Original Article

Changes of dopamine D2, alpha1 adrenergic receptor expressions and developmental stages of seminiferous tubule in rat testis after methamphetamine administration: A preliminary study

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Abstract

The alterations of dopamine D2 receptor (D2R) and α1 adrenergic receptor (α1AR) expressions and developmental stages of seminiferous tubule were investigated in twenty-two male rats that underwent one of three Methamphetamine (METH) treatment regimes: acute binge (AB), escalating dose (ED) or escalating binge dose (ED-binge), in comparison with the control. The percentages of stage II seminiferous tubule were significantly decreased in ED and ED-binge groups. Reductions of D2R immunoreactivity in spermatocytes were found in all METH treated groups, while D2R reductions in elongated spermatids were found in ED and ED-binge groups. The expressions of α1AR in spermatocytes were increased in all METH treated groups as well as in round and elongated spermatids in ED and ED-binge groups. The results indicate that METH can cause changes in D2R and α1AR expressions in the testis of METH-addicted rats, which may disrupt DA and NE functions leading to changes of seminiferous epithelium development and consequently spermatogenesis impairment.

Keywords: Methamphetamine, dopamine D2 receptor, adrenergic receptor, spermatogenesis, seminiferous epithelium

1. Introduction

Methamphetamine (METH) is a central nervous system (CNS) stimulant drug. METH can affect on CNS by changing dopamine (DA) and norepinephrine (NE) levels. Moreover, METH has been reported to have an adverse effect on the reproductive system. A previous study demonstrated that METH altered serum testosterone (T) concentration and increased apoptotic cells in the seminiferous tubule in mice (Yamamoto *et al*., 2002). METH has also been reported to decrease proliferation of spermatogonia in rat testis (Alavi, Taghavi, & Moallem, 2008), induce abnormal sperm morphology, decrease sperm concentration and increase apoptotic cells in rat seminiferous tubule (Nudmamud-Thanoi & Thanoi, 2011). In our research group, we found that METH not only decreased progesterone receptors, estrogen receptor alpha and estrogen receptor beta in rat testis, but also reduced sperm concentration and normal sperm morphology (Nudmamud-Thanoi & Thanoi, 2011). In addition, METH has an effect on reproductive system by alteration of sex hormones such as estrogen and progesterone (Nudmamud-Thanoi, Sueudom, Tangsrisakda, & Thanoi, 2016). METH has also been reported to have effects on the interference function of hypothalamic-pituitary-gonadal axis (Shen *et al*., 2014), as well as activating the sympathetic nervous system (Haile, De La Garza, Mahoney, & Newton, 2013). Therefore, these two mechanisms can cause sex-hormone changes which may consequently induce reproductive system abnormality.

As mentioned above, METH is a dopamine agonist. Thus, we hypothesized that METH may have direct effects on testicular cells since many previous studies suggested that dopamine and adrenergic receptors are expressed in testis and spermatozoa (Adeoya-Osiguwa, Gibbons, & Fraser, 2006; Huo, Zhong, Wu, & Li, 2012; Otth *et al*., 2007). In addition, abnormal morphology of Sertoli cells and arrested development of spermatozoa have been reported in α1B adrenergic receptor knockout male mice (Mhaouty-Kodja *et*

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al., 2007). Other studies have also reported that NE is involved in development of Leydig cell (Huo *et al.*, 2012) and activation of sperm capacitation (Adeoya-Osiguwa & Fraser, 2005). High dose of DA has been suggested to decrease sperm motility (Ramirez *et al.*, 2009). Interestingly, our previous study also demonstrated that DOPAC, a metabolite of DA, and NE concentration in rat testis were changed after exposure to METH (Janphet, Nudmamud-Thanoi, & Thanoi, 2016). Therefore, the immunoreactivities of dopamine and adrenergic receptors in testis as well as the changes of developmental stages of seminiferous epithelium after METH exposure were investigated in this study. These will provide more information on the effects of METH on the male reproductive system.

2. Materials and Methods

2.1 Animals

Twenty-two male Spargue-Dawley rats were obtained from Nation Animal Center, Salaya, Nakorn Pathom. The animals were placed in cages (28x18x19 cm) and maintained within control temperature at 24±1 ℃ with 12 hours light and dark cycle. Before treatment, the animals were allowed to acclimatize for 5 days. The protocols of this study were approved by the Animals Research Ethics Committee of Naresuan University, Thailand.

2.2 Methamphetamine administration

D-methamphetamine hydrochloride (Lipome AG, Arlesheim, Switzerland) was used in this study. The animals were divided into four groups, comprising control group (n=6), acute binge group (n=6), escalating dose group (n=4) and escalating binge dose group (n=6). The animals in the control group received 0.9 % normal saline through intraperitoneal (i.p.) injection for 15 days. For the acute binge group, animals were injected (i.p.) with 0.9 % normal saline for 14 days and on day 15 animals were injected with 6 mg/kg METH four times (every 2 hours). In the escalating dose group, animals received METH 0.1-4 mg/kg for 14 days (three times a day at 3 hour intervals) and on day 15, animals were injected with 0.9 % saline. Animals in the escalating binge dose group were injected with METH 0.1-4 mg/kg for 14 days (three times a day at 3 hour intervals) and on day 15 animals were treated with 6 mg/kg METH four times (every 2 hours).

2.3 Hematoxylin and eosin staining for stage of seminiferous tubule analysis

The stage of seminiferous tubules was investigated by haematoxylin and eosin (H&E) staining technique. The slide was captured by image capture system joining with computer. Developmental stages of seminiferous epithelium in cross section of testis were identified according to the criteria previously described (Leblond & Clermont, 1952; Nudmamud-Thanoi, Tangsrisakda, & Thanoi, 2016).

2.4 Immunohistochemistry analysis

The expressions of dopamine D2 receptor (D2R) and alpha1 adrenergic receptor (α1AR) in testis were investigated using immunohistochemistry technique. The sectioned testis was deparafinized in xylene and rehydrated by using serial alcohol and distilled water. After that, the tissue sections were permeated by employing citrate buffer (pH 6.0) and using high temperature in a microwave for antigen retrieval. Next, the testicular tissues were doused with endogenous peroxidase blocking solution, consisting of 10% methanol, 0.3% H_2O_2 and 0.1% triton X and incubated with 5% bovine serum albumin (BSA) for blocking non- specific proteins. Testis sections were then incubated in specific primary antibodies, namely are anti- D2R (Merck Millipore, California, U.S.A) and anti α1AR (Abcam, UK), for 1 hour at room temperature and then put in a refrigerator for 12 hours. After that, sections were incubated with biotinylated secondary antibody (Vector Laboratories, Burlingame, California) for 2 hours and avidinbiotinylated horseradish peroxidase complexes (ABC kit) (Vector Laboratories, Burlingame, California, U.S.A) for an hour to enhance the signal. Finally, the specific proteins were visualized by chomogen 3, 3–diaminobenzidine (DAB) substrate (Vector Laboratories, Burlingame, California). In addition, rat brain sections were processed to investigate the expression of D2R and α1AR used used as positive controls.

The immunoreactive detected slides were captured by image capture system joining with computer. Ten seminiferous tubules per section were randomly selected under light microscope. The quantity of specific protein expressions in testes were counted.

2.5 Data analysis

The data were analyzed by one-way ANOVA and followed by LSD post-hoc test. The statistical significances were determined as $p < 0.05$. The results were expressed as mean ± SEM.

3. Results

3.1 Changes of developmental stages of seminiferous tubule

The developmental stages of seminiferous tubule in rat can be classified into fourteen stages as shown in Figure 1. After METH exposure, the developmental stages of seminiferous tubule were changed (Figure 2). The percentages of stage II were decreased in ED and ED-binge groups (p<0.05) when compared with the control. Significant decreases of stage V and stage XII were observed in the ED group and the ED-binge group, respectively (p<0.05). On the other hand, significant increases (p<0.05) of stage XI and stage XIII were found in the ED-binge group and the ED group, respectively.

When the developmental stages of seminiferous epithelium in rat were classified into three categories including early stage (I-V), middle stage (VI-VIII) and late stage (IX-XIV), the results showed that the early stage (I-V) was reduced in all METH-treated groups and reached significance in the ED group when compared with the control (P<0.05) (Figure 3).
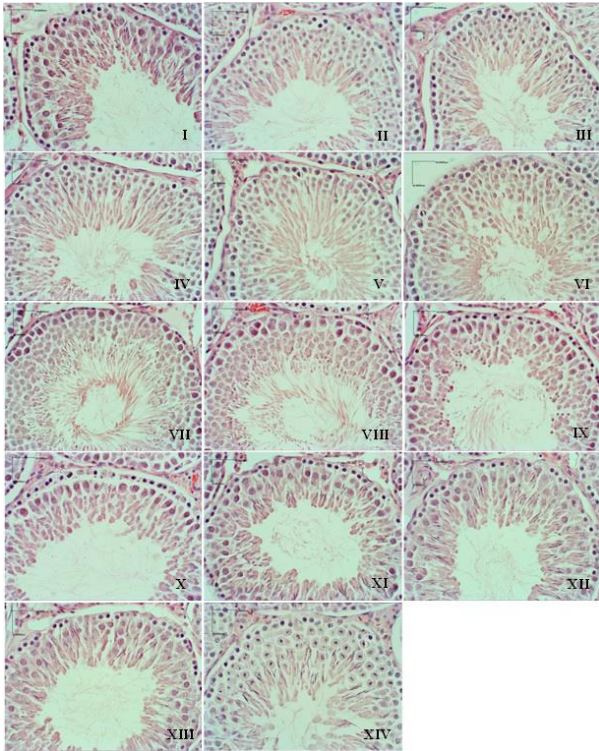
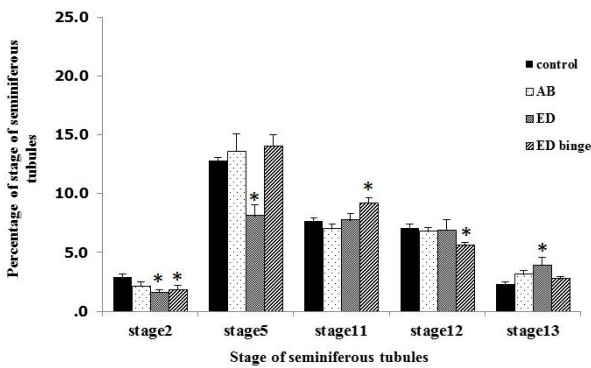Figure 1. The developmental stages of seminiferous tubule in rat.

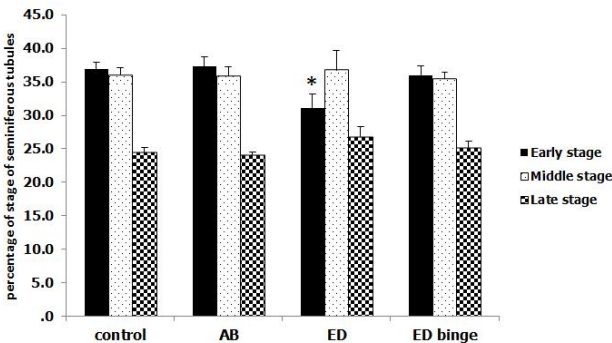Figure 2. The developmental stages of seminiferous tubule after METH exposure.

Figure 3. The developmental stages of seminiferous epithelium in rat were classified into three categories including early stage (I-V), middle stage (VI-VIII) and late stage (IX-XIV).

3.2 The expression of D2R immunoreactivity

The immunohistochemical staining demonstrated D2R localization in all spermatogenic cells, as well as in Sertoli cells (Figure 4). The immunoreactivity of D2R in each group is shown in Figures 5C-F, while the expression of D2R in rat brain was shown in Figure 5B as a positive control.

The quantitative data of D2R expression (Figure 6) demonstrated that the percentages of D2R expression in spermatocyte were significantly decreased in all METH treated groups (P<0.05) when compared with the control. D2R expression in round spermatids was significantly decreased in ED and ED-binge groups while that in Sertoli cells was significant decreased only in the ED-binge group. Conversely, the expression of D2R in elongated spermatids was significantly increased in both ED and ED-binge groups (P<0.05). However, there were no significance differences in percentages of D2R expression in spermatogonia.
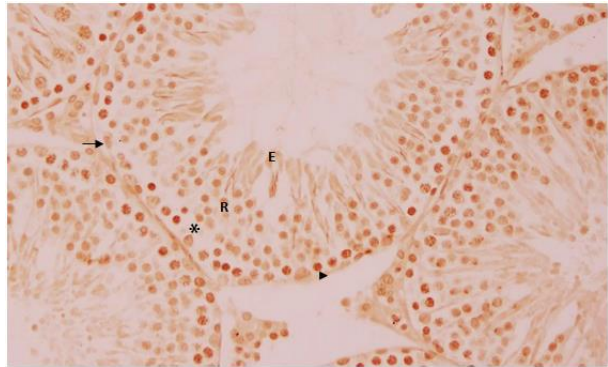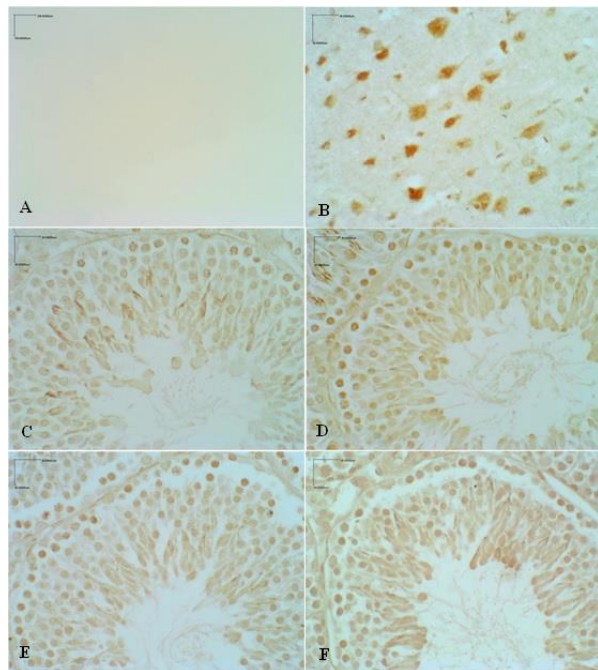

Figure 4. The immunohistochemical staining.

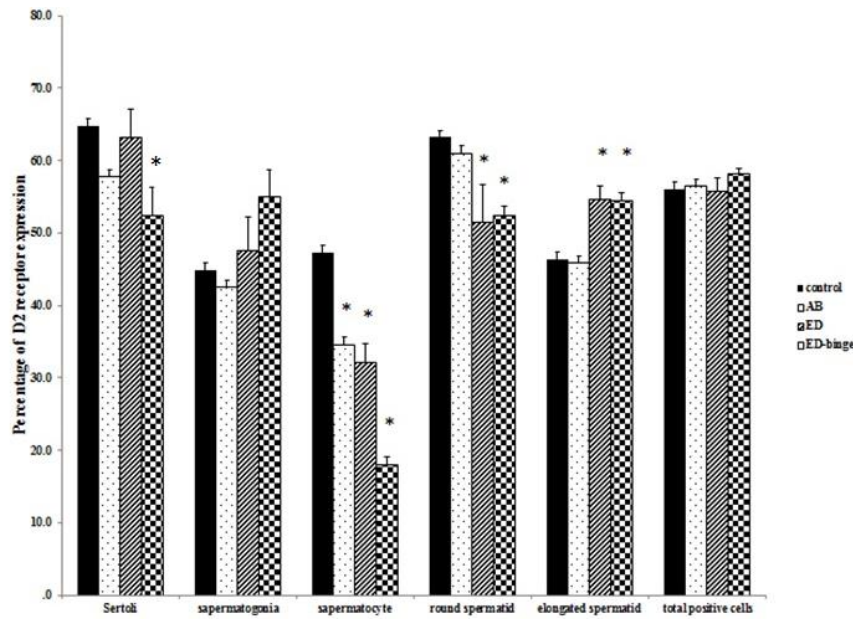Figure 5. The immunoreactivity of D2R in each group.

Figure 6.   The quantitative data of D2R expression.

3.3 The expression of α1AR immunoreactivity

        The qualitative result of α1AR immunoreactivity is shown in Figure 7. The immunoreactivity of α1AR in each group was shown in Figure 8C-F, while the immunoreactivity of α1AR in rat brain section is shown in Figure 8B as a positive control.

        The quantitative data of α1AR immunoreactivity are illustrated as percentage of positive cells in Figure 9. The percentages of α1AR immunoreactivity in spermatocytes were significantly increased in all METH-treated groups ($P<0.05$) when compared with the control. Moreover, the α1AR immunoreactivity in round and elongated spermatids were significantly increased in both ED and ED-binge groups. The percentage of α1AR immunoreactivity in Sertoli cells was increased in all METH-treated groups but just reached significance only in the ED-binge group ($P<0.05$). In contrast, a significant decrease of α1AR immunoreactivity in spermatogonia was found only in the ED-binge group when compared with the control.

4. Discussion

4.1 Effects of METH on changes of seminiferous tubules stages

        Changes of developmental stages of seminiferous epithelium in rats addicted with METH showed a decrease in the early stage of development (stage I-V) in the present study. These changes could reflect the effect of METH on spermatogenesis arrest as it has been reported that in the early stage of seminiferous epithelium development (stage I-V) consisting with the proliferation of spermatogonia by mitotic division and spermatogonia differentiation from type A to type B (Leblond & Clermont, 1952). Moreover, METH can cause fluctuate change in the development of seminiferous
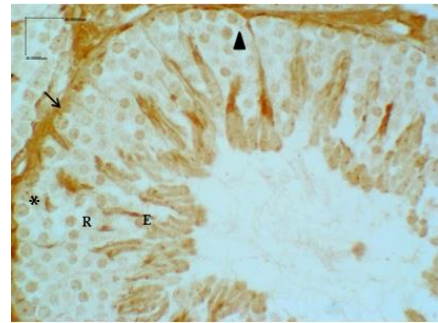

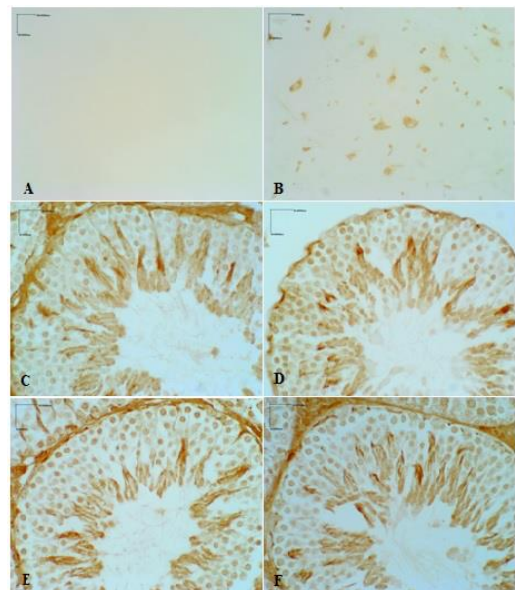Figure 7.   The qualitative result of α1AR immunoreactivity.
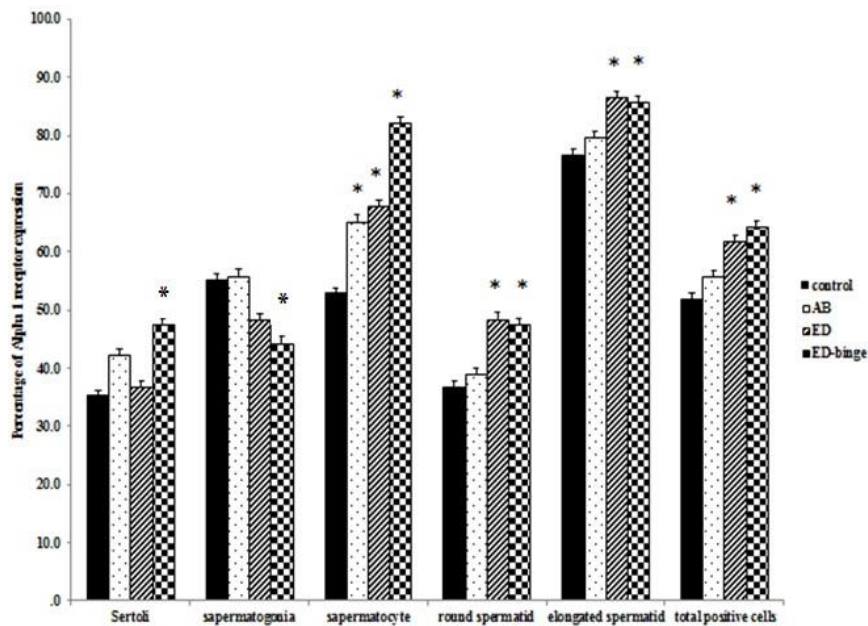

Figure 8.   The immunoreactivity of α1AR in each group.

Figure 9.   The quantitative data of α1AR immunoreactivity.

epithelium in the late stage (stage XI-XII), which may induce abnormal sperm quality such as sperm motility, sperm morphology and sperm concentration, similar to the report of Nudmamud-Thanoi *et al.* (2016). In addition, the results from our previous study suggest the effects of METH on catecholamine (NE and DA) concentrations in testis (Janphet *et al.*, 2016) which may affect the level of testosterone release (Mayerhofer, Steger, Gow, & Bartke, 1992; Stojkov-Mimic *et al.*, 2015) leading to abnormal sperm production. In addition, the spermatogenesis is regulated by endocrine and testicular autocrine/paracrine factors through Leydig cells and Sertoli cells (Huleihel & Lunenfeld, 2004). Besides, Leydig cells can modulate the progression of spermatogenesis reflected in the stages of seminiferous tubule development (Paniagua *et al.*, 1988). Moreover, there is a report on the effect of METH causing alterations of progesterone and estrogen receptor expressions on Sertoli and spermatogenic cells (Nudmamud-Thanoi *et al.*, 2016). As mentioned above, these toxic effects of METH on testis should contribute to abnormal spermatogenesis, which can be reflected in the changes of stages of seminiferous epithelium development. In this study, the immunoreactivities of D2R were investigated and the results showed the expressions of D2R in rat seminiferous tubules were observed in all spermatogenic cells and Sertoi cells. It is in agreement with prior study which found the expressions of D2R in rat testis and rat spermatogenic cells (Otth *et al.*, 2007). The expression of D2R outside the CNS could illuminate the interaction between nervous system and reproductive system. It may play an important role in proliferation and/or differentiation of the male germ cells as the present study showed the expressions of D2R in all germ cells in pre-meiotic phase and post-meiotic phase of the seminiferous tubule. After METH exposure, the expressions of D2R in rat seminiferous tubules were changed. The reductions of D2R expression were found in Sertoli cells that could lead to abnormality of sperm development (Griswold, 1998). A decrease of D2R expression in spermatocyte and

round spermatid in chronic METH-treated groups may indicate that D2R could play an important role in METH uptake into the cell as a decreased dopamine activity in methamphetamine abusers has been reported (Wang *et al.*, 2012) with a consequent reduction in the neuroprotective impact of D2R (Ares-Santos, Granado, & Moratalla, 2013), which could allow METH uptake into the cell. Additionally, since METH has the effect on DA concentration in testis this may be derived from sympathetic innervation (Gnessi, Fabbri, & Spera, 1997) leading to the changes of its receptor expression. A decrease of D2R in METH abusers, as revealed by receptor down-regulation due to pharmacological effects of METH, may induce an increase of extracellular DA concentration (Wilson & Kish, 1996). Since spermatogenesis is a complex process and is not well understood, it may need different levels of dopamine in different stages of development of germ cells. Therefore, up and down regulations may occur to retain homeostasis. Accordingly, DA and D2R may play an important role in spermatogenesis; METH-induced changes of D2R in spermatogenic cells and Sertoli cells may consequently lead to abnormal spermatogenesis.

In the present study, the expression of α1AR in Sertoli cells was increased in the ED-binge group. An increase of α1AR in Sertoli cells after METH exposure may have an effect on the function of Sertoli cells in spermatogenesis since this process is regulated by testosterone and FSH levels, which modulated by Sertoli cells (Griswold, 1998). A decrease of α1AR expression in spermatogonia with an increased expression of α1AR in spermatocyte, round and elongated spermatids in chronic METH-exposed animals may reflect abnormal regulation in the function of catecholamine in sperm development since it has been reported that NE and adrenergic receptors have been involved in sperm capacitation and acrosome reaction (Cornett & Meizel, 1978; Way & Killian, 2002, 2006) and sperm motility (Sliwa, 1994). Therefore, alterations of α1AR expressions after METH administration in spermatogenic cells and Sertoli cells may result in abnormal

spermatogenesis and low sperm quality. Taken together, these results demonstrate that METH can affect the male reproductive system, especially sperm development via the activation of neurotransmitter changes including DA and NE. It may affect either directly on spermatogenic cells during spermatogenesis or indirectly on Sertoli and Leydig cells communication.

5. Conclusions

In summary, the present study revealed that METH can cause alterations of D2R and α1AR expressions in Sertoli and spermatogenic cells, as well as changes in the frequency of developmental stages of seminiferous tubules. These results suggest that METH can disturb the actions of catecholamine in testis by changing secretion levels of neurotransmitters and their receptor expressions. These changes may lead to spermatogenesis impairment and sperm dysfunction. Specific investigation should be carried out in the near future.

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