Therapeutic Effects of Pomegranate and Costus Extracts against Testes Toxicity Induced by Carbon Tetrachloride in Adult Albino Rats

Taha, E.A. a* and Al-Osery, H.A.M. b

a Faculty of Science Jazan University, Biology Department, Saudi Arabia.

Abstract

Traditionally various human diseases are treated with Costus (COT) and Pomegranate (POM). In the present study protective effects of costus and pomegranate ethanolic extract were evaluated against CCl4-induced oxidative damages in rat testis. A total of 30 adult male albino rats (140–160 g) were randomly categorized into six equal groups. The first group was control group. Second group received CCl4 1 ml/kg intraperitoneally (50% in olive oil) for 8 weeks (3 times per week). The tested groups (group 3 and 4) were administered either POM or COT with 500mg/kg b.wt by gavage 3 times per week for 4 weeks. While the fifth and sixth groups were the combination CCl4 and two test extracts. The experiment continued for 12 weeks. At the end of the experiment, the animals were euthanized. The testes of all rats were harvested for light microscopic examination. Light microscopy showed vacuolization in the basal portion of the seminiferous tubules in CCl4 treated group. Intercellular dehiscence between the spermatogenic cells was significant in CCl4 treated group compared to control group. Besides, CCl4 caused germ cell loss, inhibition of mitosis, structural deterioration of the Sertoli cells in seminiferous tubules of rat testes, empty germ cells and shrinkage of tubule structures. Supplementation with costus roots and pomegranate extracts (500mg/kg body weight) attenuated the toxicity of CCl4 in testis tissues through improvement in the histological structure towards the normal. The results clearly displayed that costus and pomegranate treatment augments the antioxidants defense mechanism and provides the evidence that it may have a therapeutic role in free radical mediated injury in testes tissue.

Citation:

1. Introduction

Carbon tetrachloride (CCl4) is a colorless, liquid organic compound which has well-known hepatotoxic and nephrotoxic activities (Horn et al., 2006). Scarpelli and Iannaccone (1990) reported that CCl4 used in the fumigation of grains, as an insecticide in agriculture, in dry cleaning and in filling fire extinguishers. Therefore, certain industrial workers are at risk of CCl4 related toxicity. Metabolic activation of CCl4 by cytochrome P450 system to the free radicals is reported to enhance oxidation of lipids, proteins and resulting in widespread membrane damage (Sheweita et al., 2001). Oxidative stress is known to show detrimental effects on the testis function via the induction of peroxidative damage to the plasma membrane (Visser
and Heyns, 2003). CCl4 triggers oxidative damages through free radicals production (Bruckner et al., 2002). Many authors reported that, free radicals of CCl4 bind with polyunsaturated fatty acid (PUFA) of sperm membrane to produce alkoxy and peroxy radicals that, in turn, generate lipid peroxides, that are highly reactive, change sperm concentration, alters hormonal levels, reduces enzyme activity and finally induce injury or necrosis (Sikka et al., 1995; Ogeturk et al., 2005). Free radicals causes reduction in GSH contents and alteration of reproductive hormones, necrosis of spermatocytes/spermatids and degeneration in seminiferous tubules (Guo et al., 2005; Horn et al., 2006).

Plant-based medicines have a respectable position today, especially in developing countries where modern health services are not sufficient. Indigenous remedies are gaining popularity in both rural and urban areas because they are effective, safe and inexpensive. Information collected from ethnic groups or indigenous traditional medicine has played an important role in the discovery of new products from plants as chemotherapeutic agents (Katewa et al., 2004). Medicinal plants have always been a good source to find new remedies for human health problems. Recently, a wide range of these plants have been screened for antioxidant and anticarcinogenic properties (Upadhyay et al., 2010). *Punica granatum L.* (Punicaceae), commonly called pomegranate, recently described as nature’s power fruit, is a plant used in folkloric medicine for the treatment of various diseases (Abdel Moneim, 2011). Negi et al. (2003) reported that pomegranate peel extracts exert anti-oxidant and anti-mutant activities in vitro due to their content of polyphenols (tannins, ellagic and gallic acids). *Saussurea lappa* (Costus) is a traditional herbal medicine that has been used to treat asthma, inflammation, rheumatism, coughs, tuberculosis, and many other diseases (Shah, 1982; Choi et al., 2012). It has also been used as an analgesic, digestive, aphrodisiac, and diuretic (Yaeesh et al., 2010). Previous studies have demonstrated that *S. lappa* possesses several pharmacological properties, such as antioxidative (Saleem et al., 2013), anticancer (Kim et al., 2012) and hepatoprotective effects (Yaeesh et al., 2010).

Due to antioxidant properties of costus and pomegranate, it is being used in the current study to determine whether the compounds showing protective effect against the toxicity caused by CCl4 in reproductive system of male rats.

### 2. Materials and methods

#### Preparation of Ethanolic extract of pomegranate peel (POM) and costus (COT)

The pomegranate peel was manually removed, shade-dried and powdered. Three kilograms of shade dried pomegranate peel and costus rhizomes, coarsely powdered, charged into aspirator bottles and allowed to soak in absolute ethanol (100%) for 72 hours at room temperature. The extracts were filtered, concentrated using rotary vacuum to get the solid mass. The residue yielded were 16.7 %and 11.16% for pomegranate peel and costus respectively. The extract was dissolved in distilled water for oral administration.

#### Experimental plan

Six-week-old male albino rats weighing 160 ± 20 g they were fed on standard laboratory diet with water ad libitum and housed in plastic cage at room temperature and were exposed to natural day and night cycles. The rats were acclimatized to laboratory condition for 7 days before begining of experiment. For chronic toxicity eight week experiment was designed. 30 male albino rats were randomly divided into seven groups (5 rats of each group). Administration of CCl4 (0.5 ml/kg b.w., 40% CCl4/olive oil) was
intraperitoneally (i.p.) twice a week for eight weeks. After CCl4 toxicity, the rats were administered Pomegrenate or costus extracts (500 mg/kg b.w.) orally twice a week for eight weeks.

**Experimental protocol**
Following dosing plan was adapted for the study.

**Group 1:** the normal control received only feed

**Group 2:** Olive oil (0.5 ml/kg b.w., i.p.) + distalled water (0.5 ml/kg b.w. orally)

**Group 3:** ethanolic extract of costus (500 mg/kg b.w., orally)

**Group 4:** ethanolic extract of pomegrenate (500 mg/kg b.w., orally)

**Group 5:** CCl4 twice a week (0.5 ml/kg b.w., i.p., 40% CCl4/olive oil)

**Group 6:** CCl4 twice a week (0.5 ml/kg b.w., i.p.) + costus extract (500 mg/kg b.w., orally)

**Group 7:** CCl4 twice a week (0.5 ml/kg b.w., i.p.) + pomegrenate extract (500 mg/kg b.w., orally)

**Histopathological study of tissue**
At the end of the experimental period (12 weeks), the animals were given Ether anesthesia and dissected from ventral side. Organs were perfused with ice cold saline and excised. Testes of the treated rats were taken and fixed in 10 % formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. These specimens were cleared in xylene, embedded in paraplast and prepared blocks for further microtomy 5–6 μm thin slides were prepared with microtome; wax was removed, stained with hemotoxilin-eosin and photographed under light microscope Lillie (1965).

**Figure 1.** Representative section of control, Pomegrenate and costus treated group (500 mg/kg) group showing normal architecture of the seminiferous tubules. (A) showing, seminiferous tubules(S) associated with complete spermatogenic series (H&E, X200). The peripheral layer of cells is composed of primary spermatocytes (1ry, secondary spermatocytes (2nd ) followed by a zone of spermatids (st) and finally spermatozoa (sz) about to be released into the lumen. (H&E, X400)
**Figure 2.** CCl4 treated group (A) showing vacuolation and deterioration the germinal cells are abnormal and complete destruction is seen in all components of the cells. (B) seminiferous epithelium exhibiting cytoplasmic vacuolization (V). The spermatogonia are shed into the lumina of tubules. (Arrow) and pycnotic nuclei (head arrow), (C) deterioration of seminiferous tubules (arrow) ; germinal layers, basement membrane is absent and desquamated germ cells (black star) in tubular lumen (D) displayed pachytene spermatocytes (arrow), chromatolysis (white star). (H&E, X400)

![Figure 2](image)

**Figure 3.** Testis of CCL4 group receiving Pomegrenate and costus extracts. showing normal appearance of seminiferous tubules (s) with normal spermatids (st) and Spermatozoa (sz). (A) CCl4-pomegrenate group. (B) CCl4- costus group (H&E, X400)

![Figure 3](image)
3. Results

Microscopic assessment of control group revealed normal seminiferous tubules, sperms with normal morphology, and concentration in Figure 1. Histological structure of germ cells was found to be normal in appearance. There were no marked changes in testicular histology relative to controls in the vehicle as well as POM and COT extract treated groups. Thus, normal spermatogenesis, well preserved Sertoli cells and well delineated tubular basement membrane were observed in control, POM and COT- treated groups (Figure 1a and 1B). The interstitium between tubules and leydig cells was also intact (Figure 1a and 1B). However, in the CCl4-treated group, differences were observed in histology of testis as compared with control group. Complete atrophy of seminiferous tubules was exhibited , degenerative changes in most of the germ cells such as loss of germ cells, abnormality of germinative epithelium, interruption in meiosis, sperm with abnormal shape and concentration, The spermatogonia are shed into the lumina of tubules and delocalization of seminiferous tubules. Partially the ground substance within the interstitium also disappeared and replaced by fibroblast and inflammatory cells. These changes were markedly reduced with oral administration of costus and pomegranate revealing a marked repairing of testicular abnormalities. POM and COT treated groups as shown in Figure ures 3(A) and 3(B) demonstrated maximum antioxidant and healing effects against CCl4 induced damage showing sperm with normal morphology and concentration as compared with control group.

4. Discussion

Considerable attention was given to the involvement of oxygen free radicals in various diseases. There is no doubt that reactive oxygen species (ROS) play an important role in pathological changes in many organs (Sahreen et al., 2013). Testicular oxidative stress appears to be a common feature in infertility, which suggests that, there may be benefits to develop better antioxidant therapies for relevant cases of hypo spermatogenesis (Turner and Lysiak, 2008; Yousef and Salama, 2009). Sies (1993) reported that Several endogenous protective mechanisms have evolved to limit ROS effect and the damage caused by them. However, when the formation of ROS is excessive, additional protective mechanisms of dietary antioxidants may be of a great importance. Therefore, many natural agents possessing antioxidative properties have been proposed to prevent and treat infertility and reproductive hormonal imbalance induced by oxidative stress (Kandasamy et al., 2010).

In the present study, CCl4 treatment showed marked degeneration and alterations of germ cells; The histological
changes in testes of rats administered CCl4 are in agreement with Khan and Ahmed (2009) who reported that CCl4 administration caused testicular atrophy, degeneration of germinal layer, decrease in testosterone, gonadotropins (FSH, LH) and increase in prolactin and estradiol in male rat. Sahreen et al. (2013) displayed that CCl4 resulted in the oxidative damage to testicular proteins in rats. CCl4 requires bioactivation by phase I cytochrome P450 system to form reactive metabolic trichloromethyl radical (CCl₃*) and peroxy trichloromethyl radical (*OOCCl₃). These free radicals can bind with polyunsaturated fatty acid (PUFA) to produce alkoxy (R*) and peroxy radicals (ROO*), that, in turn, generate lipid peroxides, that are highly reactive, change enzyme activity and finally induce injury or necrosis (Weber et al., 2003). Yousef and Salama (2009) reported that oxidative stress results from the production of oxygen radicals in excess of the antioxidant capacity caused degenerative effects in many tissues. The target of free oxygen radicals related acute or chronic toxicity resulting from CCl4 is not only liver but also other tissues including heart, testis, lung, kidney, brain and blood (Abraham et al., 1999). Male infertility is inducers of oxidative stress, which leads to an increase in germ cell apoptosis and subsequent hypospermatogenesis. Moreover, reactive oxygen species and oxidative damage of bimolecular may contribute to male infertility by reducing sperm function (Atessahin et al., 2005).

Debnath and Mandal (2005) studied the histomorphology of testis after exposure to toxic chemicals and revealed shrinkage of the tubular diameter and testicular atrophy leading to degenerative changes in the germinal epithelium. Similar destructive effects were also accounted in the present study in CCl4 treated rats. Data of the present study revealed that CCl4 may hamper continuing proliferative behavior of testicular cells thus obstruct reproduction. Deformities in spermatogenesis and partial degeneration of germ and Leydig cells have been displayed. In the current study, the proposed plan aimed to assess and examine the possibility of Costus and Pomegrenate to protect and reduce oxidative damages caused by CCl4 in testis tissue of male albino rats. Groups administered ethanolic extract of costus rhizomes demonstrated a quality active spermatogenesis, thin basement membranes and normal seminiferous tubules in most of the part of testis. The present results support the findings of Gupta (2010) who stated that the rhizomes and roots of costus clears toxins from the body. Also Bavara et al. (2008) evaluated the antioxidant potency of an ethanol extract of Costus speciosus root in alloxan-induced diabetic male.

Moreover, Vijayalakshmi and Sarada (2008) investigated different parts of Costus speciosus for their polyphenol content and antioxidant activity. Chakraborty (2009) showed the antioxidant activity of chloroform extract of Costus speciosus leaves for its free radical scavenging activity. Thus, the curative role of costus on CCl4 induced testis destruction may be due to its patent antioxidant activity as demonstrated by Saha et al. (2013).

Minimizing the testicular toxicity of CCl4 by POM extract treatment may be due to Polyphenols present in pomegranate. Polyphenols are the most abundant antioxidants in most diets. As reviewed by Middleton et al. (2000), polyphenols exert antioxidant activities. Polyphenols like catechin or quercetin can directly scavenge ROS, such as superoxide radical, hydrogen peroxide, or hypochlorous acid, which can be very deleterious by damaging lipids, proteins and DNA (Binsack et al., 2001). Pomegranate is among the richest fruit in polyphenols (Chalfoun-Mounayar et al., 2012), which exert many health-promoting effects, including the ability to increase intercellular antioxidant levels, decrease capillary permeability and fragility and
scavenge oxidants and free radicals (Binsack et al., 2001). Many studies in vitro and in vivo concerning pomegranate extracts and its individual constituent's support that they are potent antioxidants (Kim et al., 2002; Toi et al., 2003).

Because of its potent antioxidant activity, pomegranate considers one of the commonly used natural antioxidants. Pomegranate fruit, juice, and peel extracts is a rich source of polyphenols and hence posses a potent antioxidant properties (Murthy et al., 2002; Singh et al., 2002). The effectiveness and safety of its isolated antioxidants have been tested (Cedra et al., 2003). Murthy et al., 2002 added that methanolic extract of the peel has shown a higher antioxidant potential than that of seeds and could prevent CCl4-induced hepatotoxicity.

In conclusion, According to our results, pomegranate peel extract and Costus rhizomes have shown curative abilities against CCl4 induced testicular toxicity.

**Highlight**

The rats were divided to six groups (5/group). Control group, CCl4 intoxicated group. The tested groups (group 3 and 4) were administered either POM or COT ; fifth and sixth groups were the ombination CCl4 and two test extracts.

At the end of experimental period testes were fixed for histopathology.

male rats treated with CCL4 alone showed marked histopathological changes

male rats treated with either costus or pomegranate exhibited therapeutic role for injury in testes tissue caused by CCl4

**Recommendations**

According to the above results the authors recommend using of costus roots and pomegranate as antioxidant agent for treating testes injury.

**Competing interests**

The authors declares that there is no competing interests

**Reference**


