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EFFECTS OF CHRONIC PAIN ON SOME REPRODUCTIVE ASPECTS IN ADULT MALE ALBINO RATS

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ABSTRACT

Background: Chronic pain is a self-reinforcing pathological state in which plastic changes in the stress system may contribute to the patient's suffering and pain-related disability. Psychological stress has been perceived clinically as a potential risk factor affecting male fertility. However, its potential effect on spermatogenesis and testicular function has received little attention compared with the role of testicular oxidative-stress mechanisms. **Objective:** The present work was designed to evaluate the effects of chronic pain on some reproductive aspects in adult male albino rats. **Material and methods:** Twenty adult male albino rats of local strain weighing 135 - 150 g were chosen to be the model of the present study. They were divided into two equal groups: Group I (Control group) received no treatment, and Group II (Chronic pain group) were subjected to induction of chronic pain by daily subcutaneous injection of 0.5 ml of 2.5% formalin into the plantar surface of one hindpaw for two weeks. Blood samples were withdrawn, serum was separated for determination of serum fasting glucose, corticosterone, testosterone, and prolactin levels. Rats were killed and pelvi-abdominal cavities were opened to obtain testes, epididymis and prostate for studying histopathological changes. **Results:** Chronic pain was associated with significant changes in behavior and hormonal profile of the reproductive functions in addition to histopathological disturbances. **Conclusion:** Chronic pain has a drawback effects on the behavioral and reproductive functions. So, a great importance of treating pain must be paid to avoid its drawback effects on the body functions.

KEYWORDS: Chronic pain, stress, male sex hormones, testes, epididymis, prostate.

INTRODUCTION

Chronic pain is a self-reinforcing pathological state in which plastic changes in the stress system may contribute to the patient's suffering and pain-related disability (Gatchel et al., 2007).

When facing prolonged uncertain and uncontrollable threat, the organism modifies its level of metabolic activity to adapt to environmental demands that may eventually lead to maladaptive responses inducing a series of stress-related pathophysiological strain. Such a state has been referred to as allostatic load and may contribute to the triggering, amplification, and/or persistence of the pain state (Borsook et al., 2012).

One of the potential consequences of allostatic load is an over-activation of the hypothalamic-pituitary-adrenal axis which may be critical when adapting to chronic pain as uncertainty about upcoming pain, exacerbation of pain by anticipatory anxiety and negative emotions (Roy et al., 2009 and McEwen & Kalia, 2010).

It has been reported that prolonged pain may constitute an allostatic load in individuals showing more stress vulnerability which induces long-lasting plastic changes that in turn affect the health state (Vachon-Presseau et al., 2013). Prolonged or repetitive stress makes the stress response a pathological one, leading to adjustments in homeostasis which include pathological effects on metabolism, vascular function, tissue repair, immune function and the nervous system (Herman, 2013).

It has reported been that chronic pain syndrome has detrimental effects on the quality of life including the aspect of sexual dysfunction (Teuchert et al., 2017).

Psychological stress has been perceived clinically as a potential risk factor affecting male fertility. However, its potential effect on spermatogenesis and testicular function has received little attention compared with the role of testicular oxidative-stress mechanisms (Vachon-Presseau et al., 2013).

The present work was designed to evaluate the effects of chronic pain on some reproductive aspects in adult male albino rats.

MATERIALS AND METHODS

The experimental protocol and animal handling were approved and performed according to the guidelines of animal use of the Ethical committee of Faculty of Medicine - Al-Azhar University. Twenty adult male albino rats of local strain weighing 135 - 150 g were chosen to be the model of the present study. They were left for two weeks in the laboratory room before any experimental interference for acclimatization with free access to water and rat chow pellets. Rats were kept in suitable cages (40 x 30 x 30 per 5 rats) at room temperature with the natural light-dark cycle. Rats were divided into two equal groups:

Group I (Control group): received no treatment.

Group II (Chronic pain group): were subjected to induction of chronic pain by daily subcutaneous injection of 0.5 ml of 2.5% formalin into the plantar surface of one hindpaw for two weeks. Each rat was then placed in a cage (Kaneko and Hammond, 1997).

Observation to determine nociceptive responses began after placing the rat into the cage and continued for the next hour. Nociceptive response was indicated by licking of the injected paw, little or no weight placed on the injected paw, or the injected paw elevated and was not in contact with any surface (Kaneko et al., 2000).

At the end of the experimental period, blood samples were withdrawn from the retro-orbital plexus into test tubes. Serum was separated and stored frozen at -20^{o}C until assayed for determination of the levels of serum fasting glucose (Maughan, 1982), corticosterone (Solberg et al., 2001), testosterone (Demetriou, 1987), and prolactin (Liu et al., 1994). Then, rats were killed and the pelvi-abdominal cavities were opened to obtain testes, epididymis and prostate for studying the histopathological changes.

Statistical analysis: Data input and analysis were done using SPSS computer program. All results were expressed as mean ± standard error. Mean values of the different groups were compared using a one-way analysis of variance (ANOVA). Least significant difference (LSD) post hoc analysis was used to identify significantly different mean values. P value < 0.05 was accepted to denote a significant difference.

RESULTS

Rats of the control group displayed an average degree of motor activity inside their cages. On manipulation, they tried to escape and showed slight aggression towards the person. On the other hand, rats of the experimental group showed typical signs of stress during the first week of the experiment. They showed exaggerated aggression which was great enough to the extent that manipulation became difficult. On manipulation, the animals showed aggression in the form of escape, vocalization (crying), and trying to bite the manipulator's hands. This was followed by gradual decrease in their motor activity to the extent of docility which continued till the end of the experiment. However, other external signs of stress (escape, vocalization, and trying to bite the manipulator's hands) persisted till the end of the experiment.

Results of the present work showed that induction of pain led to significant increase in serum glucose level from 99.7 ± 3.2 mg/dl to 140.1 ± 2.4 mg/dl (+40.52 %), significant increase in serum corticosterone level from 21.4 ± 1.91 ng/ml to 29.5 ± 1.2 ng/ml (+ 37.8 %), significant decrease in serum testosterone level from 4.22 ± 0.36 ng/ml to 3.02 ± 0.31 ng/ml (- 28.43 %), significant increase in serum prolactin level from 4.98 ± 0.48 ng/ml to 7.81 ± 0.11 ng/ml (+ 56.82 % Table 1).

In the control group, sections of the testes revealed normal testicular tissues. No atrophic changes, congestion or polymorphnuclear (PNL) infiltration were noted. The epididymis revealed normal epididymal tissue without atrophic changes, congestion or PNL infiltration. Also, the prostate revealed normal prostatic tissue lined by tall columnar epithelium, no congestion or PNL infiltration (figures 1 - 3).

In the experimental rats, the testes showed partial atrophic changes within the acini. Some of the testicular acini showed spermatogenic arrest. The stroma showed increased vascularity (congested stroma) and polymorphonuclear infiltration with minimal Leydg cells hyperplasia, in addition to partial thickening of the testicular capsule. The epididymis showed thickened wall and marked congested vessels and stroma with minimal PNL infiltration. The prostate showed normal capsule, mild congestion and PNL infiltration. The prostatic acini showed thick folded pesudostratified columnar epithelium with increased acinar secretions indicating hyperactivity (figures 4 - 8).

Table 1: Changes of the measured parameters in the tested groups (Mean ± SE).

Groups / Parameters	Group I (n=10)	Group II (n=10)	% changes
Blood glucose (mg/dl)	99.7 ± 3.2	140.1 ± 2.4*	+ 40.52 %
Corticosterone (ng/ml)	21.4 ± 1.91	29.5 ± 1.2*	+ 37.8 %
Testosterone (ng/ml)	4.22 ± 0.36	3.02 ± 0.31*	- 28.43 %
Prolactin (ng/ml)	4.98 ± 0.48	7.81 ± 0.11*	+ 56.82 %

- Group I: control. - Group II: Pain group.
- n: No. of rats in each group. ***** Significant.

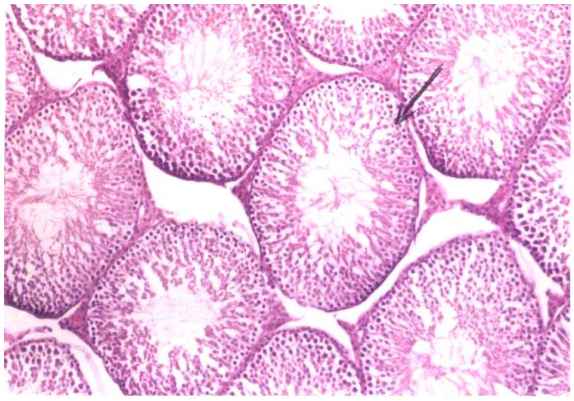

Fig 1: Normal testicular morphology showing normal seminiferous tubules (arrow) in the control group (Hx & E - X 400).

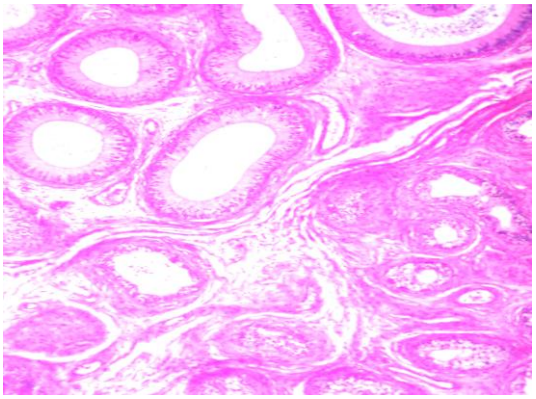

Fig 2: Normal morphology of the epididymis (Hx & E - X 400).

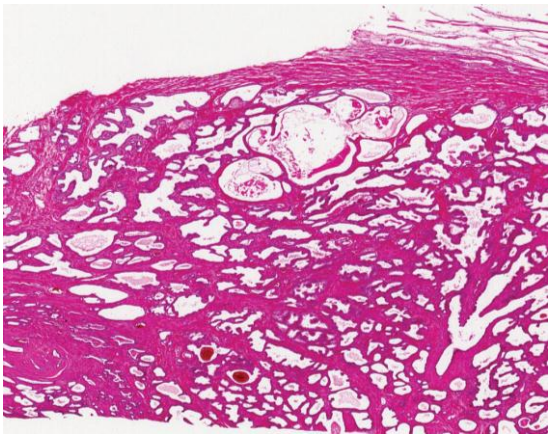

Fig 3: Normal morphology of the prostate (Hx & E - X 400).

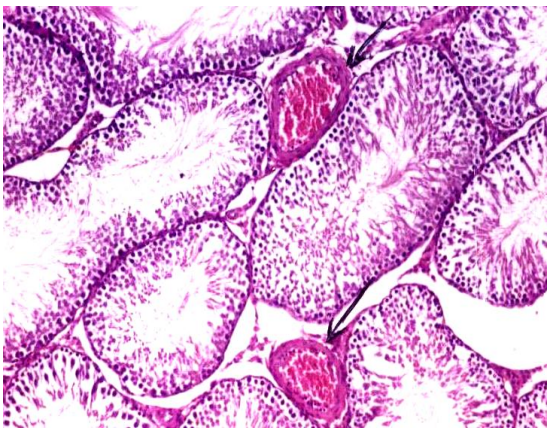

Fig 4: Congested interlobular blood vessels (arrows) of the testes in the pain group (Hx & E - X 400).

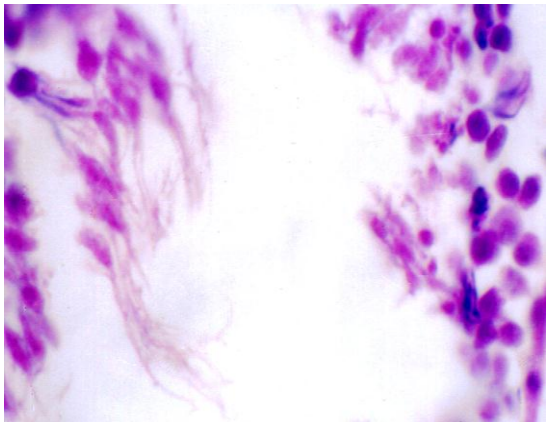

Fig 5: Arrested spermatogenesis in the pain group (Hx & E - 400x).

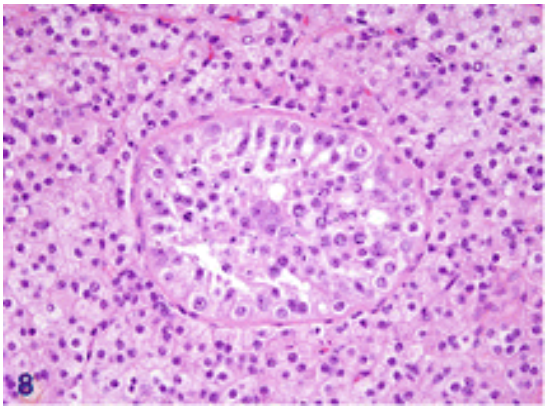

Fig 6: Interstitial cell hyperplasia and lack of mature spermatids in the pain group. (Hx & E - 400x).

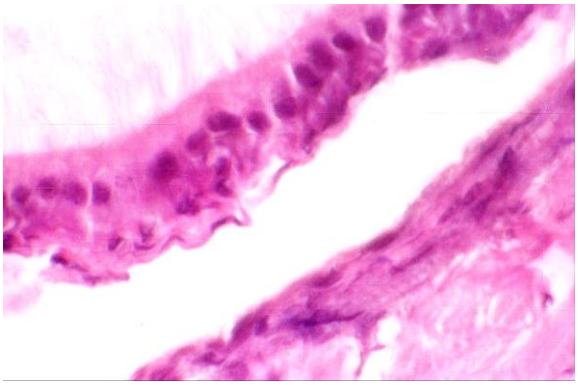

Fig 7: Thickened wall and congestion of the epididymis in the pain group (Hx & E - 400x).

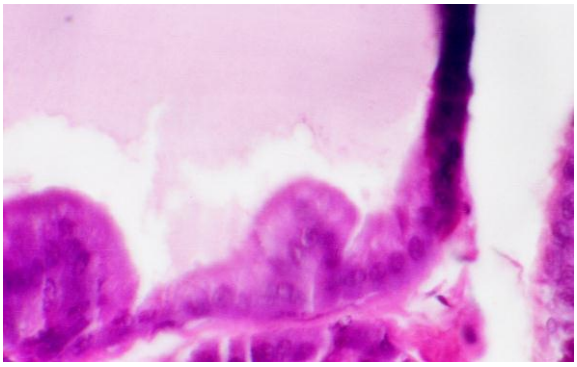

Fig 8: Thick folded epithelium with increased acinar secretions of the prostate in the pain group (Hx & E - 400x).

DISCUSSION

Pain is a universal phenomenon causing tremendous human suffering and compromising the quality of life for countless individuals. Pain is the number one reason that people seek for medical appointments and costs (Symons et al., 2015). Psychological stress has been perceived clinically as a potential risk factor affecting male fertility (Vachon-Presseau et al., 2013).

Results of the present work showed that induction of pain led to marked behavioral changes in the form of exaggerated aggression followed by gradual decrease in their motor activity to the extent of docility. These were in addition to disturbed metabolic and endocrinal responses indicated by elevated blood glucose, corticosterone and prolactin levels associated with reduced testosterone level.

These results agreed with Barrett (2015) who reported that pain is associated with disturbed animal behavior ranging from mild to severe aggression which differs from one species to other. Changing from acute to chronic pain leads to changing animal behavior from aggression to decreased motor activity, and concluded that pain can have very significant effects on the behavior of humans and animals.

It has been reported that animals who are exposed to painful stimuli exhibit pain behaviors such as scratching, limited mobility and exploring, vocalization, withdrawal from the painful stimulus or other self-protective maneuvers (Matson et al., 2010).

The duration of the nociceptive stimuli also influences the behavioral expression of pain and should be considered when evaluating pain behavior. Intermittent long duration stressors appear to be more effective than short-term stressors (Piesla et al., 2009).

The formalin test is used routinely only in rodents where an injection of dilute formalin into the dorsum or plantar tissue of a paw causes a behavioral response consisting of licking, flinching and shaking of the injected paw (Berge, 2011).

In the present work, exposure to pain led to significantly elevated blood glucose level. This result wais in agreement with Bellows et al. (2016) who reported that stressful situation results in both hyperglycemia and glucosuria. Gulbahar et al. (2010) has reported that post operative blood glucose level significantly increased compared with the preoperative basic values. Rhoads (2012) has reported that, during recovery of major surgery, hyperglycemia develops and explained this by reduced muscle and fat glucose uptake, leading to elevated glucose levels. Homeria et al. (2012) has also reported that elevated blood glucose level in response to stress depends on intensity of stress. In addition, it has been reported that chronic stressful situations are associated with hyperglycemia and worse glycemic control among those who do not cope effectively with stress (Tony et al., 2011).

In the present work, hyperglycemia could be due to excessive secretion of stress hormones specially corticosteroids. Cortisol, as a stress hormone, plays an important role in glycogenolysis and gluconeogenesis which are stimulated by catecholamine secretion due to high sympathetic activity in response to stress (Sprague and Arbelaez, 2011).

In the present work, exposure to pain led to significantly elevated serum corticosterone level. This result was in agreement with Tamburella et al. (2013) who reported that exposure to restraint stress resulted in increased serum corticosterone representing neuroendocrine stress responses, and suggests that the stressors act by inducing a disruption in cellular mechanisms governing neuronal plasticity and disturbances in the HPA axis.

Increased corticosterone levels could be explained by activation of the HPA axis. The stress response starts with the activation of parvocellular secretory neurons of the paraventricular nucleus in the hypothalamus. These neurons release CRH into the superior hypophyseal

portal vein, which stimulates the release of ACTH from the anterior pituitary, and finally the release of corticosterone from the adrenal gland into circulation (Dziedzic et al., 2014).

Results of the present work showed that exposure to pain led to significantly decreased serum testosterone level. This result was in agreement with Dhanabalan et al. (2011) who reported that restraint stress raised the serum level of corticosterone, and significantly suppressed the testicular level of steroidogenic acute regulatory protein and serum testosterone level. Also, in the testis and epididymis, restraint stress raised the levels of lipid peroxidation and hydrogen peroxide and significantly suppressed the activities of antioxidant enzymes. Brunton (2013) has also reported that prenatal stress is associated with a significant reduction in the circulating levels of the sex steroids, testosterone and estradiol. Reduced testosterone levels are of particular interest since, in rats, prenatal stress exposure blocks the normal prenatal surge in the circulating testosterone levels that occurs in male fetuses in late gestation.

It has also been reported that suppression of the hypothalamo-pituitary-gonadal axis in response to stress results in decreased gonadotropin-releasing hormone, gonadotropin hormones (FSH and LH), and gonadal sex steroids (testosterone, progesterone, and estradiol), in addition to downstream effects on the reproductive system. In addition, stress or high glucocorticoid concentrations in male rats inhibit LH-mediated testosterone secretion in cultured Leydig cells and rduced sperm motility (Everds et al., 2013 & Teuchert et al., 2017).

Results of the present work showed that exposure to pain led to significantly increased serum prolactin level. This result was in agreement with Zografos et al. (2009) who reported that the stress response involves the HPA axis and the release of growth hormone and prolactin. In addition, the circulating catecholamines, cortisol and growth hormone, as part of the stress response, result in elevated blood glucose levels (hyperglycaemia).

Increased level of prolactin causes spermatogenic arrest, impaired sperm motility and altered sperm quality (Kaiser, 2012). Prolactin levels are well known to be affected by psychological stress, with long-term psychological stress leading to significantly increased serum prolactin levels (Lennartssen et al., 2014). Increased prolactin levels can also affect testosterone secretion and sexual behavior and activity. The magnitude of the prolactin response seems to be related to the magnitude of the response of the HPA axis stress (Lennartssen and Jonsdottir, 2011).

Stimulation of the HPA axis is associated with inhibition of the HPG axis by glucocorticoids via the inhibition of GnRH at the hypothalamic level. The main regulators of the HPA axis include CRH, glucocorticoids and ACTH. The stress system seems to have many negative effects on male reproductive function. The HPA axis might also have a direct effect on the testes, as CRH receptors have been identified in male reproductive tissues (Kalantaridoua et al., 2010).

Results of the present work revealed different morphological changes of the reproductive organs including atrophic changes, congested vessels and minimal PNL infiltration. These were in agreement with Everds et al. (2013) and Rezaie et al. (2017) who reported that exposure of male rodents to stress resulted in tubular degeneration in the testes, atrophic changes (apoptosis, atrophic epithelium and decreased secretion) seen in the seminal vesicles, and prostate as a qualitative finding. These findings could be explained by decreased testosterone secretion.

It has been reported that, in male rodents, reproductive organ weights and histologic changes associated with stress are due to decreased testosterone secretion caused by suppression of GnRH. The most sensitive and frequently seen changes are decreased weights of accessory sex organs (seminal vesicles and prostate). The secretory function of these accessory sex organs is highly dependent on androgen concentrations and the decreased organ weights are primarily the result of decreases in the secretory products (Chapin and Creasy, 2012).

It has been reported that the histological study showed that the brunt of painful stress was on the testes in addition to organs with secretory function (prostate). The sloughed spermatids, seen in the testes, may be due to disturbed testosterone concentration level (Ashworth et al., 2011). Prostatic infiltration by the inflammatory cells might be due to stimulation of the immune system as it was found that the immune system is affected by stress. It has been reported that the relation between stress and the immune system may help in understanding the changes and the degree of polymorphonuclear infiltration as stress initially stimulates then depresses the immune system response (Sandercock et al., 2011).

In conclusion, the present work demonstrated that pain has a great drawback effects on the behavioral and reproductive functions. So, of great importance paying attention for treating pain successfully to avoid its drawback effects on the body functions and improving quality of life.

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