Study on the Protective Effects of Bee Glue (Propolis) Against the Histological Changes of Dacarbazine in Male Albino Mice

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ABSTRACT
The objective of this research was to define the histological testicular effects of dacarbazine on male mice, which is one of the anticancer drugs, and the use of bee glue (Propolis) as a one of the natural therapeutic substances, which were mentioned in the Holy Quran in limiting such effects. To achieve the objective of the study were treated male albino mice, which were divided into four main groups for five consecutive days as follows: G1 control group, G2 treated with Propolis (50 ml/kg b wt.), G3 treated with dacarbazine DTIC. (3.5 mg/kg b wt.), and G4 the dual Treatments with Propolis & Dacarbazine and divided into three categories: a) Treated Propolis 2h before Dacarbazine. b) Treated both Propolis and Dacarbazine at the same time. c) Treated Propolis 2h after Dacarbazine. The testicular tissue of mice treated with dacarbazine showed mild degenerative changes, disintegration and separation with necrosis in the majority of the seminiferous tubules, inflammatory infiltration cellular, sloughing and depletion of endothelium germ cells, reduction in the of seminiferous tubules of different spermatocytes and spermatozoa, deterioration of the links between Sertoli cells(S.C) and neighboring spermatocytes. of Leydig cells(L.C) showed an irregular shape and lost the nuclear shape. On the other hand, the mice received the dual treatment with Propolis and Dacarbazine (T2A, T3A &T4A) showed clear positive response and normal testicular tissue and normal seminiferous tubules with intact epithelial lining, seminiferous tubules where restored their regular interrelationship endothelium of germ cells appeared normal spermatocytes and spermatozoa content increased in all the lumina of the seminiferous tubules, (S.C) resting on the basement membrane, together with spermatocytes and spermatogonia and the links between (S.C) and different spermatocytes have been restored, the number of (L.C) have increased with normal shape and nuclei as compared with mice treated with (DTIC) alone..

KEYWORDS: Propolis, dacarbazine, Histological changes , testis,, mice

INTRODUCTION
Cancer is a fatal disease, and as the chemotherapy drugs may not be effective against certain cancer cells, and that the effectiveness of these drugs may degrade as a result of the evolution of drug resistance in cancer cells, in addition, regarding anti-cancer drugs, several studies have confirmed that despite its effectiveness on cancer cells, they have negative effects on normal cells represented by: stimulate the production of free radicals, which reflects the strong impact (potent mutagen, teratogen and carcinogen) on normal cells. Hence, the researchers focused on the possibility of using natural compounds such as: complementary factors in the treatment of cancer due to the ineffectiveness of the drugs currently available or to their adverse effects. There are many efforts to treat cancer by various natural and synthetic materials, because of the unwanted side effects
of caused by chemotherapy and drug resistance factors, which highlight on complementary medicine as an alternative solution, [1;2:3].

In recent years, many scientists worldwide have conducted researches to find an anti-proliferation composite for cancer cells and natural diets have been known beneficial to human health since long time. The aim of chemoprevention is to prevent the start of the process of carcinogenic or to dampen this process in its early stages and so to exclude the development of the tumor, which is able to invade nearby tissues in order to spread [4]. From this point, evidences regarding the integration of antioxidants have been increased with certain types of chemotherapy due to its effectiveness in reducing the neoplastic toxicity and its reduction of free cracks resulting from chemical processing [5].

Since propolis is a natural substance in food and healing, it has been used for thousands of years in traditional medicine all over the world due to its strong antioxidant and free radicals sweeping components such as flavonoids, vitamins, aromatic acids, fatty acids, esters and ketones. Propolis also has -regarding vital- a strong antibiotic, antibacterial, anti-fungal, anti-tumor and anti-inflammatory activities [6;7;8;9;10].

Therefore, the present study aims to find out histological changes induced by the treatment of one of the drugs used in cancer chemotherapy, Dacarbazine, on testicular tissue and the effectiveness of propolis in the reduction of these changes.

**MATERIALS AND METHODS**

**Animals used:**

This research experiments conducted on male albino mice (*Mus Musculus*, 2n = 40) of the MFI strain, Aged 10-12 weeks, obtained from the animal house of the King Fahd Medical Center located at King Abdulaziz University in Jeddah.

**Dacarbazine (DTIC):**

Dacarbazine is chemotherapy used in cancer patients; its trade name is known as DETICENE, and have been purchased from (Medac, Germany).

**Propolis:**

Bee glue, a material collected by bees from leaves' buds and has numerous benefits, and was obtained from Wild Honey Company in Riyadh, Saudi Arabia.

**Experimental Desin:**

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<th>Table 1: Shows the number of mice and the amount of doses of different groups experience</th>
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C:Control, P: Treatment with Propolis, T1: Treatment with Dacarbazine, P+T1: Treatment with Propolis & Dacarbazine

18 male mice were used in this study, which was divided into 4 main groups as follows:

Group 1 treated with a distilled water
Group 2 treated with a 50mg/kg dose of bee glue (Propolis) [11;12].
Group 3 treated with a 3.5mg/kg dose of Dacarbazine [13].
Group 4 treated with a combination of Propolis at a dose of 50mg/kg and Dacarbazine at a dose of 3.5mg/kg

Groups were further categorized into 3 categories as follows:

Category (a): receiving sequentially combined treatment with Propolis 2h before Dacarbazine
Category (b): receiving simultaneous treatment with Propolis and Dacarbazine at the same time.
Category (c): receiving sequentially combined treatment with Propolis 2h after Dacarbazine.

after 24 hours of the last treatment, the animals were sacrificed, and testis were taken out and were fixed in a solution buffered neutral (10%formalin), then the slides were stained with haematoxilyn-eosin [14;15], and were examined by an optical microscope Olympus BX51, For the study of the natural installation of the control group and the treatment groups histological changes.
Results:

Histological structure of the testis:

Testis is composed of a large number of somniferous tubules which sperm is made up of its mature walls surrounding this tubules intertubular connective tissues there is interstitial cells known as Leydig cell which secrete hormones responsible for the appearance of secondary sexual characteristics. there in the wall somniferous tubules cells represent stages formation spermatozoon even up to its final form this process is known the process of spermatogenesis, somniferous tubules appear as round or oval formulations each encapsulated with superfine basement membrane a wall each of which contains several layers of cells arrange from the outside to the inside:

1- Spermatogonia small crowded cells, located around the perimeter of the inside tubules.
2- Primary spermatocytes its the largest cells with large nuclei.
3- Secondary spermatocytes its the smallest of the former about half the size, its nuclei dyed dark-colored.
4- Spermatids its smaller than the previous, its nuclei more concentrated and assemble in groups.
5- Spermatozoa located in the cavity of tubules, always convergent and relate with especially large cells Its called Sertoli cells, spermatozoon has elongated head, a long thin tail, nucleus is located in the head which narrowed tip created for acrosome(Figs 1a,b).

Treatments with Dacarbazine:

When Examination the transverse -sections of testicular tissue of male mice treatment dose(T1A) observed disintegration and spacing Seminiferous tubules from each other within the testis sector(Figs 3a,b), alienation in epithelial germ cells for Some of these tubules(Figs 3b,c,d), inflammatory infiltration(Figs 3a,e), sertoli cells (S.C) complete dissolution and Laceration links between sertoli cells and some of the spermatocytes with the advent of large vacuolization between spermatocytes (Figs 3a,b,e), emergence of a local death in some areas(Figs 3d,e), decrease in the number of leydig cells (L.C) with taken irregular shapes also its nuclei began to lose its natural shape and take different forms(Figs 3a,b,c,d), sperms scarceness in a lot of cavities Seminiferous tubules(Figs 3a,e).

The dual Treatments with Propolis & Dacarbazine:

Histological examination of the male mice testis Dual treatment (T2A ,T3A&T4A)marked improvement, It has been seen when examining transverse -sections the extent response of testicular tissue in to this treatment Where restoration Seminiferous tubules interdependence with each other within the testis sector And it appeared epithelial germ cells normal. And increased content of testicular spermatozoa, improvement in the number of spermatocytes, it returned her natural layered arrangement inside Seminiferous tubule, as restore sertoli cells its contact with both other germ cells and spermatocytes, the leydig cells have been observed to increase the number and size, nuclei return to normal in terms of form and place (Figs 4,5,6 ).
Fig. 1: Group I: Microscopic images of transverse section from mice testes of the control (C) shows: many of Seminiferous tubules with clear cavities and covered with a clear basilar membrane, Sertoli cells (S.C), Leydig cells (L.C), stratification of spermatocytes in its various stages inside Seminiferous tubules: 1. Spermatogonia  2. primary spermatocytes  3. secondary spermatocytes  4. spermatids.  5. Spermatozoa fig 1a (X 200) fig 1b (X 400)

Fig. 2: Group II: Microscopic images of transverse sections from mice testes of the treatment dose (Propolis) shows: Seminiferous tubules, Sertoli cells (S.C), Leydig cells (L.C), stratification of spermatocytes in its various stages inside Seminiferous tubule

Fig2a (X 200)  
Fig2b (X 400)  
Fig2c (X 1000)
Fig. 3: Group III: Microscopic images of transverse sections from mice testes of the treatment dose (T1A) shows: Combination of abnormal seminiferous tubules (3a,b), Spacing between Seminiferous tubules (3a), Few in the density of basement membrane and its alienation in some places (indicated by the arrow), with note the expansion in the cavity between Seminiferous tubules, notice alienation epithelial germ cells (3b,c,d), few in the density of primary & secondary spermatocytes inside seminiferous tubule with few in the density and dispersed the spermatids and unstructured installation (3a). Manifestations of inflammation And nominated cellular (3a,e), Laceration links between spermatids (S,C) and its neighboring Sperm cells (3b), emergence of a local death in some areas (indicated by the arrow) (3d,e), morphological changes in Leydig cells (L,C) and its nuclei taken side place of the cell (indicated by the arrow) with few in the density (3a,b,c,d), sharp decrease in the number of sperms (3a,e).

Fig 3a (X 200)
Fig 3b,c (X 400)
Fig 3d,e (X 1000)
Fig. 4, 5, 6: Group IV: Microscopic images of transverse sections from mice testes of the treatment dose (T2A, T3A, T4A) shows: High response of testicular tissue to this treatment in terms of restoring seminiferous tubules natural form and its layered arrangement to a large extent and restore contact links between Sertoli cells (S.C), Leydig cells (L.C) to restore the natural form with increase in the density, increase the testicular content of sperms.

Discussion:

The study showed that the treatment with the drug Dacarbazine for five consecutive days resulted in histological changes with serious damage in the testicles of male rats, where the disintegration and spacing of seminiferous tubules was noted in an abnormal form, sloughed epithelial germ cells for this tubules, a decrease in testicular content for each spermatogonia and spermatocytes with the occurrence of morphological abnormalities of these cells, laceration of links between Sertoli cells and spermatocytes which is next to it and lack in the number of Leydig cells that are taken irregular shapes. Such pathological changes are consistent with what was monitored by other researchers in their previous studies as a result if their use of Dacarbazine drug [16], and other anti-cancer drugs, where they noticed that the treatment by anti-cancer drugs have had either a loss [17;18], or a loss and sloughed [19] or damage to epithelial germ cells of seminiferous tubules [20;21;22].

As it was noticed in this study, a clear reduction in the testis content for each of the spermatocytes in general, spermatogonia cells and especially primary spermatocytes. This result is consistent with what was indicated by other studies about that the treatment by one of anti-cancer drugs such as Cyclophosphamide has caused a shortage in the number of spermatogonia cells and primary spermatocytes in male rats [23]. Also it was noted in this study, a shortage in the numbers of spermatids and spermatocytes inside the cavity of seminiferous tubules and these results have been supported by [19;24;25;26;27] who have noticed a significant shortage in spermatids and a remarkable dampening to the process of spermatogenesis which resulted a
decrease in the concentration of Spermatozoa inside the cavity of Seminiferous tubules as a result of treatment by Cisplatin drug, also by [28] at treatment of ifosfamide drug, and [29] who noted that the treatment by the drug Gemcitabine has caused an obstruction to the process of Spermatogenesis resulting a deformation of spermatocytes as well as Spermatozoa, plus a shortage in their number. Some of the side effects of the most of chemotherapy treatment are that they lead to a breach in the process of Spermatogenesis and a sharp drop in the number of Spermatozoa and their vitality [30].

Scott et al. [25] has explained the cause of this reduction in the number of cells, Spermatids and Spermatozoa in male rat testes’ tissue, so he explained saying that the treatment with anti-cancer drugs had caused dampaning and a quick stop to cell-cycle of germ cells and spermatocytes, and stimulated changes in the ripening process which resulted a clear reduction in the number of cells and then an overall reduction in all types of cells in the testis.

Accordingly, the observable spermatids and spermatozoa are only in seminiferous tubules the less by getting affected where spermatogonia cells and spermatozoa are still natural to some extent and still have the ability to return, split and to make differentiation [31]. Also it was noted in that study the emergence of large gaps (vacuolization) between spermatocytes and this result consistent with what he observed on the vacuolization in the areas between spermatogonia cells and primary spermatocytes or between spermatogonia cells and Sertoli cells, and the cause was in the degeneration and detachment processes which occur in the spermatocytes as a result of drug treatment, and common cold was noted as well in the inflammatory infiltration with the emergence of a rupture and congestion of blood vessels [18;32;33].

This study has also showed abnormal morphological changes to primary & secondary spermatocytes as well as their nuclei and Chrominance element. [34] has explained that the appearance of the various changes in primary & secondary spermatocytes can be attributed the fact that these cells originated and derived mainly from spermatogonia cells that are already damaged. As for [35], they’ve explained the cause of the damage and installation crash of spermatocytes, by that it is caused as a result of chemical reaction between the active group in the drug and nucleic acids such as DNA within a chromosome, or as a result of the cellular and genetic effects of the drug and its metabolic outputs on DNA or essential proteins for the cells maturation process [36;37].

This study has also showed the affected Sertoli cells by the laceration of links between Sertoli cells and spermatocytes, which is next to it, and lack in the number of Leydig cells that are taken irregular shapes. Such results are consistent with the findings of [18;22;28;38;39]. The stages of growth and maturation of germ cells into sperms are an important function of Sertoli cells, and that many previous studies have shown that the treatment by anti-cancer drugs such as Dacarbazine lead to the creation of a significant decrease in testicle content and serum from Testosterone, (FSH), (LH) hormones as well as the impact on the functions of sertoli cells &leydig cells [16;19;20;24;40;41;42]. Therefore, it is possible that the effect ofDacarbazine on sex hormones rates, which are responsible for maintaining and completing the process of Spermatogenesis is one of the important indirect causes that led to the emergence of sertoli cells & leydig cells in an abnormal form resulting a decrease in the number of spermatozoa in the cavity of Seminiferous tubules in testicular tissue [43].

Perhaps it is important to note that the changes occurring in the process of lipid peroxidation and glutathione (GSH), which are usually accompanied by a decrease in antioxidant enzymes activities such as GPx and GR enzymes, so these changes, are considered as toxic manifestations for testes as a result of the treatment with anti-cancer drugs. Studies have shown that in natural tissues and organs, there must be a state of equilibrium between the production of active oxygen species ROS and between anti-oxidants, which operates as a scavenger for this free radical. This is because of that the physiological side requires the presence of active oxygen species in small and reasonable quantities because it is necessary for fertilization and acrosome reaction [44]. The increased production of active oxygen species within the tissue may lead either to breaks the defense mechanisms of antioxidant or may exceeds the capacity of the antioxidant defense system. In addition, the plasma membrane of spermatozoa is usually rich in polyunsaturated fatty acids, and the cytoplasm this spermatozoa contains small amounts of scavenging enzymes, making the sperm more vulnerable to attack by active oxygen species (excessive ROS) [45;46].

Since previous studies have confirmed that the drug Dacarbazine is one of the cytotoxic and genotoxic agent [16]. It is also considered as a strong oxidant factor, by the fact that treatment with chemotherapy drugs such Dacarbazine lead to the launch of free radical such as reactive oxygen represented species in the hydrogen peroxide (H2O2), superoxide and hydroxyl free [OH-] known by its ability to destroy DNA and other biological molecules in the cell [47]. The chemotherapy is working to reduce the rates of antioxidant substances and enzymes in different tissues [48], and since these substances are known for their ability to remove cellular toxicity induced by treatment of chemical compounds, including drugs that, through their work as scavenger for free radical. Therefore, treatment with Dacarbazine drug will result in a shortage in the mechanical protection within cells and an increase in the oxidation processes which results the release of free radical that are known by their destructive ability towards the cells and then the tissues. Therefore, it is possible that this cellular and toxic effect of the drug is one of the direct causes for the emergence of such changes on the testicular tissue (under study).
As for the dual cooperation between Propolis and Dacarbazine drug, the results of histological examination to male mice testis have shown the noticeable improvement which seemed by seminiferous tubules restoring their interdependence with each other and the appearance of epithelial germ cells, each with a normal to a large extent, the increase in testicular content of sperm cells and sperms, the disappearance of common cold and Cellulitis signs.

The restore of Sertoli cells to their interdependence with germ cells and other spermatocytes and the increasing the numbers of leydig cells and restoring their natural shape. Such finding are consistent with several previous studies that monitored the effect of propolis on testicular tissue, the male rats treatment recorded with a dose rate of (50 mg/kg) of propolis besides a dose rate (34 mg/kg) of aluminum chloride (AlCl3) for 70 days. In a study of male rats treated with Chlorpyrifos (CPF) dose rate (9 mg/kg) for 70 consecutive days, an increase in the abnormalities in sperm and a decrease in their number was observed, and when using dual treatment with propolis by a dose rate of (50 mg/kg), it was notice that propolis reduces the toxic effects resulting from the treatment with CPF in male rats [12].

As a recent study explore the protective effect of propolis on Doxorubicin (Dox)-induced testicular damage, histopathologic examinations revealed that Treatment by propolis prevented its changes without diversion its anticancer activity, and increased serum testosterone level [50]. Also Chrysin (CR), which is one of a flavonoid that naturally exists in propolis can mitigated the side effects of acute Paracetamol (PRC) reproductive toxicity in male which caused decreased the sperm motility, and increased dead sperm rate, abnormal sperm cell rate [51].

The logical interpretation of the role played by the propolis in reducing histological pathological changes caused by treatment with a Dacarbazine drug on testicular tissue is that propolis may have been affected in some way on the sex hormones, both in terms of its effect on leydig cells or sertoli cells thus improve their functions that are affected as a result of drug treatment. Or it may affect the secretion of pituitary gland included hormonal formation process (FSH), (LH) by activating spermatogenesis process as evidenced by several recent studies [50;52;53], through the work on urging sertoli cells to secrete primary estrogen hormone to convert spermatids to spermatozoa, while (LH) is working on leydig cells to control the formation of steroid hormones (including the production of the hormone testosterone) which has an important role in the completion of Spermatogenesis process [54]. In addition, the fact that propolis is a strong potent antioxidant agent in preventing injury to the testicular tissue, through its role on the scavenger free radical, reduction lipid peroxidation level and height in catalase activity and glutathione concentration [52; 55; 56], and the antioxidant activity of propolis can be associated with its active components which are associated with of anti-cancer and anti-oxidant activities [57;58;59;60] of:

- Group of vitamins: most notably vitamin E, C, B1, B2, B6 which proved by previous researches their ability as antioxidants materials in reducing the cellular and genetic toxicity effects of chemotherapy by doing scavenged a wide range of oxygen and reactive nitrogen and other free radical species that are soluble in the fats of cells membranes and to stop their prevalence [61; 62;63;64;65;66].
- Flavonoids, they have multiple biological effects such as: anti-inflammatory, anti-allergy, anti-viral, anti-cancer, anti-oxidant and have vital protective effects in vivo and in vitro [67], the previous studies have been shown that flavonoids antioxidant ability is much stronger than vitamins as vitamin C, E [68]. It prevents oxidation through interaction with the cell membrane’s proteins and fats and make a vital physical changes in the membranes of cells and modulating the redox [69] and has a high antioxidant capacity scavenging free radical and prevent their harm [70], in addition, they greatly enhance the antioxidant enzymes activities (glutathione peroxidase, catalase, superoxide dismutase) [10;71;72] and increase the glutathione levels within cells. So the flavonoids are considered as an important food supplement in the treatment of various diseases associated with oxidative [51; 73].

Therefore, the current study recommends using propolis when the chemical treatment is being conducted for cancer patients as an alternative factor or adjunct therapy and complementary for anti-cancer therapies.

REFERENCES


