ejpmr, 2018,5(2), 257-263

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

NATURAL CONTRACEPTIVE FOR MALE RATS

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Article Received on 24/11/2017 **Article Revised on 14/12/2017** **Article Accepted on 04/01/2018**

ABSTRACT

Many plants have therapeutic actions for some diseases and also, it has adverse effects which can be employed to solve another some problems. This study is aimed mainly on the adverse effect of *Citrullus-colocynthis* on fertility. Where, fertility control is essential and concerned with national public health. **Methods:** Thirty male rats were divided into 3 groups, 1st group was kept as control, 2nd and 3rd groups received *C-colocynthis* extract orally (50 mg, 150 mg /100g B W respectively) day after day for 70 days. **Results:** There was an increase in hepatic lactate dehydrogenase (LDH), protein thiols (Pr-SHs), reduced glutathione (GSH), glutathione peroxidase (GPx) and superoxid dismutase (SOD). Also, there was an increased in hepatic total protein, albumin, globulin and adenosine triphosphate (ATP) in liver homogenate as compared to the control. The testes weight and the values of deformed head sperm showed an increased. In treated groups the results showed a decreased in sperm number, motility and the number of life sperm in compared with control. Histopathological examination showed some changes in seminiferous tubule with some degenerative changes. **Conclusion:** *C-colocynthis* extract had anti-fertility effects on male rats in addition to its antioxidant effects and increasing of ATP and liver protein content.

KEYWORDS: Seeds, Extract, Fertility, Public health, Sperm.

INTRODUCTION

National public health concerned with an increasing growth rate of the world's population, so, the control of fertility became essential.[1]

In addition to action of *Citrullus colocynthis as* antidiabetic, antioxidant, hypolipidimic action[2,3] and anti-inflammatory effect.[4] *Citrullus colocynthis* seed and pulp aqueous extracts increased the activities of these liver function enzymes (ALT, AST and ALP).[5]

Many foreign and endogenous substances can be detoxified by glutathione peroxidase.[6] Also, it was playing an important role in the detoxification of H_2O_2 and lipid peroxide.[7] Glutathione-S-transferase conjugated with electrophilic metabolites of drugs.[8] Hydrogen peroxide was decomposed directly to water and oxygen in the presence of catalase and reducing agent such as GSH.[9]

The reduced glutathione concentrations in hepatic cells are highly quite and hepatotoxicity of many xenobiotics must be preceded by depletion of GSH.[10] GSH can react chemically with ROS in a number of ways. It was reducing H_2O_2 directly to water with the formation of GSSG, in most cells this reaction catalyzed by selenium-dependent GPx.[11] Also, it is act to maturation, spermatogenesis and the maintenance of accessory sex organs and play active role in development and growth of male reproductive organs.[12,13]

Ovarian weights were decreased and there was a decreased in viable fetus's number in female rats receiving Citrullus colocynthis. So, exposure of female rats to Citrullus colocynthis L. for long term caused adverse effects on fertility and the reproductive system.[14,15] No pathological changes were seen in any of the maternal organs, but in some cases, concepti were seen as necrotic masses. In more recent work proteins capable of inducing abortions and necrosis of placental trophoblasts have been isolated from *Citrullus colocynthis* seeds.[16]

Oral administration of ethanolic extract of *Citrullus colocynthis* seeds is more essential to evaluate and studying the effect of extract on male fertility by estimation of testes weight, life sperm, sperm cell concentration, sperm motility and epididymal sperm abnormalities is the aim of this study.

METHODS

Study design

Thirty male albino rats (200–220 g) were selected for the present study. Rats were obtained from the National Research Centre Cairo, Egypt. These rats were fed with basic diet containing barley and carrots and allowed to free access of tap water and kept under constant

environmental conditions at room temperature. The male rats were divided into 3 groups (10 in each), the first group received orally (50 mg/100 gm BW), the second group received (150 mg /100 gm BW) of ethanolic extract of *Citrullus colocynthis* seeds (day after day) for 70 days and the third group was kept as control. Seed extract may be the preferred route for therapeutic application.[17]

Preparation of *Citrullus colocynthis* seeds extract

The seed powder was defatted by n-hexan. Then, defatted seeds were extracted using methanol 70%. This extraction process was repeated three times and dried at low temperature (< 40°C) under 100 mmHg pressure in a rotary evaporator. The dried extracts were stored in zero temperature till use in further experiment. For oral administration, the extract was reconstituted in distilled water.[18]

Preparation of liver homogenate

Animals were killed by cervical dislocation and then livers were rapidly removed, weighed and homogenized. The homogenate was divided into two aliquots. The first one was deproteinized with ice-cooled 12% trichloroacetic acid and the obtained supernatant, after centrifugation at 1000 xg, was used for the estimation of GSH and ATP contents. The second aliqout was centrifuged at 1000 xg and the resultant supernatant was used for estimation of LDH, Pr-SHs, total protein and albumin levels.

Lactate dehydrogenase (LDH) was determined kintically using a test reagent kit according to the method of Buhl and Jackson.[19] Pr-SHs were determined according to the method of Koster.[20] Total protein level was determined using a test reagent kit based on the method of Weichselbaum.[21] Albumin level was determined using a test reagent kit based on the method of Doumas.[22] Liver ATP was determined using a test reagent kit according to the method described by Adams.[23] Glutathione peroxidase (GPx) activity was determined according to Kumar.[24] Superoxide Dismutase (SOD) activity was determined according to Jemec.[25] The estimation of GSH level was determined according to Öktem[26] by using Rat GSH ELISA Kit, El-Aab, Catalog No: E0294r. All dry chemicals and solvents were obtained from Sigma Chemicals.

Studying the effect of the alcoholic extract of *C-colocynthis* seed on male fertility

After 70 days (the period for covering all the spermatogenesis.[27] Male rats were killed and the testes and seminal vesicles of each rat were dissected out, weighted and examined macroscopically.

Epididymal spermatozoal examination

Epididymal contents was obtained immediately by cutting the tail of epididymis and squeezing it gently to obtain the fresh undiluted semen in a clean Petri dish to proceed the following examination:

A-Progressive motility

A small droplet of undiluted semen was added to one drop of sodium solution 2.9-3% on warm slide. Several fields were examined under microscope and the incidence of progressive motile sperms were estimated and recorded.[28]

B- Sperm cell concentration

The pipette of haemocytometer for counting erythrocytes was used. The undiluted semen was withdrawn up to mark 0.1 and pipette was then field up to mark 101 by normal saline stained with eosin. The contents of the pipette were examined by holding the ends of the pipette between the thumb and the index fingers for shaking vigorously. The cover slid was placed over the counting chamber and a drop of diluted semen was spread between the Haemocytometer chambers and its cover.[28]

The sperms in 5 large squares (80 small squares) were counted using high power of microscope (40x). The sperm cell contraction (in mm^3) was estimated by multiplying the counted number of sperms by 10 (depth) and 1000 (dilution).

C- Epididymal sperm abnormalities

A drop of epididymal contents of each rat was mixed with an equal drop of eosin-nigrosin stain. The semen was carefully mixed with the stain, this film were spread on clean and grease free slides. Two hundred sperms were randomly observed per slide under high power lens of the light microscope and percentage of abnormal sperms was recorded.

Macroscopic examinations

Epididymal spermatozoal examination:

Epididymal contents were obtained immediately by cutting the tail of epididymis and sequeezing it gently to obtain the fresh undiluted semen in a clean petri dish to proceed the following examination:

a- Progressive motility.
b- Sperm cell concentration.
c- Epididymal sperm abnormalities.

Histological studies

Rat testes of all experimental groups were selected and processed by paraffin blocks were sectioned 5 µm thickness and histological stained with hematoxylin and eosin (H&E) stain for light microscopic examination.[29]

Statistical analyses

All obtained data were represented as mean ± SE. Differences between the mean value were statistically analyzed by using one way analysis of variance (ANOVA) utilizing computerized statistical program (InStat) $P < 0.05$ was considered statistically significant.

RESULTS

The results were showed lactate dehydrogenase (LDH) activity, protein thiols (Pr-SHs) level, reduced glutathione (GSH), glutathione peroxidase (GPx) activity

and superoxid dismutase (SOD) activity were significantly increased in hepatic cells (tab 1). Also, there were markedly increased in liver total protein content, Liver albumin and liver globulin content as compared to the control group in rats treated with (50mg and 150mg /100 gm BW) of an alcoholic extract of *Citrullus colocynthis* seeds (tab 2).

Male fertility

After 70 days of an alcoholic extract of *Citrullus colocynthis* seeds treatment to mal albino rats in a dose of (50 mg/100 gm BW) and (150 mg /100 gm BW). The male rats were anaesthetized and sacrified then the mean values of testes weight were recorded in table (3), the epididymis was obtained and crushed for collecting the seminal fluid and examined microscopically for estimating the motility of sperm, sperm cells concentration $1mm^3$ and deformed head sperm. In *Citrullus colocynthis* seeds treated albino males the mean values of testes weight showed a significant increased. Regarding to sperm cell concentration of the treated group the results showed a significant decreased in number compared with control. The percentage of progressive motility showed significant decreased in treated group in comparison with the control group. Results also showed a significant decreased in the number of life sperm in treated group than in control group, while the values of deformed head sperm were significantly increase in treated group than in non-treated group.

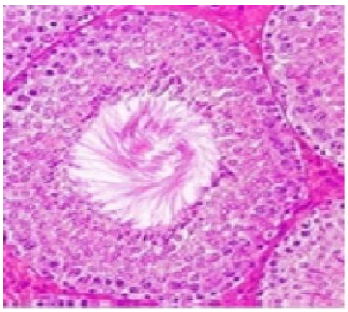

Fig. (1) Testes of normal rats group

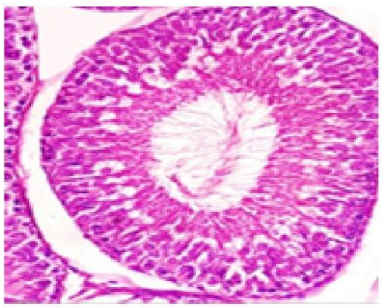

Fig. (2) Testes of rats treated with 50 mg/kg BW

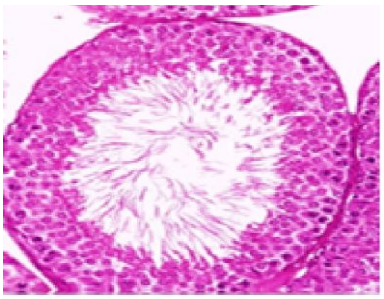

Fig. (3) Testes of rats treated with 150 mg/kg BW

Histopathology

In the histological study of testis in normal group seminiferous tubules showing organization of cells at all stages of spermatogenesis with clear maturation of spermatozoa occurred near the lumen (Fig 1), but in treated rats, seminiferous tubule showing with some degenerative changes. Also there was an increasing in interstitial space between the seminiferous tubules and decreasing in the number of spermatocytes and spermatogonia (Fig 2 and 3).

Fig (1) Normal group: Leydig cells are present with normal seminiferous tubules and lumen is filled with sperm.

Fig (2) (Testes of rats treated with 50 mg/kg BW): Showed some degenerative changes of seminiferous tubule with increasing interstitial space between them and decreased in spermatocytes and spermatogonia number.

Fig (3) (Testes of rats treated with 150 mg/kg BW): Showed degenerative changes with disruption of spermatogenesis. Lumen appear with less number of sperms and interstitial space was increased. Also, spermatogenic number was decreased and irregular epithelium loosened at some places.

Table (1): Effects of orally doses of (50mg and 150mg) of an alcoholic extract of *C-colocynthis* seeds on the levels or activities of LDH, Pr-SHs, GSH, GPx and SOD on liver rats.

	Control	Low dose (50mg)	High dose (150mg)
LDH (U/g protein)	271.6 ± 4.33	309.1 ± 2.01*	326.4 ± 7.29 **
Pr-SHs (umol/g protein)	80.6 ± 2.00	93.8 ± 1.65*	99.5 ± 1.81 **
GSH (mg/g protien)	9.6 ± 0.31	12.2 ± 0.38*	15.1 ± 0.52 **
GPx (ug/ g protein)	235.9 ± 5.44	261.2 ± 3.14*	282.1 ± 2.46 **
SOD (ug/g protein)	111.5 ± 1.92	130.9 ± 1.45*	141.6 ± 2.26 **

* Significantly different from control at $P < 0.05$.

** Significantly different from control at $P < 0.05$.

Table (2): Effects of orally doses of (50mg and 150mg) of an alcoholic extract of *C-colocynthis* seeds on the level of total protein, albumin and globulin in liver homogenate.

	Control	Low dose (50mg)	High dose (150mg)	P
Total protein (mg/g.wet.tissue)	85.8 ± 1.88	95.8 ± 2.41*	103.5 ± 3.36 **	* P < 0.05 ** P < 0.001
Albumin (mg/g.wet.tissue)	43.5 ± 1.96	51.5 ± 1.56*	55.8 ± 1.35 **	* P < 0.01 ** P < 0.001
Globulin (mg/g.wet.tissue)	41.3 ± 1.75	45.0 ± 1.17*	48.1 ± 1.03 **	** P < 0.001

Table (3): Showing mean values of testes weights and spermatozoa examination during oral administration of *C-colocynthis* at a dose level (50mg and 150mg) to male rats for 70 days.

Parameters	Control	Low dose (50mg)	High dose (150mg)
Testes weight	2.82 ± 0.16	3.45 ± 0.13	5.37 ± 0.30 *
Life sperm	1.17 ± 0.13	0.94 ± 0.09	0.50 ± 0.07 *
Sperm cell con.	910 ± 28.7	766.9 ± 34.8	416.9 ± 21.5 *
Sperm motility	90 ± 3.18	71.8 ± 3.13 *	41.2 ± 1.93 **
Deformed head sperm	14.1 ± 0.58	26.7 ± 1.71 *	56.2 ± 2.01 **

* Significantly different from control at P < 0.05.
** Significantly different from control at P < 0.05.

Table (4): Effects of orally doses of (50mg and 150mg) and of an alcoholic extract of *C-colocynthis* seeds on level of adenosine triphosphate (ATP) in liver homogenate.

	ATP (U mol/g. protein)
Control	3.29 ± 0.17
Low dose (50mg/100 gm)	4.89 ± 0.22*
High dose (150mg/100 gm)	6.30 ± 0.33**

* Significantly different from control at P < 0.05.
** Significantly different from control at P < 0.05.

DISCUSSION

Although LDH is classified as a non-specific enzyme that can reflect injury to extrahepatic tissue[30], Increase in liver LDH activity by *Citrullus colocynthis* extract might be due to the intracellular accumulation of Ca^{+2}, which results in activation of phosphfructokinase and anaerobic glycolsis leading to lactate formation.[31] On the other hand, treatment with *Citrullus colocynthis extract* resulted in an increase in glucokinase, glucose-6-phosphate and phosphofructokinase values and a decrease in hexokinase value.[32] The ingestion of *Citrullus colocynthis* seed stimulated the activities of LDH.[33]

Also, increase the level of Pr-SHs in liver, as well as GSH level, GPx and SOD activities in liver. These effects due to their active constituents (*glucosides, flavonoids, alkaloids, tinnins and saponins*).[34] Antioxidant activity is one of the most important mechanisms for preventing or delaying the onest of major degenerative diseases of aging, including cancer, heart disease, catracts and congnitive dysfunction.

The antioxidants are believed to exert their effects by blocking oxidative processes and free radicals that contribute to the causation of these chronic diseases.[35] *C-colocynthis* exhibit antioxidant[36], where they were reported that the *citrullus colocynthis* extract was particularly induced the GPx, SOD and Glutathione-S-Transferase gene transcripition and increase GSH level in liver of treated rats.[37]

Oxidative stress is a key factor in many human diseases.[38] Reactive oxygen species (ROS) have the potential to damage nucleic acids, proteins and biomembranes. When cellular defense mechanisms fail, severe dysfunction or cell death can result, events that are part of the respective pathogenic process. There was accumulating evidence that plant derived antioxidants may reduced or prevented oxidative stress and had a beneficial influence on animal and human health.[39]

Our results in agreement with the results of *Dhanasekar and Sorimuthu*[36], reported that, oral administration of *Citrullus colocynthis* seed extracts at a concentration of 150 mg/kg BW for 30 days showed an increase in reduced glutathione, superoxide dismutase, glutathione peroxidase and glutathione-S-transferase in the liver and kidney.[40]

These results agreed with that reported by *Yuan and Kitts*[41], observed significant reductions in malondialdehyde and associated increases in GSH-Px after adminstration of antioxidants extract. Gebhardt[42] has examined the hepatoprotective and chemopreventive effects of *C-colocynthis* using a number of model systems. The present studies found that the *Citrullus colocynthis* extract increase the level of liver Pr-SHs, as

well as GSH, GPx and SOD activities. Therefore, *Citrullus colocynthis* extract have a free radical scavenging effects.[43] Extract of *C. colocynthis* has highest antioxidant and free radical scavenging effect.[44] Upon treatment with ethanolic extract of *C-colocynthis* seeds as well as gliclazide to alloxan induced diabetic rats restored the level of antioxidant enzymes to normal.[45]

The results of the present study showed that there was an increase in liver protein level where this is render to high content of protein in seeds of *C-colocynthis* and these confirmed with[46], who reported that the ingestion of *C-colocynthis* seeds would increase in liver protein levels.

Our results observed that an increase in liver protein and adenosine triphosphate levels in seed extract treated rats. It can be speculated that no hepatic toxicity or damage was observed or noticed during the administration of *C-colocynthis* extracts. The present results were confirmed with other studies[47], who found that, some components existing in the extracts of *C-colocynthis* which promote liver RNA and protein synthesis as was observed in the hepatoprotective activity.[48]

The results illustrated that the ATP content of *Citrullus colocynthis* extract inducted rats liver was more than that of control. In general, the increases in ATP during the *Citrullus colocynthis* extract administration were mainly for maintenance of tissue energy, which likely accomplished through increases glycolysis, indicated by an increased a key enzymes of the glycolytic pathway in agreement with Oyedapo and Araba.[47]

The effect of oral treatment of *C-colocynthis* in a dose of 150 mg/100 gm BW in our work on male fertility in rats showed marked changes in the tests, epididymis and accessory glands. The mean values of tests weight showed significant increase in compared with control. In addition, significant effect was observed in the progressive motility, life and dead sperms and concentration of sperms. Concerning the spermatozoal examination in treated rats, the life sperms percentage, sperm cell concentration and sperm motility showed significant decrease in treated rats in compared with control, while, deformed head sperm in treated rats were increased as compared with control. Our results agreed with those results mentioned by *Dixit*[49] who noticed that oral administration of *C- colocynthis* fruit extract to male dogs resulted in testicular lesions and mass atrophy of spermatogenic elements. *Stepka and his colleagues*[50] found that daily oral administration of the fresh juice Momordica (*Citrullus colocynthis*) leaves to a group of female mice reduced the fertility rate.

Sperm motility and density, number of pups, fertility, and circulatory levels of testosterone were observed in all treated groups. The weights of testes, epididymis, seminal vesicle and prostate were significantly decreased.[15,51]

C. Colocynthis showed an antiandrogenic nature, thereby reduced reversible infertility in male albino rats.[44,52] Ingestion of *Citrullus colocynthis* lead to testicular lesions and mass atrophy of spermatogenic elements.[53] Also, it was induced harmful effect on testes function.[51]

CONCLUSION

The present study clearly demonstrated that *C. colocynthis* seeds have anti-fertility effects on male albino rats with no side effects where it had antioxidant effects and increasing ATP and liver protein content. Also, it caused some histopathological changes were observed in seminiferous tubules of the testis. However, further studies are required for understanding its mechanism of action.

ACKNOWLEDGMENT

The author record their great thanks to all departments of pharmacognosy and pharmacology, Faculty of Pharmacy, Port Said University, Port Said, Egypt, for helping and facilitating the performance of this work.

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