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# MONOSODIUM GLUTAMATE INDUCED PROSTATE DAMAGE: THE ROLE OF BERBERINE AND AQUEOUS EXTRACT OF GREEN ALGAE FOR TREATMENT IN MALE RATS

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# 6- SUMMARY

The prostate gland is one of the important secretory glands in the male reproductive system as; it secretes enzymes, amines, lipids and metal ions essential for the nourishment and protection of sperms during ejaculation for potential ovum fertilization, the prostate gland secrete proteases such as prostate-specific antigen (PSA), prostatic acid phosphatases (PAPs), immunoglobulins and zinc. From the main organs in the male reproductive system are the testes which are responsible for the production of male gametes (sperms) and male sexual hormones in a process called steroidogenesis which refers to the enzymatic reactions leading to the production of male steroid hormones such as testosterone.

Physiologically low and controlled levels of reactive oxygen species (ROS) and nitric oxide are secreted for normal sperm function. The presence of high levels of ROS in the cell such as; hydrogen peroxide ($H_2O_2$), hydroxyl radicals ($OH^-$) and superoxide anions ($O_2^{-2}$) can oxidize lipids, proteins, carbohydrates and DNA, ultimately resulting in the blockade of normal cell metabolism. To oppose the adverse effect of ROS, the cells stimulate the activation of antioxidant enzymes such as catalase, superoxide dismutase (SOD), glutathione system enzymes including; glutathione peroxidase (GPx), Glutathione-S- transferase (GST), Glutathione reductase which is responsible for reducing the glutathione. When the cellular antioxidant capacity is unable to cope with the pro-oxidants this can eventually leads to elevation in of free radicals (ROS), this imbalance results in occurrence of oxidative stress. This event has been shown to be involved in various pathogenic processes including aging, cancer and inflammation. ROS react with the polyunsaturated fatty acids (PUFA) in cell membrane and subsequently leads to lipid peroxidation that generates other reactive molecules, such as thiobarbituric acid reactive substances (TBARS) and malondialdehyde which can form DNA adducts that, if not adequately repaired, can lead to point mutations in tumor suppressor genes. These reactive molecules may also generate inflammatory stimuli to propagate the effect.

The chronic inflammation is from the pathologies that can occur either in testis or prostate gland; an inflammatory stimulus leads to the recruitment of leukocytes and mast cells to the site of damage, consecutively this will leads to increase in release in and accumulation of reactive oxygen species (ROS) at the site of damage which produce cytokines such as TNF-α , IL-1 β that will activate the transcription factor NF-κ B which activates the expression of many genes involved in the inflammatory process such as induced nitric oxide synthase ( i-NOS), cyclooxygenase -2 (COX-2) , adhesion molecules Furthermore; it will also increase the cytokines production as IL-1 β, IL-2 and TNF- α. The i-NOS expression stimulate the release of nitric oxide (NO) in turn, which will subsequently up-regulate the expression of P53 gene at the site of

inflammation. Testicular or prostatic inflammation could lead to dysfunction of these organs through the loss in membrane morphological integrity, impaired cell functions, along with impaired sperm motility and induction of sperm apoptosis. Non-bacterial prostatitis (CNP) is the most common form of the prostatitis syndromes (inflammation of prostate gland which is characterized by chronic, idiopathic pelvi-perineal pain and leukocytes expressed in their prostatic secretions).

Monosodium Glutamate (MSG) is one of the food flavors which increases the sapidity of food and produces a flavor that cannot be provided by other foods. MSG has a toxic effect on different organs in the body including the testis by causing a significant oligozoospermia and increases abnormal sperm. Berberine, is a naturally derived lipophilic isoquinoline alkaloid, it present in plants of the genus *Coptis, Hydrastis* and *Berberis*. Previous studies showed that berberine had the ability to inhibit reactive oxygen species (ROS) generation. The marine green algae (*Ulva lactuca*) is one of the marine algae rich in non-enzymatic antioxidant components, such as ascorbic acid, reduced glutathione, and has higher contents of polyphenols and flavonoids. These natural products were chosen in this study for their pharmacological effects which have been extended to include cardio-protective, anti-diabetic, anti-inflammatory effects, anti-oxidative and anti-cancer actions.

The present study aimed to induce prostatic dysfunction in rats by oral administration of monosodium glutamate (MSG) which is known as a pro-oxidant inducing oxidative stress and chronic inflammation. And in turn, we studied the adverse effect of MSG on testicular tissue of the rats. We used two different types of natural products which are berberine (BBR) and aqueous extract of *Ulva lactuca* (UL) to study the safety of their oral administration on healthy rats and then we empirically investigated their treatment potency against MSG induced testicular and prostatic oxidative stress and inflammation

Sexually mature fourty eight sexually mature Sprague-Dawley male rats randomly divided into six groups, the sham control group which are healthy rats(n=6) , berberine control group (BBR) n=6; BBR was orally administrated to rats in a dose of 50 mg/kg body weight daily for 30 days, aqueous extract of Ulva lactuca (UL) control group n=6; UL was orally administrated to rats in a dose of 100 mg/kg body weight daily for 30 days, monosodium glutamate prostate dysfunction induced group (MSG) (n=10), monosodium glutamate was orally administrated to rats in a dose of 15 mg/kg body weight daily for 45 days. Treatment by BBR group (n=10); MSG was orally administrated to rats in a dose of 15 mg/kg body weight daily for 45 days, followed by administration of BBR to the rats at a dose of 50 mg/kg body weight daily for 30 days. Treatment by UL group (n=10); MSG was orally administrated to rats in a dose of 15 mg/kg body weight daily for 45 days, followed by administration of UL to the rats at a dose of 100 mg/kg body weight daily for 30 days. At the end of the animal treatment or induction designed period, rats were sacrificed.

Blood and tissues samples were collected from the whole study groups, routine seminal parameters were assessed by CASA, plasma testosterone and free PSA were determined by ELISA techniques, Pro-oxidant, antioxidant enzymatic and non-enzymatic parameters, total and prostatic acid phosphatases were spectrophotometrically determined. Relative expression of pro-inflammatory genes such as TNF-α, COX-2, IL-1β, i-NOS and P53 were elucidated by PCR and DNA-agarose gel electrophoresis.

The results of our study showed that the administration of MSG to normal rats adversely affect the seminal dynamic and physiological parameters such as spermatozoa concentration, spermatozoa motility ratio and spermatozoa count per ejaculate when compared to sham control group. Moreover, histological examination of the testicular tissue of the rats treated with MSG also supported the occurrence of damage in seminiferous tubules which have irregular shapes and size, loss of spermatogenic which leads to shrinkage of the tubules and consequently widened interstitial tissue. The administration of MSG decreased the level of serum testosterone when compared to sham control group however, it increased the level of prostate specific antigen (PSA) and activities of acid phosphatases in blood when compared to control. Also its administration increased the prostatic and testicular pro-oxidants such as TBARS and NO when compared to control. Furthermore, it increased the activity of xanthine oxidase (XO) enzyme in comparison to control; XO directly catalyze the production of reactive oxygen species (ROS). MSG significantly decreased the activity of antioxidant enzymes which catalyze the production of antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) along with reduced glutathione (GSH) in comparison to sham control group in both tested tissues. At molecular level, MSG showed up-regulation in expression of testicular and prostatic pro-inflammatory genes such as TNF-α, i-NOS, COX-2, IL-1β and P53 in comparison to control. This indicated that MSG induced chronic inflammation which is caused by oxidative stress and increase in ROS.

The administration of UL to healthy rats at a dose of 100 mg/kg showed that it significantly elevated the activity of antioxidant enzymes such as GPX, GST and SOD in prostatic tissue however, it did not show significant change in their activities in testicular tissue when compared to respective sham control. Furthermore, the administration of UL to healthy rats decreased prostatic TBARS level however, it was not altered in testicular tissue. Testicular and prostatic NO content showed slight increase when compared to corresponding sham control groups. Moreover, it did not show significant alteration in serum parameters such as; testosterone, PSA, total and prostatic acid phosphatases in comparison to sham control. As for the seminal dynamic and physiological parameters, the administration of UL for normal rats displayed significant increase in spermatozoa concentration, spermatozoa motility ratio and spermatozoa count per ejaculate when compared to control. Furthermore, the testicular

histological examination showed normal architecture as that of control. At molecular level, the administration of UL to healthy rats did not show significant effect on testicular relative expression of COX-2, IL-1β and P53 genes when compared to sham control one. However, it down regulated the prostatic TNF-α relative expression and, it slightly increased the prostatic COX-2 and testicular i-NOS and TNF-α relative expression in comparison to control.

In the current study we used the UL as a treatment of prostatic and testicular pathologies mediated by (MSG) oxidative damage in rats due to the high antioxidant and anti-inflammatory potencies possessed by UL at a dose of 100 mg/kg. The results shown in showed that UL successfully decreased the serum testosterone, PSA and TAPs and PAPs activities when compared to MSG prostatic pathologies induced group along with seminal dynamic and physiological parameters results which showed that, treated rats administrated with UL displayed significant increase in spermatozoa concentration, spermatozoa motility ratio and spermatozoa count per ejaculate and it successfully normalized them. Moreover, the testicular histological examination showed good population of spermatogenic cells in comparison to the adverse effect in testicular morphology mediated by MSG. Interestingly, the antioxidant status of the UL treated rats was improved, where the treatment of rats with UL significantly decreased prostatic and testicular TBARS, NO content and XO activity compared to corresponding MSG induced groups. Furthermore, the activity of prostatic and testicular antioxidant enzymes such as GST, GPX and SOD along with GSH content showed increase when compared to corresponding MSG induced groups. The rats treated with UL showed down regulation in gene expression of prostatic and testicular pro-inflammatory genes such as TNF-α, IL-1β, COX-2, i-NOS and P53 when compared to MSG induced group and it successfully normalized prostatic TNF-α, IL-1β and testicular COX-2.

In our study, we tried to investigate the effect of BBR orally administrated to rats at dose of 50 mg/kg for 30 days on healthy rats and in the treatment of prostatic and testicular pathologies induced by MSG. The healthy rats administrated with BBR showed that it did not show adverse effect on them as illustrated in our results; as it did not show significant alteration in serum parameters such as concentration of testosterone, PSA and activity of phosphatases (TAPs and PAPs) when compared to sham control group. It also showed improvement in spermatozoa concentration, spermatozoa motility ratio and spermatozoa count per ejaculate in comparison to control. On the level of oxidative stress; BBR did not show significant effect on testicular and prostatic oxidative stress parameters such as TBARS, activity of XO and anti-oxidant enzymes activities such as GST, GPX and reduced glutathione when compared to sham control group however, it significantly elevated the activity of testicular SOD in comparison to sham control group and a slight decrease in testicular NO by 38 % when compared to corresponding sham control groups. At molecular

level, testicular pro- inflammatory genes such as TNFα, COX-2 and IL-1β along with P53 relative expression were not significantly altered compared to that of sham control group. However, prostatic P53 and TNF-α showed significant decrease in their relative expression and it showed a slight up regulation of testicular i-NOS and prostatic COX-2 relative expression in comparison to corresponding sham control group.

The treatment of rats with BBR after MSG administration successfully maintained normal levels of serum testosterone. Moreover, it decreased serum PSA level, TAP and PAP activities when compared to MSG treated group. It maintained normal ranges of spermatozoa concentration, spermatozoa motility ratio and spermatozoa count per ejaculate and it decreased the histological deformities in testicular tissue due to MSG toxicity. Furthermore, it decreased the oxidative damage mediated by MSG as it decreased testicular and prostatic NO, TBARS contents and XO activity when compared to MSG induced group and successfully normalized the antioxidant enzymes activities in testicular and prostatic tissues such as GPx, GST and SOD along with reduced glutathione (GSH). At molecular level, treatment of rats with BBR down regulate the relative expression of testicular and prostatic TNF-α, i-NOS, COX-2, IL-1β and P53 when compared to MSG induced group and also it successfully returned back the expression of prostatic IL-1β and TNF-α to normal pattern.

In conclusion, the oxidative stress and chronic inflammation induced by MSG can eventually leads to prostatic dysfunction and testicular deformities that can develop infertility in male rats. Molecular investigation of pro-inflammatory genes and biochemical parameters measured such as oxidative stress enzymatic and non-enzymatic parameters can be used as diagnostic tools in laboratory investigations of the oxidative stress induced prostatic dysfunctions. Berberine and aqueous extract of *Ulva lactuca* can be used as natural remedies of oxidative stress as they could efficiently reverse the adverse effects induced by MSG due to high antioxidant and anti-inflammatory potencies. As, they possess high content of flavonoids and polyphenolic compounds. These treatments effectively improve the oxidative homeostasis of prostate gland and testicular tissue, increase serum testosterone and decreased the PSA and acid phosphatases in blood.

At the end of this study, it is recommended to prevent intake of food containing MSG and it is preferred to substitute it with another flavor. Moreover, it is recommended to use natural products such as *Ulva lactuca* extracts and berberine as substituents for drugs in treatment of various diseases as; they are rich in antioxidants and anti-inflammatory agents and they are safe to be used.