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Research paper

Protective role of ginseng extract against oxidative stress, reproductive and some biochemical parameters alterations induced by doxorubicin in male rats

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Abstract

This study investigates the modulating effect of ginseng against testicular toxicity, oxidative stress and changes in some biochemical parameters induced by doxorubicin. Twenty male rats were divided into four groups. The 1st group received distilled water orally (control group), The 2nd group received doxorubicin (5 mg/kg b.wt. intrapertenoineal) once a week for eight weeks, The 3rd group received ginseng extract (200 mg/kg b.wt.) daily for eight weeks and the 4th group received doxorubicin with ginseng extract by the same doses as in the 2nd and the 3rd groups respectively. At the end of the 8th week, blood and semen samples were taken for biochemical and semen analysis, respectively. The doxorubicin treated group had significantly higher serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), Creatine kinase (CK) and lactate dehydrogenase (LDH) along with lower levels of total protein, albumin and globulin. In addition, a significant decrease in antioxidant enzymes (SOD, CAT, GSHPx), and glutathione (GSH) associated with higher level of malondialdehyde (MDA) were observed. At the same time, the group that took doxorubicin with ginseng did not differ from control group in terms of these parameters. Male fertility study showed changes in testosterone and semen analysis in both groups treated with doxorubicin, while the group that took doxorubicin with ginseng showed an improvement towards control levels of these parameters. Thus ginseng supplement can reduce the negative effects of doxorubicin- induce.

Keywords: *Antioxidant Status; Biochemical Parameters; Doxorubicin; Ginseng; Semen Analysis.*

1. Introduction

Doxorubicin is antibiotic drug isolated from Streptomyces peucetius var. caesius (Arcamone et al. 1969, Frederick et al. 1990) which is first generation of anthracycline, a potent broad spectrum chemotherapeutic agent used for the treatment of several types of cancer (Sakr et al. 2013, Chen et al. 2016). It is used in treatment of breast, uterine, ovarian, bladder and lung cancers (Marinelo et al. 2018). Meanwhile, doxorubicin induce serious problems such as male infertility due to its harmful effect on testicular tissue, apoptosis and it is also decreases DNA synthesis with toxicities to different organs like kidney, heart and liver (Kato et al. 2001, Shivakumar et al. 2012, Yang et al. 2017). Many studies have been done to reduce doxorubicin toxicity by using numerous physical, chemical and/or biological techniques (Hellmann 1999). It was found that some herbal nutrients reduce the toxic effect of doxorubicin through its antioxidant effect (Shihong et al. 2014). Medicinal herbs were used to treat a wide range of health problems such as cancer (Jimenez et al. 2003). Ginseng is one of the most popular herbs in both Western and Eastern countries which known as a traditional Asian medicine for stimulation of sexual function (Hong et al. 2002). Commonly, Ginseng is used in herbal medicine (Park et al. 2013) as it contains high iodine content and saponins (Jang et al., 2011). Ginseng saponins (ginsenosides) have been studied and found to be responsible for some medicinal effects, specifically antihypertensive, anticancer, anti-stress and anti-diabetic effects (Issa & El-Sherif 2017).

Panax ginseng is one of the most valued medicinal herbaceous plant belonged to the Araliaceae family (Kamel & Lotfy 2006). Panax ginseng (Asian ginseng) is one of three main types of ginseng; the rest are American and Siberian ginseng. Panax ginseng was grown both in Korea and China. There are white Panax and red Panax. Red ginseng is considered more potent than the white (Nair et al. 2012). Clinically, Korean red ginseng enhances sexual functions (Jang et al. 2008).

Hypothesis usage of ginseng extract as antioxidant may reduce the adverse effect that resulted from doxorubicin administration (as anticarcinogenic drug) in rats. The objective of the present work is to evaluate the effect of doxorubicin on male fertility and biochemical parameters in rats and modulating effect of ginseng extract.

2. Material and methods

2.1. Drug

2.1.1. Doxorubicin (Adricin) A product of EIMC united pharmaceuticals, Egypt (50 mg doxorubicin hydrochloride/ vial)

2.1.2. Ginseng Extract (Korean red ginseng extract) was obtained from Pharco Pharmaceuticals, Alexandria, Egypt, each capsule contain 100 mg of ginseng extract

2.2. Experimental rats and experimental design

Twenty male albino rats, 5months old, 180-200g body weight were used in this study. Rats were housed in metallic cages under hygienic condition and divided into 4 groups (5rats/each). The 1st group received distilled water orally (control group), the 2nd group (doxorubicin group) received doxorubicin (5 mg/kg b.wt. intraperitoneal) once a week for eight weeks (Brilhante et al. 2011), the 3rd group (ginseng group) received 200mg ginseng extract/kg b.wt. using stomach tube daily for eight weeks (Lee. et al., 2019), 4th group received doxorubicin and ginseng extract by the same dosage of 2nd group and 3rd group respectively. At the end of experiment, blood samples obtained in plain tubes to separate serum for biochemical analysis, antioxidant capacity evaluation and testosterone level determination. After that, rats were scarified and dissected to obtain epididymis for semen analysis.

2.3. Biochemical evaluation

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to (Reitman & Frankel 1957), Lactate dehydrogenase (LDH) was determined according to (Buhl & Jackson 1978), Creatinine kinase (CK) was determined according to (Horder & Elsner 1991), Total protein was determined according to (Doumas et al. 1981), Albumin was determined according to (Bauer 1982) , Globulin was calculated as follow: Globulins =total protein –albumin.

2.4. Antioxidant state evaluation

Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GSH-Px) were determined according to Nishikimi et al. (1972), Sinha (1972) and Paglia & Valentine (1967), respectively. Glutathione (GSH) concentration was determined according to (Beutler et al. 1963). Malondialdehyde (MDA) was determined according to (Nielsen et al. 1997).

2.5. Male fertility evaluation

Content of epididymis tail of each rat was collected by squeezed gently in sterile watch glass containing 1ml sodium citrate solution 2.9% for semen analysis according to (Bearden & Flaquary 1980). Serum testosterone hormone level was estimated by radioimmunoassay according to (Wilson & Foster 1992).

2.6. Statistical analysis

The obtained data was analyzed by using computerized SPSS program (version 25) according to Tambane &Dunlop (2000).

3. Results

3.1. Biochemical results

Regarding the changes in biochemical parameters, there was a significant increase in AST, ALT, LDH and CK activities along with a significant decrease in total protein, albumin and globulin levels in doxorubicin group in comparison with control group. Meanwhile, there was a significant decrease in AST, ALT, LDH and CK activities associated with non-significant changes in total protein and albumin levels in doxorubicin plus ginseng group when compared with doxorubicin group. Interestingly, non-significant changes were reported in total protein, albumin and globulin levels in doxorubicin plus ginseng group in comparison with control group. Albumin/Globulin ratio showed non-significant changes between different groups. Ginseng treated group showed non-significant changes in enzymes activities under investigation and protein profile when compared to control group as shown in Table 1.

3.2. Antioxidant state results

Concerning to antioxidant capacity, there was a significant increase in MDA level accompanied with a significant decrease in GSH concentration and CAT, SOD and GSH-Px activities in doxorubicin group in comparison with control group. In doxorubicin plus ginseng group, there was a significant increase in SOD activity and a significant decrease in MDA level along with non-significant changes in GSH concentration, CAT and GSH-Px activities when compared with doxorubicin group. At the same time, there were non-significant changes in MDA level, GSH concentration and CAT, SOD, and GSH-Px activities in doxorubicin plus ginseng group in comparison with control group. Importantly, Ginseng treated group showed a significant increase in GSH concentration and CAT, SOD, and GSH-Px activities along with non-significant changes in MDA level when compared with control group as shown in Table 2.

3.3. Male fertility results

With respect to semen analysis and testosterone hormone level, doxorubicin group showed a significant decrease in sperm count, sperm motility percent, live sperm percent and testosterone level along with a significant increase in total sperm abnormality percent when compared to control group. On the other hand, there was a significant increase in sperm count, sperm motility percent and live sperm percent associated with a significant decrease in total sperm abnormality percent in doxorubicin plus ginseng group in comparison with

doxorubicin group. Testosterone level in doxorubicin plus ginseng group showed non-significant changes when compared with control group and doxorubicin group. Importantly, the rats treated with ginseng alone showed a significant increase in sperm count, sperm motility percent, live sperm percent and testosterone level accompanied with a significant decrease in total sperm abnormality percent when compared with control group as shown in Table 3.

Table 1: Serum Biochemical Parameters (Mean±SE) in Doxorubicin, Ginseng and Combined Groups after Eight Weeks

	Control	Doxorubicin	Ginseng	Doxorubicin + Ginseng
AST (u/l)	78.32±1.38^{b}	94.53±1.53^{a}	76.78±1.21^{b}	83.12±1.59^{b}
ALT (u/l)	79.56±1.15^{b}	93.24±1.54^{a}	75.83±1.67^{b}	86.33±1.89^{b}
LDH (u/l)	168.17±2.16^{b}	226.06±2.62^{a}	164.21±2.32^{b}	176.23±2.54^{b}
CK (u/l)	132.34±3.22^{b}	165.87±4.44^{a}	129.16±3.59^{b}	140.23±3.73^{b}
Total Protein (g/dl)	6.67±0.32^{ab}	4.16±0.25^{c}	7.83 ±0.89^{a}	5.67±0.61^{bc}
Albumin (g/dl)	3.85±0.32^{b}	2.32±0.35^{c}	4.37±0.55^{a}	3.21±0.96^{bc}
Globulin (g/l)	2.82±0.44^{ab}	1.84±0.55^{c}	3.46±0.32^{a}	2.46±0.33^{b}
A/G ratio	1.37±0.24^{a}	1.26±0.20^{a}	1.26±0.18^{a}	1.30±0.22^{a}

Means in the same row with different superscript letters (a, b, c) are significantly different at P <0.05.

Table 2: MDA, CAT, SOD, GSH and GSH-Px in Doxorubicin, Ginseng and Combined Groups after Eight Weeks.

	Control	Doxorubicin	Ginseng	Doxorubicin + Ginseng
MDA (µmol/L)	15.83±0.75^{bc}	24.45±0.94^{a}	11.32±0.45^{c}	19.89±0.73^{b}
CAT (u/ml)	23.57±1.21^{b}	11.34±1.27^{c}	27.35±1.81^{a}	19.98±1.41^{bc}
SOD (u/ml)	84.62±0.83^{b}	69.36±0.89^{c}	99.43±0.64^{a}	81.32±0.78^{b}
GSH (mmol/L)	79.14±4.51^{b}	58.35±4.73^{c}	93.45±3.92^{a}	69.55±5.84^{bc}
GSHPx (u/ml)	97.14±5.31^{b}	77.21±3.62^{c}	112.26±3.4^{a}	88.22±4.73b^{c}

Means in the same row with different superscript letters (a, b, c)are significantly different at P < 0.05.

Table 3: Semen Analysis and Testosterone Level in Doxorubicin, Ginseng and Combined Groups after Eight Weeks.

	Control	Doxorubicin	Ginseng	Doxorubicin + Ginseng
Sperm count (x10^{6}/ml)	1.95±0.46^{b}	1.36±0.41^{c}	2.34±0.45^{a}	2.09±0.48^{b}
Sperm motility (%)	80.87±0.89^{b}	65.05±0.38^{c}	88.44±0.58^{a}	78.26±0.54^{b}
Live sperm (%)	86.123±0.56^{b}	75.47±0.87^{c}	90.55±0.61^{a}	80.34±0.33^{b}
Total abnormality (%)	14.23±0.23^{b}	37.23±0.69^{a}	10.0±0.42^{c}	16.43±0.59^{b}
Testosterone (ng/ml)	3.48±0.56^{b}	1.98±0.82^{c}	4.23±0.62^{a}	2.71±0.45^{bc}

Means in the same row with different superscript letters(a, b, c) are significantly different at P <0.05.

4. Discussion

Concerning biochemical parameters, Rats that received doxorubicin showed significant elevation in AST, ALT, CK and LDH activities. These findings corroborate the findings of previous studies (Mohamed et al. 2015, Abdalla et al. 2016, Jun et al. 2017) in rats received doxorubicin. These result supported by Singh et al. (2008) who showed that doxorubicin induce toxicity and leakage of these enzymes. Increased activities of CK and LDH enzymes may be attributed to destruction of myocardial cells that induced by doxorubicin (Patil & Balaraman 2009, Al-Sowayan & Nadia 2014) as a result of oxidative stress occurred after doxorubicin administration (Fadya et al. 2019, Haybar et al. 2019). Additionally, a significant increase in AST and ALT activities may be due to their increased leakage from damaged and necrotic hepatocytes as a result of doxorubicin toxicity (Injac et al. 2008). Notably, Yagmurca et al. (2007) found that hepatic injury is attributed to doxorubicin ability to produce excess free radicals and lipid peroxides and to suppress free radicals scavenging capacity and antioxidant defensive mechanism.

Ginseng group revealed non-significant reduction in AST, ALT, CK and LDH activities in comparison with control group. Meanwhile, there was a significant decrease in AST, ALT, LDH and CK activities in doxorubicin plus ginseng group when compared with doxorubicin group. These findings are consistent with that obtained by Shihong et al. (2014) who reported that ginseng normalized liver and other organ functions and the serum enzyme activities (AST, ALT, CK and LDH). The obtained results may be due to ginseng protects cell membrane fatty acids from decomposition induced by free radicals (Okada & Zhang 1998). Also, Akash et al. (2018) showed that ginsenoside Re (one constituent of ginseng) protect cardiac cells from oxidative damage as a result of its ability for scavenging of free radicals.

In the present study, doxorubicin induced significant reduction in serum total proteins, albumin and globulins. These results are consistent with (Sridevi 2011, Pugazhendhi et al. 2018, Vesna et al. 2018). Reduction in total protein and albumin may be due to damage of hepatocytes as a result of doxorubicin toxicity (Osama et al. 2019).

Ginseng induced non-significant increase in serum total proteins, albumin, globulins and A/G ratio compared with control group. These results are consistent with (Hess et al. 1982). They found that ginseng induced non-significant elevation in total protein, albumin and globulin. Also, Eskandari et al. (2017) found that ginseng induced non-significant elevation in total protein and globulin in rats. On the other hand, Doxorubicin plus ginseng group showed non-significant changes in total protein and albumin levels in comparison either with control group and doxorubicin group. These results may be due to that ginseng has protective effect on hepatic cells and improved serum albumin (Song et al. 2004).

Regarding antioxidant state, Doxorubicin administrated rats showed significant elevation in MDA beside significant reduction in SOD, CAT, GSH and GSH-Px. Similar results were observed in previous studies (Mohamed et al. 2015, Faten et al. 2018, Haybar et al. 2019). Xipeng et al. (2017) showed that doxorubicin increased MDA coupled with reduction in SOD, CAT, GSH and GSH-Px in rats. These findings may be attributed to the generation of free radicals and the induction of oxidative stress induced by doxorubicin administration (El-Maddawya & Abdel-Naby 2019, Injac et al. 2008).

Rats received ginseng alone showed significant increase in serum SOD, CAT, GSH and GSH-Px associated with non-significant decrease in MDA compared with control group. Similar results were reported by Fu & Ji (2003) and Kyu et al. (2013). Wan et al. (2007) showed that ginseng extracts induce elevated in antioxidant enzymes. Furthermore, these results are consistent, partially, with the previous results by Kim et al. (2016) who showed a reduction in MDA beside increase in SOD, CAT, GSH and GSH-Px in rats administrated with ginseng. Based on human study, Kim & Park (2003) reported that the activity of SOD and CAT increased after administration of ginseng extract for eight weeks.

Doxorubicin plus ginseng group showed non- significant changes in MDA and GSH levels and activities of CAT and SOD enzymes in comparison with control group. Meanwhile, this group revealed a significant increase in SOD activity associated with a significant decrease in MDA level compared with doxorubicin group. These results in accordance, partially, with those reported by Young et al. (2019) who reported that ginseng enhance the activity of the antioxidant enzymes (SOD, CAT, GSH and GSH-Px) on doxorubicin-induced toxicity in rats. The improving effect of ginseng may be explained by what was reported by Kitts et al. (2000) that ginseng extract have the ability to scavenge superoxide radicals and inhibit lipid peroxidation through transition metal chelation.

Concerning male fertility evaluation, Rats that received doxorubicin displayed significant reduction in sperm count, motility present, a live sperm present and testosterone level beside significant elevation in sperm abnormality percent. These results are in consistent with those achieved by El-Maddawya & Abd-ElNaby (2019). They reported a significant decrease in sperm count, motility %, and live sperm %, a significant increase in sperm abnormalities with a significant decrease of the testosterone level in doxorubicin treated rats. These results are supported by Yokochi & Robertson (2004). They reported that doxorubicin induced male infertility. Notably, Hou et al. (2005) found that a single dose of doxorubicin administration to the immature rat resulted in acute cytotoxic effect on testis. In addition, Semet et al. (2017) showed that doxorubicin induced drop in testosterone level leading to reduction in sperm production and sperm characters. The obtained results are in harmony with Vendramini et al. (2010) who showed reduction of sperm concentration and motility and an increase of sperm anomalous forms in doxorubicin-treated rats. They attributed these results to testicular toxicity occurred by doxorubicin that induced apoptosis for mitotically dividing spermatocytes and intermediate spermatogonia.

Rats that received ginseng alone showed significant increase in sperm cell concentration, sperm motility %, and a live sperm % and testosterone hormone coupled with significant decrease in sperm abnormalities %. The obtained results are supported by that obtained by Wan et al. (2007) who recorded that ginseng induced elevation in sperm cell concentration, sperm motility, alive sperm and testosterone hormone in rats. In addition, ginseng increases the production of spermatozoa and testosterone levels (Eskandari et al.

2017). Keeping with this line, Zakai et al. (2011) reported that ginseng improved seminal picture and attributed these results to direct effects of ginseng on the central nervous and reproductive systems. Salvati et al. (1996) and Hosseini et al. (2012) added that ginsenosides has stimulatory effect on spermatogenesis in testis.

Rats that received ginseng plus doxorubicin showed significant increase in sperm count, sperm motility %, live sperm present and testosterone level associated with a significant decrease in sperm abnormality % in comparison with doxorubicin treated rats. These findings are in harmony with that reported by Jong et al. (2002) who reported that Coadministration ginseng may be partially protective against doxorubicin-induced testicular toxicity and fertility in mice. To clarify these results, it is important to note that increases in the levels of antioxidant enzymes leading to improve male fertility and biochemical parameters (El-Saieed 2003). It is worth mention that ginseng is a powerful antioxidant and has an extensive range of functions including induction of spermatogenesis and activation of either glial cell line-derived neurotrophic factor (GDNF) or cyclic adenosine 3´, 5´-monophosphate (cAMP)-responsive element modulator (CREM) in rat testes (Van Kampen et al. 2003, Kang et al. 2006).

5. Conclusion

It could be concluded that doxorubicin affects the male fertility and biochemical parameters. Using antioxidant (ginseng) induces ameliorations in adverse effect of doxorubicin. So, it is good to use antioxidant with doxorubicin.

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