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# Targeting Heme Oxygenase-1 in Hypothyroidism Induced Reproductive Dysfunction in Adult Male Rats

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## Abstract

*Background:* Hypothyroidism can impair reproductive function leading to infertility.

*Aim of the Study:* The present study was designed to investigate the possible modulating effect of Heme Oxygenase-1 (HO-1) induction on hypothyroidism-induced reproductive dysfunction in rats.

*Material and Methods:* Rats were categorized into four groups, 10 rats each. Group I was control, group II (PTU): Received oral 0.05% propyl-thiouracil (PTU) for 8 weeks, group III (PTU + hemin ) received co treatment of oral PTU and hemin (30μmol/kg) by i.p. injections three times per week for 8 weeks and group IV, co administered with oral PTU and zinc protoporphyrin IX (ZnPP) (20 μmol/kg) by i.p. injections three times per week for 8 weeks and served as (PTU + Znpp) group. Serum samples were taken to asses thyroid function and serum sex hormones level. Semen samples were taken to detect sperm count and quality, while testicular tissue homogenates were used to evaluate oxidative stress markers, steroidogenic enzymes and c-Jun NH2-terminal kinases (JNK) activation level together with DNA fragmentation in testicular tissue.

*Results:* Compared to PTU group, hemin-induced HO-1 up regulation improved the oxidative stress state with enhancement of reproductive hormone secretion, which was parallel to abrogation of semen analysis parameters. Further hemin blocked the PTU induced activation of JNK and DNA fragmentation. On the contrary, inhibition of HO-1 by ZnPP further aggrevated all of the PTU induced changes.

*Conclusion:* These findings suggested the potential strategy of targeting of HO-1 in improving the hypothyroidism induced reproductive dysfunction.

***Key Words:*** *Hypothyroidism – Reproductive dysfunction – Heme oxygenase-1 – c-Jun NH2-terminal kinases.*

## Introduction

**THYROID** Hormones (THs) have vital role in regulation of lipid and carbohydrate metabolism, oxygen consumption, normal growth and development [1]. Besides, THs can regulate the testicular growth and maturation and control the proliferation and differentiation of sertoli and leydig cells during development of testis [2]. Hypothyroidism had been shown to be associated with impairment of sex hormones levels and testicular function and even may lead to infertility [3]. The mechanisms underlying hypothyroidism induced testicular dysfunction is still unclear. However, previous studies suggest oxidative stress, [4] and apoptosis [5] as major culprits.

The JNK signaling pathway, one of the three representative mitogen-activated protein kinase signaling pathways, plays an important role in apoptosis [6]. There are three isoforms of JNK (JNK1, JNK2 and JNK3), JNK1 and JNK2 are expressed in most tissues, while JNK3 is selectively expressed in the heart and nervous system tissues [7]. Recent study assumed that JNK activation may have a role in reproductive dysfunction in hypothyroidism [8] which needs to be further elucidated.

Heme oxygenase-1 is a microsomal initial rate-limiting enzyme which catalyzes the oxidative degradation of heme into biliverdin, bilirubin, and carbon monoxide [9]. These heme degradation by-products were suggested to be the key mediators through which HO-1 can exert its anti-oxidant, anti-inflammatory, and anti-apoptotic effects [10]. Hemin, a potent HO-1 inducer, have shown promising prospective in protecting against oxidative and inflammatory tissue injuries including the testicular tissue whereas, its inhibition by specific HO inhibitor such as ZnPP had been involved in worsening of testicular function in various experimental models [11-14]. Moreover, it had been shown that HO-1 expression can protect against cell death through the JNK signaling pathway in various pathological conditions [15-17]. Thus we considered whether HO-1 induction might have a role in reg-

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ulating the pro apoptotic JNK in hypothyroidism model which may represent a potential mechanism for attenuating the reproductive dysfunction in this model.

Therefore, the present study was conducted to evaluate the possible modulating effect of HO-1 induction on reproductive dysfunction in PTU induced hypothyroidism in rats highlighting its possible involved mechanisms.

## Material and Methods

*Chemicals:*

Hemin and ZnPP were purchased from Sigma-Aldrich (Sigma, UT, USA); 6-n-propyl-2-thiouracil (PTU) was obtained from (Amoun Pharmaceutical Chemicals Co., Egypt). All other chemicals were obtained from Sigma (St. Louis, MO, USA). All other chemicals were of high analytical grade and commercially available.

*Study design:*

This study was conducted in accordance with the guidelines for the animal experimental protocols of Tanta Faculty of Medicine from May 2018 to September 2018. It was carried out on forty male albino rats (weight: 200-250g). The rats were housed in clean cages, five rats per each cage, at 23±2ºC with 12h light/dark cycle and had free access to food and water.

The rats were randomly divided into four groups (10 rats each).

- *Group I (control group):* In which animals received distilled water orally without any treatment.
- *Group II (PTU):* In which rats received PTU in drinking water to reach a final concentration of 0·05% [18] and treatment continued for 8 weeks to cover a complete spermatogenic cycle in rats [1,19].
- *Group III (PTU + hemin):* Beside oral PTU ,this group received hemin at a dose of (30 μmol/kg) [20] by i.p. injections three times per week for 8 weeks.
- *Group IV (Znpp treated hypothyroid group):* This group was co treated with oral PTU and ZnPP-IX (20 μmol/kg) [20] by i.p. injections three times per week for 8 weeks.

*Blood collection and tissue sampling:*

After completing the treatment protocol, rats were sacrificed by cervical decapitation after deep anesthesia with sodium pentobarbital. Blood samples were collected and serum was separated for determination of Tri-iodothyronine (T3), Thyroid stimulating hormone (TSH), Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Testosterone (T). Laparotomy was done, then testes and epididymis were removed and cleared from adherent tissues, washed in ice-cold 1.15% KCL and then dried and weighed. The epididymis was used for assay of semen analysis, while testis from each rat was separated and one piece was homogenized in ice-cold 1.15% KCL, the resulting homogenate was centrifuged at 3000r.p.m for 10min at 4ºC to be used in assessment of testicular oxidative stress markers, steroidogenic enzymes and apoptotic markers, while the other piece was used for microsomal separation to estimate HO-1 activity. Total protein content in testicular homogenates and microsomes were estimated according to Lowry et al., [21].

*Serum T3 and TSH assay:*

Estimation of serum T3 and TSH levels were assayed using kits purchased from Calbiotech INC (CBI), USA, following the guidelines of the manufacture.

*Measurement of oxidative stress markers:*

4-Hydroxynonenal (4-HNE) is a highly reactive diffusible end product of lipid peroxidation, and a key mediator of oxidant-induced cell signaling and apoptosis [22]. The level of 4-HNE in testicular homogenate, as a marker for oxidative stress, was assessed using ELISA kits supplied by MyBioSource (San Diego, CA, USA). Meanwhile testicular Total Antioxidant Capacity (TAC) was determined using Ferric Reducing Antioxidant Power (FRAP ) assay based on method of Benzi and Strain [23].

*Estimation of microsomal HO-1 activity in testicular tissue:*

Isolation of renal microsomes was conducted based on the method of Schenkman and Cinti [24], then measurement of microsomal HO-1 activity was carried out by modification of Tenhunen et al., [25] method.

*Measurement of serum sex hormones:*

Determination of serum level of FSH, LH and T were carried out using their respective ELISA kits supplied by (EIAab Co. Wuhan, China) for LH and FSH and (Cusabio, Wuhan, China) for T hormones.

*Detection of testicular steroidogenic enzymes activity level:*

Testicular 3β-Hydroxysteriod Dehydrogenase (3β-HSD) and 17β-hydroxysteriod dehydrogenase (17β-HSD) activities were assayed based on the methods of Talalay **[26]** and Jarabak et al., **[27]** respectively.

*Semen samples and sperm function analysis:*

Semen samples were collected from cauda epididymis and used for estimation of sperm cell count, motility, viability and abnormality. The cauda epididymis was dissected out, weighed, immediately minced in 2mL of physiological saline, and then incubated at room temperature to allow migration of sperm from epididymis to the fluid **[28]**.

Sperm motility was assayed by putting a drop of the suspension on pre-warmed (37ºC) microscopic slide. The percentage of motile spermatozoa was evaluated in 10 random fields (X400 magnification) and the mean number of motile sperm was calculated as previously described **[29]**.

Seminal smears stained with eosin nigrosin stain were examined microscopically to determine the percentages of sperm viability (ratio of alive/ dead) and sperm abnormality based on the method of Filler **[30]**.

Sperm cell count per ml of semen was performed according to the method of Robb et al., (1978). Semen was diluted 5 times (v/v) with normal saline containing few drops of formalin (40%) to kill the spermatozoa, and counting of spermatozoa was carried out using a Neubauer haemocytometer.

*DNA fragmentation assay:*

It was analyzed based on the method described by Gercel-Taylor **[31]**.

Testicular tissue homogenates were centrifuged at 13,000 X g at 4ºC for 15min which separate the intact chromatin (pellet, B) from the fragmented DNA (supernatant, T). Pellet and supernatant fractions were assayed for DNA content using a freshly prepared diphenylamine solution and the optical density was read at 600nm. The percentage of fragmented DNA was calculated by the following formula: [Percentage of fragmented DNA = T X 100/(T + B)] .

*Assay of activation of pro-apoptotic JNK in testicular tissue:*

Testicular tissue activation of JNK was measured as the ratio of (pJNK/JNK) **[32]**, using a commercially available ELISA kit from RayBio. Company following the manufacturer's instructions.

*Statistical analysis:*

The data were expressed as the mean and Standard Deviation (SD). Statistical comparison between different groups was carried out using one way (ANOVA) followed by Tukey post hoc test using Graph Pad Instat, 32 bit for win 95/NT (Version 3.05). $p$-values <0.05 were considered statistically significant.

## Results

*Effect of HO-1 targeting on serum T3 and TSH levels of PTU induced hypothyroid rats (Table 1):*

Induction of PTU-hypothyroidism was confirmed by significant reduction in serum T3 (48.3%) and significant elevation of serum TSH levels (4.16 fold) in PTU group in comparison with the control group. Neither, induction of HO-1 by hemin or its suppression by Znpp showed any significant changes in T3 or TSH levels as shown in results of (Table 1).

*Effect of HO-1 targeting on testicular oxidative stress markers of PTU induced hypothyroid rats (Table 2):*

Results in table 2 pointed out that PTU administration resulted in provocation of oxidative stress response as indicated by augmentation of testicular 4-HNE level in PTU group with respect to the control one. To alleviate cumulative burden of oxidative stress, cells generally utilize anti-oxidant defense systems to scavenge ROS which appears to be exhausted as indicated from the significant reduction in TAC level in testicular tissue of PTU group when compared to the control one. Interestingly, co-treatment with PTU and hemin significantly abated oxidative stress, as evidenced by significant reduction in serum 4-HN and significant elevation of TAC in co treated group compared to PTU group, whereas co administration of ZnPP and PTU further aggravated these oxidative stress markers.

Additionaly, results of (Table 2) showed that, micosomal HO-1 activity recorded a significant decrease in PTU group (32.2%) compared to control one, whereas HO-1 activity was significantly increased (1.66 fold) by hemin co administration and inhibited by Znpp co treatment (24%) when compared with the PTU treated group.

*Effect of HO-1 targeting on serum sex hormones and steroidogenic enzymes activity levels of PTU induced hypothyroid rats (Table 3), Fig. (1):*

As shown in (Table 3), administration of PTU was associated with considerable reduction in serum levels of FSH, LH, and T in PTU group (52.4%, 34.5% and 35.1%, respectively) as compared to their respectives in the control group. Co administration of hemin in PTU rats resulted in significant elevation in the level of these hormones, while ZnPP therapy significantly exacerbated the diminution of their levels as compared to PTU treated rats (Table 1).

Further, the activity level of testicular pivotal androgenic enzymes (3β-HSD and 17β-HSD) were significantly decreased following induction of PTU-hypothyroidism by about (37.5% and 41.7%, for 3β-HSD and 17β-HSD respectively) when compared with the control group. On the other hand co-administration of hemin counteracted the inhibitory effect of PTU on these testicular steroidogenic enzymes, an effect which was reversed by Znpp co treatment Fig. (1).

*Effect of HO-1 targeting on relative testicular and epididyml weights (RTW, REW) and semen analysis parameters of PTU induced hypothyroid rats (Tables 4,5):*

Parallel to the observed decline in sex hormones and decreased activity of steroidogenic enzymes in PTU group, our results revealed that both RTW and REW were decreased in PTU group comparable to control one, which was associated with impairment of spermatogenesis as indicated by a significant decrease in sperm count, motility and viability (44.8%, 46.5% and 39.5%, respectively, whereas sperm abnormality was significantly increased (2.15 fold) in PTU group comparable with the control one. Interestingly, all these parameters were normalized upon hemin co treatment, an effect which was reversed by ZnPP treatment.

*Effect of HO-1 targeting on testicular DNA fragmentation and JNK activation levels of PTU induced hypothyroid rats Figs. (2,3):*

Oxidative stress has been suggested to play a role as a common mediator of apoptosis and testicular damage in hypothyroidism [1]. Therefore, we investigated the effect of PTU administration on the level of DNA fragmentation as a marker for apoptosis together with the level of activation of the pro apoptotic JNK with the possible modulating effect of HO-1 induction and suppression on their levels.

As noticed from Figs. (2,3), induction of PTU induced hypothyroidism augmented the levels of DNA fragmentation and JNK activation in PTU treated rats (2.8 and 1.6 fold, respectively) compared with the control ones. On the other hand, co administration of hemin with PTU counteracted the observed DNA damage and blocked the PTU induced activation of JNK, while these over mentioned parameters were further aggrevated by Znpp co treatment.

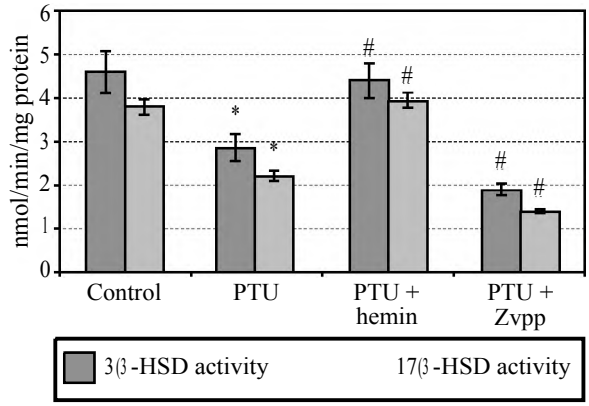


Fig. (1): Effect of HO-1 targeting on steroidogenic activity levels of PTU induced hypothyroid rats.

Data are given as mean ± SD.
*: <0.05 vs. control group. #: $p$ <0.05 vs. PTU group.

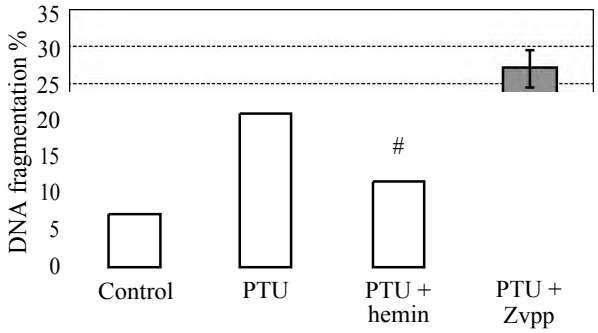


Fig. (2): Effect of HO-1 targeting on testicular of DNA fragmentation of PTU induced hypothyroid rats.

Data are given as mean ± SD.
*: <0.05 vs. control group. #: $p$ <0.05 vs. PTU group.

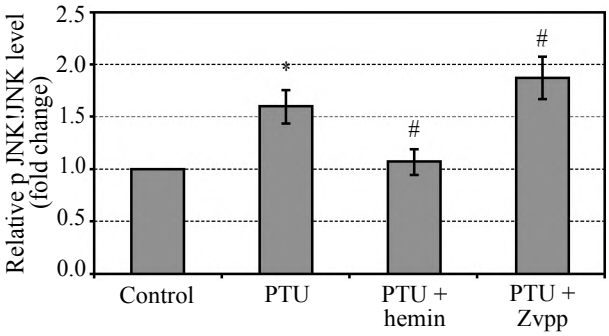


Fig. (3): Effect of HO-1 targeting on testicular JNK activation level of PTU induced hypothyroid rats.

Data are given as mean ± SD.
*: <0.05 vs. control group. #: $p$ <0.05 vs. PTU group.

Table (1): Effect of HO-1 targeting on serum T3 and TSH levels of PTU induced hypothyroid rats.

| Parameter/group | Group I (Control) | Group II (PTU) | Group III (PTU + Hemin) | Group IV (PTU + Zvpp) |
|---|---|---|---|---|
| Serum T3 (ng/dl) | 53.8±4.13 | 27.8±3.68* | 30.7±2.541 | 24.8±2.30 |
| Serum TSH ( IU/ml) | 0.67±0.05 | 2.79±0.12* | 2.69±0.08 | 2.83±0.07 |

Data are given as mean ± SD.
*: <0.05 vs. control group.
#: $p$<0.05 vs. PTU group.

Table (2): Effect of HO-1 targeting on testicular oxidative stress markers and microsomal HO-1 activity level of PTU induced hypothyroid rats.

| Parameter/group | Group I (Control) | Group II (PTU) | Group III (PTU + Hemin) | Group IV (PTU + Zvpp) |
|---|---|---|---|---|
| • Testicular 4-HN | 6.69± 1.09 | 19.49±2.02* | 5.82±0.84# | 28.±2.91# |
| • Testicular TAC mmol/mg protein | 0.17±0.02 | 0.09±0.006* | 0.21 ±0.04# | 0.06±0.004# |
| • Microsomal HO-1 activity (nmol/min/mg protein | 6.64±0.60 | 4.50±0.46* | 7.49±0.83# | 3.42±0.42# |

Data are given as mean ± SD.
*: $p$<0.05 vs. control group.
#: $p$<0.05 vs. PTU group.

Table (3): Effect of HO-1 targeting on serum sex hormones level of PTU induced hypothyroid rats.

| Parameter/group | Group I (Control) | Group II (PTU) | Group III (PTU + Hemin) | Group IV (PTU + Zvpp) |
|---|---|---|---|---|
| FSH (mIU/ml) | 1.66±0.08 | 0.79±0.05 * | 1.46±0.08# | 0.69±0.05# |
| LH (mIU/ml) | 0.84±0.05 | 0.55±0.04* | 0.76±0.07# | 0.46±0.04# |
| T (ng/ml) | 3.70±0.07 | 2.40±0.04* | 3.60±0.10# | 1.91 ±0.05# |

Data are given as mean ± SD.
*: $p$<0.05 vs. control group.
#: $p$<0.05 vs. PTU group.

Table (4): Effect of HO-1 targeting on body weight, relative testicular and epididyml weights (RTW, REW) of PTU induced hypothyroid rats.

| Parameter/group | Group I (Control) | Group II (PTU) | Group III (PTU + Hemin) | Group IV (PTU + Zvpp) |
|---|---|---|---|---|
| Body weight/g | 289.4±8.656 | 225.7±7.258 * | 230.2±6.697 | 224.8±4.849 |
| RTW (g/100gm.bw) | 1.60±0.06 | 1.37±0.04* | 1.73±0.10# | 1.24±0.03# |
| REW (g/100gmbw) | 0.407±0.04 | 0.33±0.03 * | 0.46±0.06# | 0.25±0.03# |

Data are given as mean ± SD.
*: $p$<0.05 vs. control group.
#: $p$<0.05 vs. PTU group.

Table (5): Effect of HO-1 targeting on semen analysis parameters of PTU induced hypothyroid rats.

| Parameter/group | Group I (Control) | Group II (PTU) | Group III (PTU + Hemin) | Group IV (PTU + Zvpp) |
|---|---|---|---|---|
| Sperm count ($10^6$/ml) | 78.1 ±5.3 | 43.1 ±2.9* | 82.2±4.5# | 34.6±2.9# |
| Sperm motility % | 87.1 ±4.56 | 46.6±3.95* | 89.8±4.47# | 35.2±2.70# |
| Sperm abnormality % | 7.80± 1.23 | 16.78± 1.81* | 6.69±0.68# | 19.35± 1.60# |
| Sperm viability % | 87.9±4.41 | 53.2±4.10* | 89.8±4.96# | 46.7±4.03# |

Data are given as mean ± SD.
*: <0.05 vs. control group.
#: $p$<0.05 vs. PTU group.

## Discussion

Many studies have underscored the protective effects of the HO system on testicular tissue [13,14, 33]. However, up to now and according to our knowledge, this is the first study to validate the effect of targeting of HO-1 in hypothyroidism in rats with its possible impact on reproductive dysfunction in this model.

In our study, a rat model of hypothyroidism was established by administration of PTU as indicated by a significant decrease in T3 and a significant increase in TSH levels as seen in other studies [34]. The anti thyroid effect of PTU is mediated by inhibition of thyroid hormone synthesis and blockage of the transformation of T4 to T3, thus decreasing serum level of T3 [35].

Concerning the oxidative stress in hypothyroidism, data are still conflicting ;despite decrement in ROS production was expected because of hypometabolic state in hypothyroidism [36,37], studies recorded no significant changes in lipid peroxidation [38] or even increased oxidative stress response [39].

The results obtained herein showed that PTU induced hypothyroidism triggered inhibition of antioxidation that favored lipid peroxidation ,as evidenced by increased level of 4-HNE with decreased TAC level in PTU group compared to control one as supported previously [34]. This redox imbalance may be explained by inhibition of anti oxidation by iodine deficiency in hypothyroidism [40,41], excess TSH which produces directly oxidative stress response [42] and the hypothyroidism-induced dysfunction of the mitochondrial respiratory chain that results in accelerated ROS production [43].

Intriguingly, co administration of hemin with PTU improved redox status referring to its antioxidant capacity, which agree previous findings [13, 14,44].

Upregulation of HO-1 following hemin administration provides antioxidant effects through different mechanisms including; upregulation of ferritin [45], production of a lipophilic antioxidant, biliverdin [46], induction of nuclear factor erythroid 2-related factor 2 (Nrf2) by CO which activate and regulate genes of many antioxidant enzymes [47,48].

Parallel to increased oxidative stress response in hypothyroidism, a significant decrease in the level of testicular microsomal HO-1 activity in PTU group was noticed compared to control one, whereas its level was significantly increased and decreased by hemin and Znpp treatment respectively.

In accordance, Ali et al., [36] reported a decreased activity of HO-1 in thyroidectomized model with presence of a positive significant correlation between T3 and HO-1, further, HO-1 upregulation was induced by thyroid hormone treatment [49], this documented the impact and possible regulating role of thyroid hormones on HO-1, with their ability to act in a synergistic manner to augment the response of HO-1 to chemical inducer of this enzyme [36]. From the overmentioned data, we may hypothesized that decreased HO-1 activity with its antioxidant capacity may have a role in PTU induced reproductive dysfunction as presented later, with the possible attenuating effect of HO-1 induction in this respect.

Noteworthy, our data reported a significant reduction in the serum levels of FSH, LH and T in PTU group compared to control one, which came in line with previous studies [1,35]. In contrast some studies found no change in the level of these hormones in hypothyroidism [50,51]. These controversy may be due to different treatment duration and method of induction of hypothyroidism state in experimental animals.

The observed reduction of these sex hormones recorded herein may be due to impairment of hypothalamic-pitutary gonadal axis in hypothyroidism as recorded previously. This impairment may be attributed to inhibition of hepatic sex hormone-binding globulin production by PTU with subsequent elevation of free T that inhibits the release of gonadotropin releasing hormone secretion by negative feedback mechanism [52], also the decreased pituitary LH responsiveness to LHRH due to hypothyroidism may have a role [53]. In addition, oxidative stress found herein can directly inhibit T secretion from leyding cells [54] which may be mediated by various mechanisms including; inhibition of the activities of several enzymes of the T biosynthetic pathway as recorded herein by decreased steroidogenic enzymes which was supported previously [1,55], together with reduction in the mitochondrial membrane potential required for T synthesis [56] and inhibition of the expression and activation of the steroidogenesis acute regulatory protein [57], all of which collectively decreased T production.

Notably, the level of these sex hormones was improved upon hemin co treatment which was supported previously [44]. This may be explained

by the fact that HO enzyme exists in the rat anterior pituitary gland, and a substrate and an inhibitor of this enzyme can alter the secretion of gonadotropins [58]. Another propable mechanism is the antioxidant capacity of HO-1 recorded herein which may reverse the oxidative stress induced modifications in leyding cells improving T secretion and subsequently the reproductive function in PTU treated rats [59] this came in line with our findings as hemin co treatment stimulated the activity of testicular steroidogenic enzymes which are considered the machinery for T secretion by the leyding cells [1]. Thus, the positive influence of hemin on serum level of T may be due to direct stimulatory effect of hemin on pituitary gland increasing LH release and on leydig cell enhancing steroidogenic enzymes activity.

In accordance, the results of the present study also declared a decreased RTW and REW with concomitant impairment in spermatogenesis as represented by a significant decrease in sperm count, motility and viability while a significant increase in sperm abnormality in PTU group compared to control one was noticed, this finding is compatible with previous studies [34,60].

These deleterious effects of PTU on spermatogenesis could be attributed to the decreased level of thyroid hormone which have a vital role in differentiation and maturation of germ cells [35,61], the decrement of sex hormones with their potential role in spermatogenesis [62,63]. Furthermore, oxidative stress was reported to alters the motility and the genetic integrity of sperm cells [64], besides apoptosis and DNA damage ,as presented later in this study, negatively impact the total sperm number and viability [65,66].

The observed recovery of semen analysis parameters to the control levels in hemin-treated hypothyroid rats could be attributed to the antioxidant effects of hemin, its augmentation to sex hormones together with its protection against apoptosis and DNA damage as proved by our study and supported previously [14]. In agreement of our results, Shiraishi and Naito [67] stated that increased expression of Leydig cell HO-1 preserved spermatogenesis, also HO-1 was found to be exclusively expressed in Sertoli cells that have a key role in normal spermatogenesis [68,69].

Additionally, the present work declared the augmentation of apoptosis in testicular tissue of PTU treated rats as indicated by increased level of DNA fragmention in PTU group compared to control one. This may be refered to hypothyroidism induced oxidative stress which triggered testicular DNA damage and apoptosis that negatively impact the reproductive function [1,5].

Furthermore, our results showed an increased activation of JNK in PTU treated rats, consistently, JNK activation was recorded in congenital hypothyroidism [70].

The activation of JNK may constitute another contributing factor for provocating apoptosis, as JNK had been shown to modulate the function of mitochondrial pro apoptotic and antiapoptotic proteins triggering the mitochondrial mediated apoptosis [71-73]. Additionally, Chang et al., [8] recommended the involvement of JNK signaling bathway in the pathology of reproductive dysfuction in hypothyroidism, which was explained by disruption of intracellular Ca+ homeostasis with increased intracellular Ca+ load with subsequent provocation of testicular oxidative stress response and mitochondrial dysfunction that negatively impacts the reproductive efficiency in hypothyroid rats.

Moreover, the elevated 4-HN levels recorded in this current study was reported to up-regulate JNKs and induce cleavage of caspases and apoptosis [74,75]. Thus, 4-HNE might play a crucial role in oxidative stress-mediated JNK signaling and apoptosis in this model.

On the contrary, the results obtained herein showed that HO-1 induction reduced activation of DNA fragmentation and JNK activation level in hemin treated rats compared to untreated hypothyroid ones.

These anti apoptotic effects of hemin could be attributed to its antioxidant properties as hemin treatment effectively prevented cell apoptosis caused by oxidative tissue injury in different experimental models [76,77]. Furthermore, its suppression to JNK mediated apoptosis may have a role; as many studies reported that upregulation of HO-1 was associated with reduction of the phosphorylation of the JNK and apoptosis [73,78-80]. These data collectively support the notion that the mitigating effect of hemin on hypothyroidism induced oxidative stress and JNK mediated apoptosis may constitute another possible mechanism by which HO-1 induction can improve the reproductive dysfunction in PTU induced-hypothyroidism model.

*Conclusion:*

The current study indicated that hypothyroidism adversely affected the reproductive function in rats

which was effectively prevented by hemin treatment. Such protective effect was most probably carried out by its antioxidant properities, its augmentation to sex hormone secretion, enhancement of steroidogenesis, improving spermatogenesis and its blockade to PTU-induced JNK activation and apoptosis. Consequently, our data support that HO-1 induction may present a potential therapeutic approach for hypothyroidism induced reproductive dysfunction that may open the way to translate such potential into a therapeutic reality.

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*Conflict of interest:*

We have no conflict of interest to declare.

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# إستهداف زيادة الهيم آوكسيجيناز ١ فى تحسين الخلل الإنجابى الناجم عن قصور الغدة الدرقية

الخلفية: قصور الغدة الدرقية يمكن آن يضعف وظيفة الإنجاب مما يؤدى إلى العقم.

الهدف من الدراسة: تم تصميم هذه الدراسة للتحقيق فى إمكانية تآثير الهيم آكسجيناز-١ على الخلل الإنجابى الناجم عن قصور الغدة الدرقية فى الجرذان.

المواد والطرق: تم تقسيم الجرذان إلى آربع مجموعات، كل منها ١٠ جرذان. المجموعة الآولى وتمثل المجموعة الضابطة، وآعطيت المجموعة الثانية بروبيل ثيوراسيل (٠.٠٠٥٪) عن طريق الفم لمدة ثمانى آسابيع، وآعطيت المجموعة الثالثة كلا من بروبيل ثيوراسيل والهيمين (٣٠ ميكرومول/كجم) عن طريق الحقن البريتونى ثلاث مرات فى الإسبوع لمدة ٨ آسابيع والمجموعة الرابعة إستقبلت كلا من بروبيل ثيوراسيل الفموى والبروتوبروفيرين (٢٠ ميكرو مول/كجم) عن طريق الحقن البريتونى ثلاث مرات فى الإسبوع لمدة ٨ آسابيع. وفى نهاية التجارب تم ذبح الفئران وتجميع عينات الدم لقياس وظائف الغدة الدرقية ومعدل الهرمونات الجنسية فى الدم كما تم آخذ عينات السائل المنوى للكشف عن عدد ونوعية الحيوانات المنوية، كما تم قياس مستوى الآكسدة ونشاط الإنزيمات الستيرويدية بالإضافة إلى معدل نشاط JNK ومستوى تجزئة الحمض النووى فى آنسجة الخصية.

النتائج: آظهرت النتائج آن زيادة الهيم آوكسيجيناز ١ بواسطة الهيمين آدى إلى إنخفاض ذى دلالة إحصائية فى مستوى التآكسد فى آنسجة الخصية مع تعزيز إفراز الهرمونات التناسلية، والذى كان موازيا لتحسن فى عدد ونوعية الحيوانات النووية بالإضافة إلى تقليل معدل نشاط ال JNK وتجزئة الحمض النووى فى آنسجة الخصية.

الخلاصة: هذه النتائج تشير إلى الإستراتيجية المحتملة لإستهداف زيادة الهيم آوكسيجيناز ١ فى تحسين الخلل الإنجابى الناجم عن قصور الغدة الدرقية.