increased in all experimental groups when compared to control group. When compared to fluconazole, MDA levels proved 5-bromo-2-iodo-1,3-bis(phenylthio)benzene molecule to cause less lipid peroxidation in female kidney tissue in group 4 (Table 3).

Statistically decreased MDA levels of kidney tissues in the male rats were found especially in the Group 4 when compared to group 3 ($p<0.05$). This finding indicate that 5-bromo-2-iodo-1,3-bis(phenylthio)benzene molecule did not cause lipid peroxidation in kidney tissue of male rat (Table 4).

This study is the first Süloğlu et al. (2015) reported that no toxicity data on this new compound in vitro on L929 mouse fibroblast cell lines [8]. In consistent with their findings, we found that this new compound as a potential alternative to fluconazole did not cause any lipid peroxidation in the liver tissue of female rats and in kidney tissue of male rat. This may be because of hormone (gonadal hormone) or metabolism differences between the sexes.

4.1.2. NOx levels of liver and kidney tissues in rats

NOx levels of the liver tissues in the female rats statistically decreased in both group 4 and group 5 when compared to group 3 ($p< 0.05$). 5-bromo-2-iodo-1,3-bis(phenylthio) benzene molecule at both doses was more successful at reducing liver tissue nitric oxide levels in the female rats when compared to fluconazole (Table 1). This newly synthesized compound does not cause the formation of nitrosative species in the liver tissue during drug metabolism.

There wasn't any difference among all experimental groups in terms of NOx levels of the liver tissues in the male rats ($p> 0.05$) (Table 2).

Fluconazole and both doses of the newly synthesized drug were reduced the NOx levels in the kidney tissue in female rats when compared to control ($p< 0.05$) (Table 3).

NOx levels in the kidney tissue in male rats were not changed all experimental groups except group 2 ($p> 0.05$) (Table 4).

This synthesized compound does not cause the formation of nitrosative species in both the liver and kidney tissue during drug metabolism at both sexes. It may be remarkable to note that 5-bromo-2-iodo-1,3-bis(phenylthio) benzene molecule does not cause nitrosative species formation at both doses and sexes.

4.1.3. GSH levels of liver and kidney tissues in rats

GSH is non-enzymatic antioxidants and play a role scavenging oxidative and nitrosative species in living organisms [13].

Fluconazole and both doses of the newly synthesized drug were reduced the GSH levels in the liver tissue in female and male rats when compared to control ($p< 0.05$) (Table 1and Table 2). This finding indicates that GSH may have been used to remove elevated lipid peroxidation during drug metabolism.

However, GSH levels of kidney tissue of female rats increased in group 4 and group 5 when compared to group 3 ($p< 0.05$) (Table 3). 5-bromo-2-iodo-1,3-bis(phenylthio) benzene molecule may increase kidney tissue antioxidant capacity elevating GSH level.

We found that GSH levels increased in the kidney tissue of male rats in group 5 when compared to control ($p< 0.05$) (Table 4). 5-bromo-2-iodo-1,3-bis(phenylthio) benzene molecule at this dose (0.056 mg/100 g) was found significant in terms of increasing GSH level in the kidney tissue of male rats. These increases in GSH level are parallel to tissue MDA levels for kidney tissue of male rats. 5-bromo-2-iodo-1,3-bis(phenylthio) benzene molecule may increase kidney tissue antioxidant capacity elevating GSH level.

4.1.4. MPO activity of liver and kidney tissues in rats

MPO activity of liver tissues in female rats were reduced with all three treatments (group 1, 2 and 3) when compared to control ($p< 0.05$) (Table 1). Both Fluconazole and both doses of the newly synthesized 5-bromo-2-iodo-1,3-bis(phenylthio) benzene molecule may not cause respiratory burst in the liver tissues of female rats. 5-bromo-2-iodo-1,3-bis(phenylthio) benzene molecule may not cause neutrophil infiltration.

There wasn't any difference between all three experimental group (group 1, 2 and 3) when compare to control in terms of MPO activity of liver tissues in male rats ($p> 0.05$) (Table 2). Fluconazole and both doses of the newly synthesized drug may not cause neutrophil infiltration in liver tissue of male rats.
Both doses of the newly synthesized 5-bromo-2-iodo-1,3-bis(phenylthio) benzene molecule decreased MPO activity in kidney tissue of female rats when compared to control ($p< 0.05$) (Table 3). Fluconazole and both doses of the newly synthesized drug may not cause neutrophil infiltration in kidney tissue of female rats.

There wasn't any difference between all three experimental groups except group 2.in terms of MPO activity of kidney tissue of male rats.

Süloğlu et al. (2015) suggested that Fluconazole significantly increased leukocytes and lowered neutrophils whereas 5-bromo-2-iodo-1,3-bis(phenylthio) benzene molecule did not in the liver tissue [15]. In consistent with above study, both doses of the newly synthesized 5-bromo-2-iodo-1,3-bis(phenylthio) benzene molecule may not cause respiratory burst in the liver and kidney tissues in both sexes.

Table 1. *MDA, GSH, NOx levels and MPO activity in the liver tissues of female rats*

	MDA (nmol/g tissue)	GSH (µmol/g tissue)	MPO (U/g tissue)	NOx (µmol/g tissue)
Group 1	122.8±28.8[a]	22.7±4.6[a]	0.7±0.10[a]	436.7±18.2[a]
Group 2	135.3±17.3[b]	28.4±4.2[b]	0.7±0.08[b]	426.8±36.2[b]
Group 3	77.2±4.1[c]	19.3±1.6[c]	0.4±0.02[c]	477.7±16.7[c]
Group 4	57.5±4.5[d]	18.8±1.9[d]	0.4±0.02[d]	450.2±9.4[d]
Group 5	48.6±7.7[e]	18.9±1.2[e]	0.4±0.02[e]	426.3±15.0[e]

MDA levels: a-c, a-d, a-e, b-c, b-d, b-e, c-e, c-d $p < 0.05$
GSH levels: a-c, a-d, a-e, b-c, b-d, b-e $p < 0.05$
MPO activity: a-c, a-d, a-e, b-c, b-d, b-e $p < 0.05$
NOx levels: a-c, b-c, c-d, c-e, d-e $p < 0.05$

Table 2. *MDA, GSH, AA, NO levels and MPO activity in the liver tissues of male rats.*

	MDA (nmol/g tissue)	GSH (µmol/g tissue)	MPO (U/g tissue)	NOx (µmol/g tissue)
Group 1	71.6±14.7[a]	21. 2±8.1[a]	0.6±0.1[a]	444.4±23.7
Group 2	165.9±40.6[b]	31.5±5.2[b]	0.8±0.09[b]	438.5±20.3
Group 3	87.9±11[c]	13.6±1.6[c]	0.6±0.03[c]	448.5±14.9
Group 4	79.4±9[d]	14.7±1.7[d]	0.6±0.06[d]	450.4±20.4
Group 5	82.5±7.3[e]	15.1±0.9[e]	0.6±0.04[e]	453.9±31.5

MDA levels: a-b, b-c, b-d, b-e $p < 0.05$
GSH levels: a-c, a-d, a-e, b-c, b-d, $p < 0.05$
MPO activity: a-b, b-c, b-d, b-e $p < 0.05$

Table 3. *MDA, GSH, NOx levels and MPO activity in the kidney tissues of female rats.*

	MDA (nmol/g tissue)	GSH (µmol/g tissue)	MPO (U/g tissue)	NOx (µmol/g tissue)

Group 1	157.9±27.3[a]	25.7±5.4[a]	0.4±0.10[a]	860.7±53.8[a]
Group 2	263.2±44.8[b]	24.3±2.8[b]	0.6±0.02[b]	1147.5±195[b]
Group 3	234.6±16.2[c]	27.1±3.4[c]	0.2±0.03[c]	309.0±12.8[c]
Group 4	231.2±11.6[d]	30.6±4.3[d]	0.3±0.02[d]	377.0±51.4[d]
Group 5	323.2±53.4[e]	30.1±4.5[e]	0.3±0.02[e]	351.3±34.6[e]

MDA levels: a-b, a-c, a-d, a-e, b-d, b-e, c-e, d-e. $p< 0.05$
GSH levels: a-d, a-e, b-d, b-e, c-d, c-e. $p< 0.05$
MPO activity: a-b, a-c, a-d, a-e, b-c, b-d, b-e, c-d, c-e. $p< 0.05$
NOx levels: a-b, a-c, a-d, a-e, b-c, b-d, b-e, c-d, c-e $p< 0.05$

Table 4. *MDA, GSH, NOx levels and MPO activity in the kidney tissues of male rats.*

	MDA (nmol/g tissue)	GSH (μmol/g tissue)	MPO (U/g tissue)	NOx (μmol/g tissue)
Group 1	186.0±3.6[a]	20.7±4.6[a]	0.3±0.03[a]	359.9±11.2[a]
Group 2	106.9±16.9[b]	16.8±1.8[b]	0.4±0.06[b]	953.8±61.9[b]
Group 3	223.2±34.6[c]	23.1±2.8[c]	0.3±0.03[c]	381.7±20.9[c]
Group 4	169.9±11.4[d]	23.7±3.5[d]	0.3±0.03[d]	371.2±34.3[d]
Group 5	247.2±10.3[e]	25.8±2.5[e]	0.3±0.04[e]	359.8±17.2[e]

MDA levels: a-b, a-c, a-e, b-c, b-d, b-e, c-d, d-e. $p< 0.05$
GSH levels: a-e, b-e. $p< 0.05$
MPO activity: a-b, b-c, b-d, b-e. $p< 0.05$
NOx levels: a-b, b-c, b-d, b-e. $p< 0.05$

Consequently, on females, thio halo-benzene derivative 5-bromo-2-iodo-1,3-bis(phenylthio) benzene decreases the MDA levels which are the indicators of lipid peroxidation, as well as neutrophil infiltration. It is concluded that this newly synthesized antifungal compound may not cause any oxidative damage in liver tissue, especially on females. Also 5-bromo-2-iodo-1,3-bis(phenylthio)benzene may increase tissue antioxidant capacity in rats.

4. CONCLUSIONS

This study was the first to investigate the effect of the 5-bromo-2-iodo-1,3-bis(phenylthio) benzene molecule on rat liver and kidney tissues oxidative events comparing with fluconazole. This new compound promises a remarkable future in the treatment of the fungal infections In conclusion, on males, 5-bromo-2-iodo-1,3-bis(phenylthio) benzene decreases the MDA levels which are the indicators of lipid peroxidation, as well as neutrophil infiltration. It is concluded that this newly synthesized antifungal compound may not cause any oxidative damage in kidney tissue, especially on males. Also 5-bromo-2-iodo-1,3-

bis(phenylthio)benzene may increase tissue antioxidant capacity in rats. The point to be considered is to reveal the effects of 5-bromo-2-iodo-1,3-bis(phenylthio)benzene molecule on other seconder tissues. These findings may be supported by clinical trials.

CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

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