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Protective effect of L-carnitine and L-arginine against busulfan-induced oligospermia in adult rat

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Summary

Busulfan is an anticancer drug caused variety of adverse effects for patients with cancer. But it could cause damage to the male reproductive system as one of its adverse effects. This study aimed to investigate the protective effect of L-carnitine and L-arginine on semen quality, oxidative stress parameters and testes cell energy after busulfan treatment. Adult male rats were divided into four groups: control (Con), busulfan (Bus), busulfan plus L-arginine (Bus + L-arg) and busulfan plus L-carnitine (Bus + L-car). After 28 days, the semen was collected from the epididymis and the testes were assessed. Sperm count, motility and velocity were measured by CASA, and smears were prepared for assessment of sperm morphology. Serum and testes supernatants were separated for DNA metabolites, oxidative stress and cell energy parameters. Testes tissues also subjected for caspase-3. The results showed significant improvement in sperm morphology, motility, velocity and count in the groups treated with L-arginine and L-carnitine and accompanied with an increase in MDA, GSSG and ATP, reduction in GSH, AMP, ADP, NO and 8-OHDG also recorded. These results are supported by caspase-3. Conclusions: Administration of L-arg and L-car attenuated the cytotoxic effects of busulfan by improving semen parameters, reducing oxidative stress and maintaining cell energy.

KEYWORDS

Busulfan, l-arginine, l-carnitine, oligospermia

1 | INTRODUCTION

Busulfan is a bifunctional antineoplastic agent that belongs to the class of alkyl sulfonates (Iwamoto et al., 2004). As an alkylating agent, it works by sticking to one of the cancer cell's DNA strands; thus, the cancer cells undergo apoptosis (Houot et al., 2013). Busulfan is used for the chemotherapeutic drug of leukaemia, as it is relatively cheap drug. As through, after treatment, busulfan caused adverse effects in various organs, such as the reproductive tract organs. Busulfan treatment induces oligospermia and even azoospermia and testicular atrophy in young male patients, leading to sterility in some cases (Jung et al., 2015).

L-carnitine is concentrated in a high energy demanding tissues like muscles, skeletal, cardiac and reproductive system particularly in the epididymis. L-carnitine was first isolated from beef muscle in 1905. Approximately 75% of L-carnitine of the body stores is derived from the diet, whereas only 25% is synthesised de novo from lysine and methionine.

L-carnitine also has great effects on the spermatogenesis, sperm maturation and sperm motility. It is a small water-soluble particle important in fat metabolism (Dehghani, Hassanpour, Poost-Pasand, Noorafshan, & Karbalay-Doust, 2013). It plays an important role in long-chain fatty acids oxidation in mitochondria then, producing energy. In addition, adjustment of acyl-CoA/CoA ratio, store energy as acetylcarnitine and revising the toxic effects of poorly metabolised acyl groups by releasing them as carnitine esters (Ahmed et al., 2011). L-carnitine concentration in epididymal fluid and spermatozoa range from 2 to 100 mM, that is, about 2,000-fold greater than circulating

levels (10–50 μM; Chiu et al., 2004a). Free L-carnitine is taken up from the blood plasma and transported into the epididymal plasma. It is then diffused passively into the spermatozoa, where it stacks as free and acetylated carnitine. The initiation of sperm motility is proportional to the concentration of L-carnitine in the epididymal lumen (Chiu et al., 2004b). Another potential use of seminal free L-carnitine is in the diagnosis of obstructive azoospermia. The cases of post-epididymal obstruction, such as those with *vas deference* agenesis, have extremely low concentrations of carnitine (Agarwal & Said, 2004). L-carnitine is known to have antioxidant, anti-inflammatory and anti-apoptotic effects on various pathophysiological conditions (Miguel-Carrasco et al., 2008). On the other hand, reactive oxygen and nitrogen species (ROS and RNS) are implicated in the diagnosis of male infertility. Various ways have been prepared to eliminate or reduce the production of ROS and RNS in spermatozoa, particularly in vitro fertilisation. In addition, a variety of ROS/RNS generated naturally by spermatozoa, including superoxide anion, hydrogen peroxide and nitric oxide (NO). These interactive species, especially NO, are very important in regulating sperm energy as well as acrosome reaction, which needed by spermatozoa to fulfil fertilisation possibility. Recently, it has been shown that mitochondria have a great role in human sperm fertilisation probability and quality. Also, NO and NO precursors increase sperm motility by increasing energy production in mitochondria (Buzadzic et al., 2015).

L-arginine is α-amino acid that is used in the biosynthesis of proteins. It is classified as a semi-essential or even essential amino acid in some condition. It has a main role in growth stages and health state of the person. L-arginine is a precursor of NO, so it acts as a free radical scavenger and pro-oxidant enzyme inhibitor; these roles of L-arginine are attributed to NO (Tripathi & Misra, 2009).

The aim of this study was to investigate the curative effect of L-carnitine and L-arginine following busulfan administration in restoring male fertility.

2 | MATERIAL AND METHODS

2.1 | Ethics

All animal experimentation protocols were carried out under the supervision of the Ethics Committee of the national organisation of drug control and research (NODCAR), Egypt.

2.2 | Animals

In this experimental study, twenty adult male *Sprague Dawley* rats (about 180 ± 20 g) were selected from the laboratory animal house of NODCAR. Rats were maintained under standard conditions (12-hr dark/night and free access to food and tap water throughout the experiment).

2.3 | Drugs preparation

Preparation of busulfan solution: busulfan (Sigma, B2635, USA) was first dissolved in DMSO (dimethyl sulfoxide); then, an equal volume of sterile water was added to obtain a final busulfan concentration of 20 mg/ml (Khosoroshahi et al., 2012). Preparation of L-carnitine: L-carnitine (Sigma, C0283-5G, USA) was dissolved in distilled water to obtain a concentration of 350 mg/kg.bw (Ghanbarzadeh, Garjani, Ziaee, & Khorrami, 2013). Preparation of L-Arginine: L-Arginine (Sigma, A5006-100G, USA) was dissolved in distilled water to obtain final concentration 100 mg/ml (Li, Fraser, Wang, & Whitworth, 1997).

2.4 | Experiment design

Rats were randomly divided into four different experimental groups:

Group I (Control) received a single dose of DMSO (I.P.) plus 1 ml of distilled water daily by oral gavages.

Group II (Bus) received a single I.P. injection of busulfan (Bus) at a dose of 20 mg/kg plus 1 ml of the distilled water daily by oral gavages.

Group III (Bus + L-arg) received a single I.P. injection of busulfan plus 1 ml of L-arginine (L-arg) daily by oral gavages.

Group IV (Bus + L-car) received a single I.P. injection of busulfan plus 1 ml of L-carnitine (L-car) by oral gavages.

Administration of L-carnitine and L-arginine started 1 day after injection of busulfan and was continued for 28 days (Dehghani et al., 2013).

At the end of the treatment period, the rats were weighed and anesthetised using diethyl ether, then killed. The animals were dissected and blood samples were collected from Retro-orbital plexus, centrifuged immediately after collection and stored at −20°C for biochemical assay. Then, the testis and epididymis were carefully dissected out and trimmed of all fat and blood. Epididymis was removed, minced in 1 ml phosphate buffered saline (PBS, pH 7.2) (Padmanabhaiah, 2002). The testes tissues were homogenised and supernatant stored at −20°C for further assay; testicular tissues were immediately fixed in 4% formaldehyde in buffered solution for an additional 72 hr for IHC study.

2.5 | Organ relative weights

At the end of the study, rats were euthanised and organs were dissected. Testes are removed and weighed. The organ relative weights (organ weight/body weight × 100) were measured for each rat in treated and control groups.

2.6 | Semen analyses

To obtain the sperm count, the entire epididymis from the rat was minced in PBS media and incubated for 5 min at 37°C. The sperm concentration was determined by manual evaluation using a hemocytometer (Neubauer); (Padmanabhaiah, 2002).

For assessment of sperm motility, spermatozoa were recovered from the excised caudal epididymis and allowed to capacitate for 5 min in PBS media containing at 37C. Spermatozoa were scored as motile if any movement was detected. The total number of spermatozoa, the number of motile spermatozoa, velocity of spermatozoa, type of motility

and the percentage of motile spermatozoa in each treated group were calculated by computer-assisted sperm analysis (CASA); (Amann & Waberski, 2014). One drop of sperm suspension added onto the end of glass slide then smeared and subject to Eosin-nigrosin-stain procedure (purchased from Biodiagnostic, Egypt) to determine sperm abnormality.

2.7 | Biochemical analyses

Testicular tissue was weighed and homogenised to make 10% homogenate (w/v) in ice-cold 0.1 M Tris (hydroxymethyl) aminomethane-HCl (Tris-HCl), pH 7.4, using an ice-chilled glass-homogenising vessel in a homogeniser fitted with Teflon pestle (Glas-Col, USA). The homogenate was then centrifuged in a refrigerated centrifuge at 2,000 × *g* at 4°C for 15 min to remove nuclei and debris. The supernatant obtained was used as a sample for the following assays: oxidative stress (MDA, NO, GSH, GSSG) by Agilent HP 1100 series HPLC apparatus (USA).

2.8 | Determination of nitrite and nitrate by HPLC

Sodium nitrite and sodium nitrate used for the reference standard preparation with stock concentration 1 mg/ml. A standard mixture of nitrite and nitrate was used to determine the retention times and separation of the peaks at 230 nm with a mixture of 0.1 M NaCl–CH_3OH, at a volume ratio 45:55. Nitrite and nitrate concentrations were equal in the mixture solution; Nitrites and nitrate were determined according to the method of Papadoyannis, Samanidou, and Nitsos (1999).

2.9 | Determination of MDA by HPLC

1, 1, 3, 3-tetra ethoxy propane was purchased from Sigma (St. Louis, MO, USA); a precursor of malonaldehyde (MDA) was used as a standard for this assay. The amount of TBARS formed was measured by HPLC at 250 nm. The mobile phase was 82.5:17.5 (v/v) 30 mM monobasic potassium phosphate (pH, 3.6)—methanol according to the method of (Karatepe, 2004).

2.10 | Determination of GSH and GSSG by HPLC

The thiol compound of oxidised and reduced glutathione purchased from Sigma was detected by HPLC with UV detector at 210 nm using the method of (Jayatilleke & Shaw, 1993).

2.11 | Determination of ATP, ADP, AMP by HPLC

The ATP, ADP and AMP high purity standards, perchloric acid, potassium dihydrogen phosphate, potassium hydrogen phosphate and acetonitrile (HPLC grade) were purchased from Sigma Chemical Co. ATP, ADP and AMP standards (1 mg) were each dissolved in 10 ml of deionised water to obtain ATP, ADP and AMP standard stock solutions at 100 mg/m. A volume of 20 µl of each sample was taken for HPLC analysis at 254 nm using Ultrasphere ODS EC 250 × 4.60 mm column. Mobile phase A consisted of 0.06 mol/L di-potassium hydrogen phosphate and 0.04 mol/L potassium di-hydrogen phosphate dissolved in deionised water and adjusted to pH = 7.0 with 0.1 mol/L potassium hydroxide, while mobile phase B consisted of 100% acetonitrile. The elution programme was as follows: 0 min 100% A, 0% B; 2 min 95% A, 5% B; 4 min 80% A, 20% B; 5.3 min 75% A, 25% B and 6 min 100% A, 0% B. Finally, the programme took a further 1 min to return to the initial conditions and stabilise. Flow rate of the mobile phase was 1.2 ml/min (Hai, Yueming, Yunbo, & Weibo, 2006).

2.12 | Determination of testis 8-OHDG by HPLC

Isolation and hydrolysis of testis DNA were performed using the method of Lodovici, Casalini, Briani, and Dolara (1997). The hydrolysed mixture was centrifuged, and the supernatant was injected into the HPLC. The separation of 8-OHDG was performed with an LC/Agilent 1200 series HPLC apparatus (USA) using UV detectors. For chromatographic separation, we used C18 reverse phase columns in series (Supelco, 5 pm, I.D. 0.46 × 25 cm); the eluting solution was H_2O/CH_3OH (85:15 v/v) with 50 mM KH_3PO_4, pH 5.5 at a flow rate of 0.68 ml/min. The UV detector was set at 245 nm. The resulting chromatogram identified the concentration from the sample as compared to that of the standard purchased from Sigma-Aldrich.

2.13 | Immunohistochemical staining of caspase-3

The paraffinic testis was cut into 5-µm sections and launched on positively charged slides for caspase-3 IHC. Sections were dewaxed, rehydrated and autoclaved at 120°C for 10 min in 10 mM citrate buffer (pH 6). Wash used PBS (pH 7.2) and then added 0.3% H_2O_2 in methanol for 15 min for blocking endogenous peroxidase. Slides washed again in PBS, and blocking was completed by adding blocking buffer. Incubate for 30 min at room temperature. Caspase-3 Polyclonal Antibody (Cat. No. PA1-29157, Thermo Fisher Scientific Co., USA) was added after dilution by PBS (1:1,000) and incubated for 30 min. The slides were washed three times with wash buffer each for 3 min. Secondary antibody (Cat. No. 61-9520, Thermo Scientific Co., USA) was applied to tissue sections and incubated for 30 min. The slides were washed three times for 3 min with wash buffer. Metal Enhanced DAB Substrate Working solution was added to the tissue and incubated 10-min wash two times for 3 min each with wash buffer. An adequate amount of haematoxylin stain was added to the slide to cover the entire tissue surface for counterstaining (Bancroft & Cook, 1994).

2.14 | Statistical analysis

Statistical analysis of the obtained data was performed using the general linear model (GLM) produced by Statistical Analysis Systems Institute (SAS, 1989). Significant differences among means were evaluated using Duncan's Multiple Range Test.

The following linear model was applied:

$$Y_{ij} = \mu + \alpha_i + \xi_{ij},$$

Y_{ij} = Observation measured, μ = Overall mean, α_i = Effect of treatment, ξ_{ij} = Experimental error assumed to be randomly distributed ($\sigma^2 = 0$).

3 | RESULTS

As shown in Table 1, body weight, testes weight and relative testes weight were significantly decreased ($p < .05$) following administration of BU compared to the control group. However, treatment with L-arginine and L-carnitine significantly showed enhancement in the recorded of body weight, testes weight and relative testes weight compared to BU group.

As shown in Figure 1 (a–f), motility, sperm count, progressive motility, DAB and VAP were significantly decreased ($p < .05$) following administration of BU compared to the control group. However, treatment with L-arginine and L-carnitine significantly showed improvement in the recorded of motility, sperm count, abnormality, progressive motility, DAB and VAP compared to BU group.

As shown in Table 2, MDA, GSSG, NO and 8HDG were significantly increased except GSH significantly decreased ($p < .05$) following administration of BU compared to the control group. However, treatment with L-arginine and L-carnitine significantly showed enhancement in the recorded of oxidative stress markers compared to BU group.

TABLE 1 Effect of L-arginine and L-carnitine against busulfan induces oligospermia on body weight, testes weight and relative testes weight in male rats

Parameters	Groups			
	CON	BU	Bu + L-arg	Bu + L-car
Body weight (gm)	237.00 ± 12.90	208.50 ± 23.02	227.00 ± 12.73[a]	225.83 ± 7.94[a]
Testes weight (gm)	2.43 ± 0.43	0.47 ± 0.1*	2.24 ± 0.25[a]	2.26 ± 0.22[a]
Testes to body weight ratio (%)	1.07 ± 0.16	0.22 ± 0.07*	0.94 ± 0.08[a]	1.04 ± 0.05[a]

Data represents the means ± *SD*.
Significant difference from control group *$p < .05$, Significant difference between Bu + L-arg and Bu + L-car versus busulfan [a]$p < .05$.

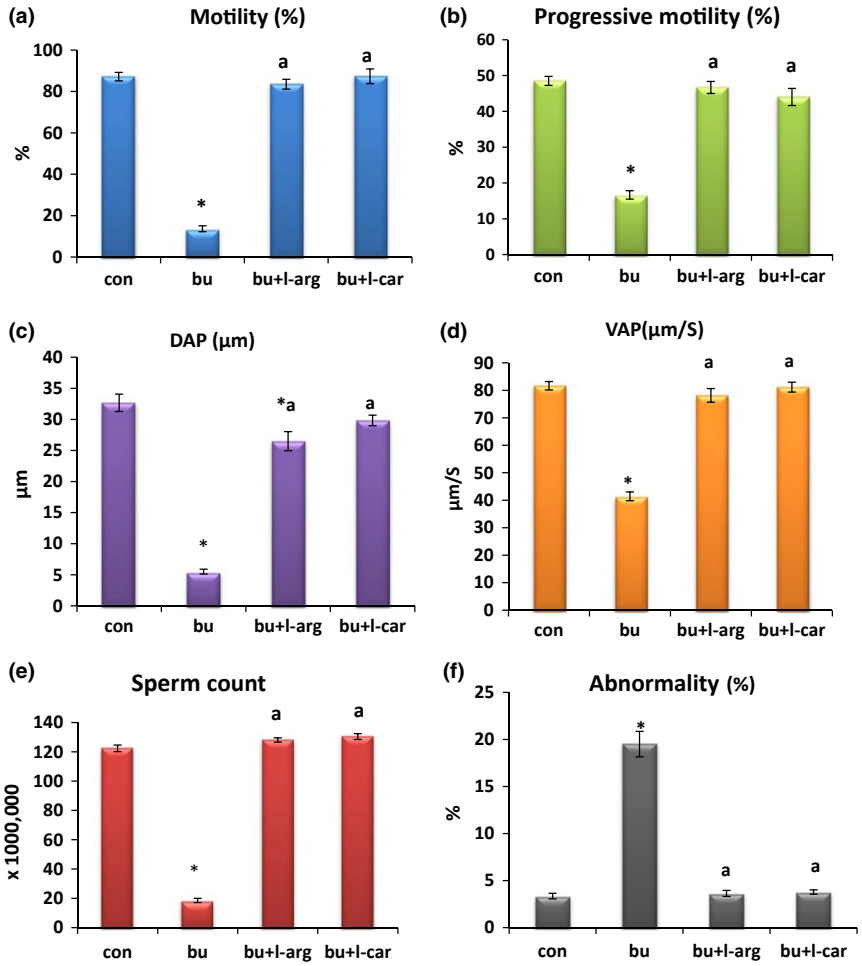

FIGURE 1 (a–f) Effect of oral administration of L-arginine and L-carnitine for 28 day on semen quality parameters (motility, progressive motility, distance average path (DAB) (µm), velocity average path (VAP) (µm/s), sperm count and abnormality). Significant difference from control group *$p < .05$, Significant difference between Bu + L-arg and Bu + L-car versus busulfan [a]$p < .05$

As shown in Table 3, AMP and ADP were significantly increased except ATP significantly decreased ($p < .05$) following administration of BU compared to the control group. However, treatment with L-arginine and L-carnitine significantly showed enhancement in the recorded of cell energy markers compared to BU group (Figures 2 and 3).

4 | DISCUSSION

Cancer is one of the major causes of death, in the face of that many cases recovers from cancer. Anticancer drugs are useful in cancer treatment, but these are accompanied by its side effects. The adverse effects of these anticancer drugs on the male fertility are often severe and may lead to male sterility. So, many studies have been developing medication or natural supplements to relieve or reduce its side effects (Jung et al., 2015). Busulfan is an anticancer drug, but it has a cytotoxic effect that showing elicited various adverse effects in the male reproductive system.

In this study, busulfan injection caused a reduction in numbers, motility and speed of spermatozoa. Busulfan also showed a reduction in testes weights, and increase in both oxidative stress parameters and DNA metabolites. These results confirmed with the previous studies (Monsefi et al., 2013; Mirhoseini et al., 2014). Busulfan-treated group also showed apoptotic effect that indicated by positive expression of caspase-3 in testis tissue. This result was in agreement with Ohira, Saito, Ando, Tamura, and Hoshiya (2014). Busulfan infertility may be attributed to release of free radicals. Free radicals affected sperm genome and caused permanent lethal sperm mutations (Dehghani et al., 2013). Another mechanism was suggested that busulfan elevated Sertoli cell marker (ck18) that caused sperm abnormality (Ahar, Khaki, Akbari, & Ghafarinovin, 2014).

In the previous vitro study, the busulfan caused a reduction in cell proliferation and obstruction of cell life that lead to apoptosis and cleavage of caspase 3 (Hassan, Hassan, & Hellstrom-Lindberg, 2001).

On the other hand, the administration of L-Arginine and L-carnitine in the present study caused amelioration in semen parameters as well as oxidative stress parameters. Reduction in expression of caspase-3 in testes tissues also evaluated in L-Arginine and L-carnitine-treated groups. L-Arginine is a nitric oxide precursor, it plays very complicated role in regulation of cell energy, and all body homoeostasis. Arginine also has antioxidant ability as it scavenge superoxide radical and suppress the oxidation process (Jarad, Al-samawy, & Al-Badran, 2011). NO also increase guanosine monophosphate production by activating soluble guanylyl cyclase which in turn increase sperm viability and motility (Kobori et al., 2015). NO play a vital role in mitochondrial biogenesis, respiration and reconstruction; it increases oxygen and substrates that needed by mitochondria (Buzadzic et al., 2015). Another study revealed that L-Arginine decreased apoptotic process by inhibiting expression of caspase-3 that leading to suppress DNA fragmentation (Piacenza, Peluffo, & Radi, 2001).

In the recent study, the improvement of semen quality that showed in L-Arginine treated group may be due to increasing mitochondrial energy production. While the higher level of NO that observed in testes homogenate of busulfan-treated rats are attributed to the reduction in sperm numbers and metabolism, this finding is supported by Eskiocak et al. (2006).

L-carnitine treatment has pronounced protective effect against busulfan infertility. In our study, L-carnitine treatment improved semen, oxidative stress and cell energy parameters. In addition, L-carnitine-treated group showed negative expression of caspase-3. The

TABLE 2 Effect of oral administration of L-arginine and L-carnitine for 28 days on MDA, GSH, GSSG, NO and 8HDG

Parameters	CON	BU	Bu + L-arg	Bu + L-car
MDA (nmol/g tissue)	17.08 ± 1.37	29.93 ± 3.6*	21.63 ± 0.83*[a]	22.68 ± 1.49*[a]
GSH (μmol/g tissue)	13.83 ± 0.88	6.87 ± 0.46*	9.72 ± 2.3*[a]	11.71 ± 0.51*[a]
GSSG (μmol/g tissue)	0.95 ± 0.19	1.78 ± 0.15*	1.12 ± 0.07*[a]	0.98 ± 0.12[a]
NO (μmol/g tissue)	13.75 ± 1.75	17.28 ± 1.73*	12.49 ± 1.29	12.19 ± 2.04
8HDG (pg/g tissue)	281.21 ± 27.72	460.86 ± 56.51*	318.27 ± 21.15[a]	313.75 ± 19.93[a]

Data represents the means ± *SD*.
Significant difference from control group *$p < .05$, significant difference between Bu + L-arg and Bu + L-car versus busulfan [a]$p < .05$.

TABLE 3 Effect of oral administration of L-arginine and L-carnitine for 28 days on ATP, AMP and ADP

Parameters	CON	BU	Bu + L-arg	Bu + L-car
ATP (μmol/g tissue)	16.14 ± 1.76	10.16 ± 0.80*	12.80 ± 0.88*[a]	12.05 ± 1.12*[a]
ADP (μmol/g tissue)	7.63 ± 1.19	9.53 ± 0.56*	7.35 ± 0.54[a]	7.07 ± 0.90[a]
AMP (μmol/g tissue)	9.24 ± 1.3	14.04 ± 0.244*	10.92 ± 0.46*[a]	10.93 ± 0.83*[a]

Data represents the means ± *SD*.
Significant difference from control group *$p < .05$, significant difference between Bu + L-arg and Bu + L-car versus busulfan [a]$p < .05$.

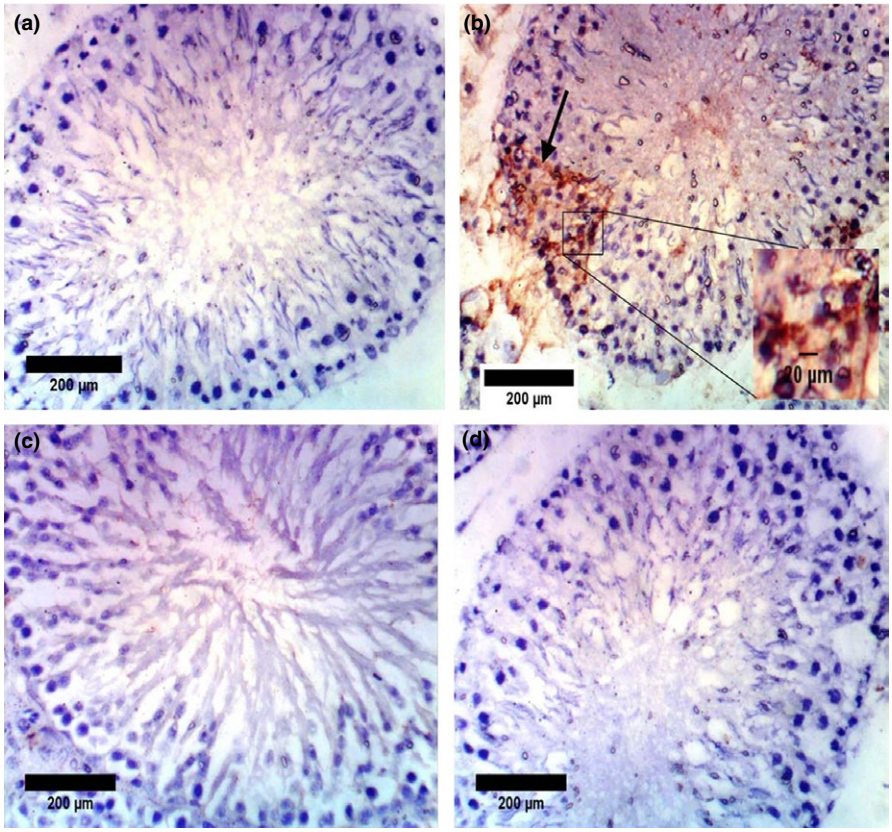

FIGURE 2 (a–d) Showing effect of oral administration of L-arginine and L-carnitine for 28 days on immunohistochemical expression of caspase-3 in male rat. (a) Immunohistochemical staining of caspase-3 in testis of rat from control group showing no expression of caspase-3 (negative immunohistochemical reaction). (b) Immunohistochemical staining of caspase-3 in testis of rat from l-arg group showing no expression of caspase-3 (negative immunohistochemical reaction). (c) Immunohistochemical staining of caspase-3 in testis of rat from Bu group showing strong positive expression of caspase-3 (immunopositivity indicated by brown colour). (d) Immunohistochemical staining of caspase-3 in testis of rat from l-car group showing no expression of caspase-3 (negative immunohistochemical reaction)

protective effect of L-carnitine may be due to its antioxidant activity, it protected the plasma membrane of spermatozoa against oxidative stress due to the toxic materials via removing the toxic acetyl-coA and substitute fatty acid in cell membrane (Vicari & Calogero, 2001). However, spermatozoa can use many substrates as an energy source, but fatty acid is the major energy source (Abdel-Hamed, El-Sayed, Iraqi, Saad, & Radwan, 2014). Acetylcarnitine suggested being energy source for spermatozoa; it produced in large amount in epididymis fluid by oxidation of fatty acid in the presence of large reservoir of L-carnitine (Ahmed et al., 2011). L-carnitine also provides Krebs cycle with free coA which essential for its regulation (Mazzilli et al., 1999). In agreement with our finding, Lenzi et al. (2004) suggested that

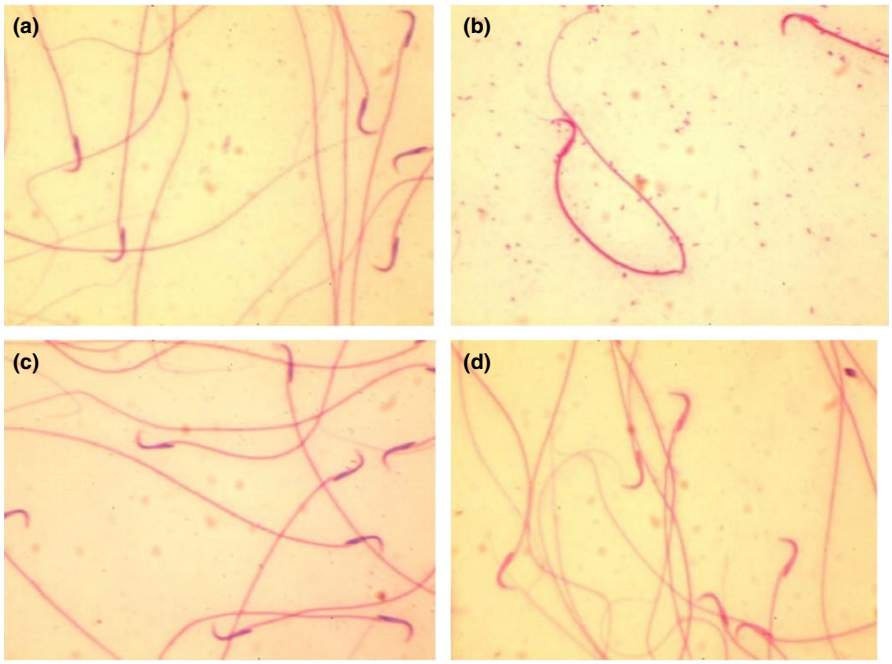

FIGURE 3 (a-d) Showing the microscopic examination of spermatozoa of rats treated with L-arginine and L-carnitine for 28 days. (a) Sperm cells of the control group that are seen to have normal head and tail, (b) Sperm cells of the BU group that are seen to have decreased in number of sperm cells with abnormal head and tail, (c) Sperm cells of the l-arg group that are seen to have normal head and tail. (d) sperm cells of the l-car group that are seen to have normal head and tail

L-carnitine caused improvement of sperm count and quality due to decrease phagocytosis of gametes and increase the number of spermatids. Another study revealed that l-carnitine enhances sperm viability and quality of chromatin by increases sperm glucose up taking as a result of its antioxidant activity (Aliabad et al., 2012).

CONCLUSION

In conclusion, it is recommended that coadministration of L-arginine or L-carnitine with busulfan attenuates the adverse effect of this drug on male fertility and protect the semen as well as testis tissue against the cytotoxic effect of busulfan.

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REFERENCES

Abdel-Hamed, O., El-Sayed, A. I., Iraqi, M. M., Saad, A. M., & Radwan, A. A. (2014). Comparative evaluation of different herbal formula and L-Carnitine on reproductive performance of male Moshtohor rabbits. In *2nd International conference on biotechnology applications in agriculture (ICBAA)*, (April), pp. 8–12.

Agarwal, A., & Said, T. M. (2004). Carnitines and male infertility. *Reproductive Biomedicine Online*, *8*(4), 376–384.

Ahar, N. H., Khaki, A., Akbari, G., & Ghafarinovin, M. (2014). The effect of busulfan on body weight, testis weight and MDA enzymes in male rats. *International Journal of Women's Health and Reproduction Sciences*, *2*(5), 316–319. doi:10.15296/ijwhr.2014.52

Ahmed, S. D. H., Karira, K. A., Jagdesh., & Ahsan, S. (2011). Role of l-carnitine in male infertility. *Journal of the Pakistan Medical Association*, *61*(8), 732–736.

Amann, R. P., & Waberski, D. (2014). Computer-assisted sperm analysis (CASA): Capabilities and potential developments. *Theriogenology*, 1;*81*(1), 5–17. doi:10.1016/j.theriogenology.2013.09.004

Aliabad, E., Mehranjani, M. S., Borzoei, Z., Talaei-Khozani, T., Mirkhani, H., & Tabesh, H. (2012). Effects of L-carnitine and L-acetyl-carnitine on testicular sperm motility and chromatin quality. *Iranian Journal of Reproductive Medicine*, *10*(2), 77–82. PMCID: PMC4163266

Bancroft, J. D., & Cook, H. C. (1994). Manual of histological techniques and their diagnostic application. Retrieved from https://books.google.com/books?id=R0prAAAAMAAJ&pgis=1.

Buzadzic, B., Vucetic, M., Jankovic, A., Stancic, A., Korac, A., Korac, B., & Otasevic, V. (2015). New insights into male (in)fertility: The importance of NO. *British Journal of Pharmacology*, *172*(6), 1455–1467. doi:10.1111/bph.12675

Chiu, M. N., Blackman, M. R., Wang, C., & Swerdloff, R. S. (2004b). The role of carnitine in the male reproductive system. *Annals of the New York Academy of Sciences*, *1033*, 177–188. https://doi.org/10.1196/annals.1320.017

Dehghani, F., Hassanpour, A., Poost-Pasand, A., Noorafshan, A., & Karbalay-Doust, S. (2013). Protective effects of L-carnitine and homogenized testis tissue on the testis and sperm parameters of busulfan-induced infertile male rats. *Iranian Journal of Reproductive Medicine*, *11*(9), 693–704. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3941323&tool=pmcentrez&rendertype=abstract.

Eskiocak, S., Gozen, A. S., Taskiran, A., Kilic, A. S., Eskiocak, M., & Gulen, S. (2006). Effect of psychological stress on the L-arginine-nitric oxide pathway and semen quality. *Brazilian Journal of Medical and Biological Research*, *39*(5), 581–588. https://doi.org/10.1590/s0100-879x2006000500003

Ghanbarzadeh, S., Garjani, A., Ziaee, M., & Khorrami, A. (2013). Effects of L-carnitine and coenzyme Q10 on impaired spermatogenesis caused by isoproterenol in male rats. *Drug Research*, *64*(9), 449–453. doi:10.1055/s-0033-1361103

Hai, L., Yueming, J., Yunbo, L., & Weibo, J. (2006). A simple and rapid determination of ATP, ADP and AMP concentrations in pericarp tissue of litchi fruit by high performance liquid chromatography. *Food Technology and Biotechnology*, *44*(4), 531–534.

Hassan, Z., Hassan, M., & Hellstrom-Lindberg, E. (2001). The pharmacodynamic effect of busulfan in the P39 myeloid cell line in vitro. *Leukemia*, *15*(8), 1240–1247. doi:10.1038/sj.leu.2402193

Houot, M., Poinsignon, V., Mercier, L., Valade, C., Desmaris, R., Lemare, F., & Paci, A. (2013). Physico-chemical stability of busulfan in injectable solutions in various administration packages. *Drugs in R and D*, *13*(1),87–94. doi:10.1007/s40268-013-0003-y

Iwamoto, T., Hiraku, Y., Oikawa, S., Mizutani, H., Kojima, M., & Kawanishi, S. (2004). DNA intrastrand cross-link at the 5′-GA-3′ sequence formed by busulfan and its role in the cytotoxic effect. *Cancer Science*, *95*(5), 454–8. doi:10.1111/j.1349-7006.2004.tb03231.x

Jarad, A. S., Al-samawy, E. R. M., & Al-Badran, A. S. H. (2011). Effect of L-arginine on spermatogenesis of the diabetic rat. *Basrah Journal of Veterinary Research*, *10*(2), 19–32.

Jayatilleke, E., & Shaw, S. (1993). A high-performance liquid chromatographic assay for reduced and oxidized glutathione in biological samples. *Analytical Biochemistry*, *214*(2), 452–7. doi:10.1006/abio.1993.1522

Jung, S.-W., Kim, H.-J., Lee, B.-H., Choi, S.-H., Kim, H.-S., Choi, Y.-K., … Nah, S.-Y. (2015). Effects of Korean Red Ginseng extract on busulfan-induced dysfunction of the male reproductive system. *Journal of Ginseng Research*, *39*(3), 243–249. doi:10.1016/j.jgr.2015.01.002

Karatepe, M. (2004). Simultaneous determination of ascorbic acid and free malondialdehyde in human serum by HPLC – UV. *LCGC Asia Pacyfic*, *7*(2), 7–9.

Khosoroshahi, A. F., Solimanirad, J., Jameie, S. B., Bakhtiari, M., Kruji, S. M., Roshangar, L., & Joghataei, M. T. (2012). An investigation into the effect of exogenous melatonin on spermatogenesis of pinealectomised busulfan induced oligospermic adult rats, 2008-2009. *Journal of Kermanshah University of Medical Sciences*, *16*(6), 435–444.

Kobori, Y., Suzuki, K., Iwahata, T., Shin, T., Sadaoka, Y., Sato, R., … Rohdewald, P. (2015). Improvement of seminal quality and sexual function of men with oligoasthenoteratozoospermia syndrome following supplementation with L-arginine and Pycnogenol?? Archivio Italiano Di Urologia, Andrologia: Organo Ufficiale [Di] Societ?? *Italiana Di Ecografia Urologica E Nefrologica/Associazione Ricerche in Urologia*, *87*(3), 190–193. doi:10.4081/aiua.2015.3.190

Lenzi, A., Sgro, P., Salacone, P., Paoli, D., Gilio, B., Lombardo, F., … Gandini, L. (2004). A placebo-controlled double-blind randomized trial of the use of combined l-carnitine and l-acetyl-carnitine treatment in men with asthenozoospermia. *Fertility & Sterility*, *81*(6), 1578–1584.

Li, M., Fraser, T., Wang, J., & Whitworth, J. A. (1997). Dexamethasone-induced hypertension in the rat: Effects of L-arginine. *Clinical and Experimental Pharmacology & Physiology*, *24*(9–10), 730–732.

Lodovici, M., Casalini, C., Briani, C., & Dolara, P. (1997). Oxidative liver DNA damage in rats treated with pesticide mixtures. *Toxicology*, *117*(1), 55–60. doi:10.1016/S0300-483X(96)03553-6

Mazzilli, F., Rossi, T., Ronconi, C., Germini, B., & Dondero, F. (1999). [Intra-spermatic L-carnitine and survival of sperm motility]. *Minerva Ginecologica*, *51*(4), 129–134.

Miguel-Carrasco, J. L., Mate, A., Monserrat, M. T., Arias, J. L., Aramburu, O., & Vázquez, C. M. (2008). The role of inflammatory markers in the cardioprotective effect of L-carnitine in L-NAME-induced hypertension.

American Journal of Hypertension, *21*(11), 1231–1237. https://doi.org/10.1038/ajh.2008.271\rajh2008271 [pii]

Mirhoseini, M., Saki, G., Hemadi, M., Khodadadi, A., & Mohammadi Asl, J. (2014). Melatonin and testicular damage in busulfan treated mice. *Iranian Red Crescent Medical Journal*, *16*(2), e14463. https://doi.org/10.5812/ircmj.14463

Monsefi, M., Fereydouni, B., Rohani, L., & Talaei, T. (2013). Mesenchymal stem cells repair germinal cells of seminiferous tubules of sterile rats. *Iranian Journal of Reproductive Medicine*, *11*(7), 537–544. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3941348&tool=pmcentrez&rendertype=abstract

Ohira, T., Saito, T., Ando, R., Tamura, K., & Hoshiya, T. (2014). Systemic histopathology of infant rats exposed to busulfan. *Journal of Toxicologic Pathology*, *27*(1), 25–29. doi:10.1293/tox.2013-0043

Padmanabhaiah, S. K. (2002). Effect of ribavirin on epididymal sperm count in rat (April 2016).

Papadoyannis, I. N., Samanidou, V. F., & Nitsos, C. C. (1999). Simultaneous determination of nitrite and nitrate in drinking water and human serum by high performance anion-exchange chromatography and UV detection. *Journal of Liquid Chromatography & Related Technologies*, *22*, 2023–2041. doi:10.1081/JLC-100101783

Piacenza, L., Peluffo, G., & Radi, R. (2001). L-arginine-dependent suppression of apoptosis in Trypanosoma cruzi: Contribution of the nitric oxide and polyamine pathways. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(13), 7301–7306. doi:10.1073/pnas.121520398

Tripathi, P., & Misra, M. K. (2009). Therapeutic role of L-arginine on free radical scavenging system in ischemic heart diseases. *Indian Journal of Biochemistry & Biophysics*, *46*, 498–502. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20361713.

Vicari, E., & Calogero, A. E. (2001). Effects of treatment with carnitines in infertile patients with prostato-vesiculo-epididymitis. *Human Reproduction*, *16*(11), 2338–2342.

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