The protective effect of propolis (bee glue) against histopathological changes induced by dacarbazine in mice kidney

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Abstract: This study was carried out to assess the protective effect of propolis against histopathological changes in mice kidney during dacarbasine (DTIC) treatment. To achieve this goal, a total of 30 male mice were allocated into four groups: 1) the control group (Gr1), 2) the second (Gr2) group received propolis (50 mg/kg bw), 3) the third group (Gr3) receiveddacarbazine (3.5 mg/kg bw), and4) the fourth group were administered dacarbazine (3.5 mg/kg bw) plus propolis (50 mg/kg bw) and divided into three categories: a) treated with propolis 2h before the administration of DTIC, b) treated with both propolis and DTIC in the same time, and c) treated with propolis 2h after the DTIC administration. All groups treated for ten consecutive days and killed after 24h from the last dose. The kidneys were removed and subjected for light microscopic study. DTIC treatment induced kidney damage, loss of its normal architecture,atrophy and distortion in Malpighian corpuscles, degeneration and necrosis in epithelial cells of renal tubules and congestion of interstitial tissues were detected. In fourth group the kidney restored the normal histological structure in both Malpighian corpuscles and Bowman's capsules, there are marked reduction in degeneration of epithelial cells of renal tubules and decrease in interstitial congestion only in the first category. While in the second and third categories showed no improvement in the kidney damage.
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1. Introduction

Dacarbazine (DTIC) is considered to be as acytotoxic alkylating agent, and can be used in the chemotherapy. It was successfully applied in curing different types of cancers, such as Hodgkin's disease, skin cancer, tumors of soft tissues, tumors of nervous cells, lymphomas (Adler et al.,2002;Lev etal.,2003; Marchesietal, 2007), as it may cause distinct decline in destroying cells that contain toxin for the patients of leukemia (Franchiet al., 1992).It was noted in the recent years that skin cancer has widely spread more than any other cancer, and dacarbazine has been taken into consideration as a golden standard in curing skin cancer. It was found that the response rate was 15% to 20%, but most responses have never been continuous (Levet al., 2004). Dacarbazine has been approved for curing skin cancer since 1970s (Alexanderet al, 2004).Dacarbazine is considered to be as one of the drugs that is prepared to be used in chemotherapy and that has the ability for alkylation. Dacarbazine, after being activatedmetabolically, attacks DNA and alkylates its Nitrogen bases, so, it inhibits the quick doubling of cancer cells growth. However, some alkylating Nitrogen bases may cause mutation leading to secondary cancers (Sanadaet al., 2004). Dacarbazine is usually used as a complementary treatment with other chemotherapys and been as anti-Hodgkin's disease in its advanced stages or been as anti-skin cancer in its advanced stages. Despite of new methods of integration in the treatment, dacarbazine is considered to be as the only reference for curing the fourth stage of skin cancer (Adler et al., 2002).When treating patients with dacarbazine, side effects were noted such as nausea and vomit for up to 90% of patients, as their case developed after 3 hours of treatment. Such side effects (symptoms) may appear in less than 12 hours after receiving treatment along with Flulike syndrome that causes rigor, fever, shortness in breath and muscles pains as well as hepatotoxicity, hair fall, erythroprosopalgia, tension. Skin reactions were also noted. (Bruceet al., 2001). Many studies suggest that having supplementations that contain antioxidants may protect the cells and tissues of patients against contrary side effects during chemotherapy with antineoplastic agents (Chakraborty etal., 2009), while components that could decrease such side effects may help improve treatment against cancer. Recently, there is a trend for searching for efficient components of plant-origin that are able to alleviate toxicity caused by chemotherapy for normal cells without intervention in antineoplastic activity (Pratheeshkumar and Kuttan, 2010). Propolis is bee glue collected by bees from leaves' buds and bark cracks of different plants. Propolis consists of 50% of flavonoids related to phenolic acids, 30% of wax, 10% of basic oils, 5% of pollen and 5% of various organic components (Piettaet al., 2002). Propolis has been widely used in medicine in the past as antioxidant,

anti-inflammatory, fortifying immune system and anti-cancer effects (Watanabe et al., 2011). In a laboratory study (Ozkul et al. 2005), it was mentioned that propolis extract has anti-cancer effects with high concentrations, as it contains flavonoids, fatty acids, aromatic acids and their esters. Such flavonoids work as inhibitors for cancer cells. Propolis has a positive effect on immune system against microbes and the growth of carcinoma, especially activating the cells of immune system (natural killer cells) and activating the prevention of the growth of cancer cells. (Sforcin, 2007). Montoro et al. (2005) mentioned that propolis protects the deterioration of DNA resulting from treating with Gamma-ray. That reflects the ability of propolis for getting rid of free radicals that harm the body. Besides, it didn't has a toxic effects when using it with high doses. (Burdock, 1998).As propolis has vital characteristics in combating microbes, antioxidant, anti-inflammatory and anti-tumor, it was chosen to be used in studying its protective effects in limiting histopathological changes in male mice's kidneys induced bydacarbazine.

2. Materials and methods

2.1. Animals used

The experiments of the research were conducted on a group of male albino mice *(Musmusculus, 2n=40)* of MFI strain between 8-9 weeks of age, with weights ranging between 30±3 g, obtained from the animal house of the King Fahd Medical Center in King Abdulaziz University, Jeddah, where the mice were placed in special plastic cages, inside a well-ventilated room, where temperature was about 22ºC±1º C approximately, and humidity ranging between 45-75%, with suitable 12 hours lighting during the daytime, and 12 hours darkness at night-time, with water provided daily, and fed with a balanced dry provender special for experimental animals that is provided by the Center.

2.2. Materials used

1) Dacarbazine (DTIC)

Has been known commercially as (Deticene), comes as powder to be dissolved in saline solution and purchased from (Medac, Germany).

2) Propolis

It was obtained from (wild honey company) Riyadh, Saudi Arabia. Purchase from Egypt.

2.3. Experiment design

Thirty male mice were divided into four groups:

1) First group G1: the control group, treated with physiological solution

2) Second group G2: was treated with poropolis (50 mg/kg body weight) (Park and Kahng, 1999)

3) Third group G3: was treated with the therapeutic dose of dacarbazine (3.5 mg/kg body weight) *(*Hardman et al*., 2006).*

4) Fourth group G4: were administered dacarbazine (3.5 mg/kg) plus propolis (50 mg/kg) and divided into three categories: a) was treated with propolis 2h before the administration of DTIC. b) Was treated with propolis plus DTIC in the same time. c) was treated propolis 2h after the admiration of DTIC.

2.4. Method of treatment

All groups that have been treated with dacarbazine was injected into the peritoneal cavity (intraperitoneal injection) (I.P) whereas the propolis was given by an oral intubation (O.I) (Park and Kahng, 1999***)*** and all groups were treated daily for ten consecutive days, after 24 hours of the last treatment, the animals were sacrificed, and kidneys were taken out to be prepared for histological study.

2.5. Histological studies

The tissues were fixed in a solution a 10% buffered neutral formalin, embedded in paraffin wax and cut 5mm thickness, and then the slides were stained with haematoxilyn-eosin *(Mallory, 1900)*, and were examined by an optical microscope Olympus BX51.

3. Results

3.1. Investigatingcross sections of kidney's tissue of *mice in control group (Gr1)*

The cortex contains the Malpighian corpuscles (M.C) and the proximal and distal convoluted tubules consists from a double-walled spherical Bowman's capsule (B.C) surrounding a capillary network or glomerulus (G). The walls of the capsule are very thin and consist of squamous epithelium (B.S). Around these corpuscles numerous of the proximal and distal convoluted tubules. The proximal convoluted tubule (P.C.T) has a relatively narrow lumen and thick walls of cuboidal or pyramidal granular cells. The distal convoluted tubule (D.C.T), on the other hand, has a large lumen and thinner walls of smaller cuboidal cells (Figure 1).

3.2. Examination of kidneys of mice of the second group (Gr2)

Our findings on second group revealed that propolis treatment showed no effect, the kidney's tissue kept its normal structures as same as control, and all components have been appeared whether (M.C) or convoluted tubules (P.C.T/ D.C.T) with their normal structure (Figure 2).

3.3. Examining the kidney's tissue in the mice receiving dacarbazine (Gr3).

It was seen that many histopathological changes occurred as represented by atrophyand distortion of (M.C) and that glomeruli's form has not been regulated (G) black arrow. Bleeding was also seen in the capillaries referring to the existence of glomerular inflammation as referred to blue arrow. There was also a defect in Bowman's capsule for a number of

nephrons, so they became narrow in some places and there could be adhesion between the lining membranes of Bowman's capsule as referred to in the red star. Such case is commonly known as glomerulonephritis, or there could be an expansion in other capsules resulting from atrophy in capillaries' network as referred to in the green arrow. Concerning the convoluted tubules (P.C.T/ D.C.T), the drug has a strong effect on damaging the lining cells of these tubules. Epithelium of tubules should hyperplasia, and they became a cone-shaped as they appeared inside the lumen. That leads to atrophy in lumen of tubules, green stars, a disorder in nucleus of these cells was also seen, regarding pyknotic nuclei. In other places, there was degeneration of walls of many epithelium cells of convoluted tubules with expanded lumen, and nucleus was also shown in the lumen (yellow arrow Figure 3A). Congestion also was investigated in the interstitial tissue' of kidney (brown arrow) with the appearance of inflammation marks in the cell represented by the existence of lymph cells (orange arrow).Vacuolar degeneration was also appeared in the interstitial tissue with congestion of blood cells. Inflammation cells have also appeared in the damaged places (green star). However, concerning convoluted tubules (P.C.T/ D.C.T), there was a hyperplasia in lining cells leading to a collapse in convoluted tubules curves (black stars) with vacuoles in the nucleus (Karyorrhexis) (yellow arrow) with the appearance of bleeding and oedema in the interstitial *tissue* surrounding convoluted tubules (Figure 3B - green arrow).

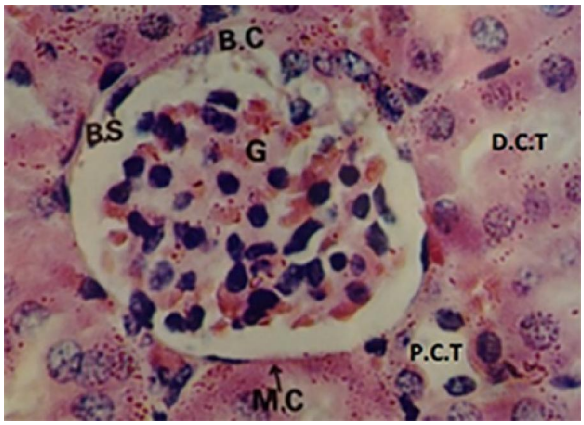

Figure 1. Transverse section of normal kidney's tissue of mice in control group (Gr1) x400.

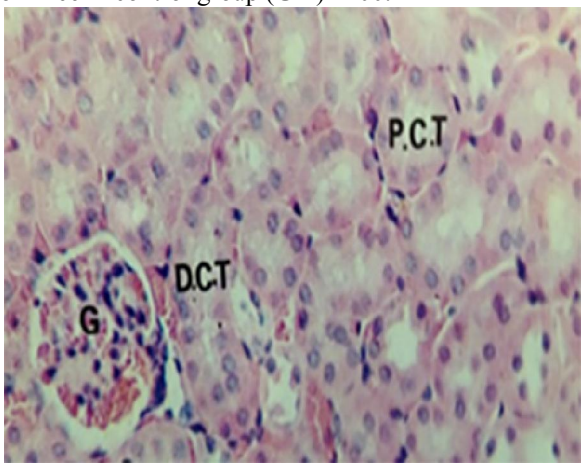

Figure 2. Transverse section of normal kidney's tissue of mice in propolis group (Gr2) x200.

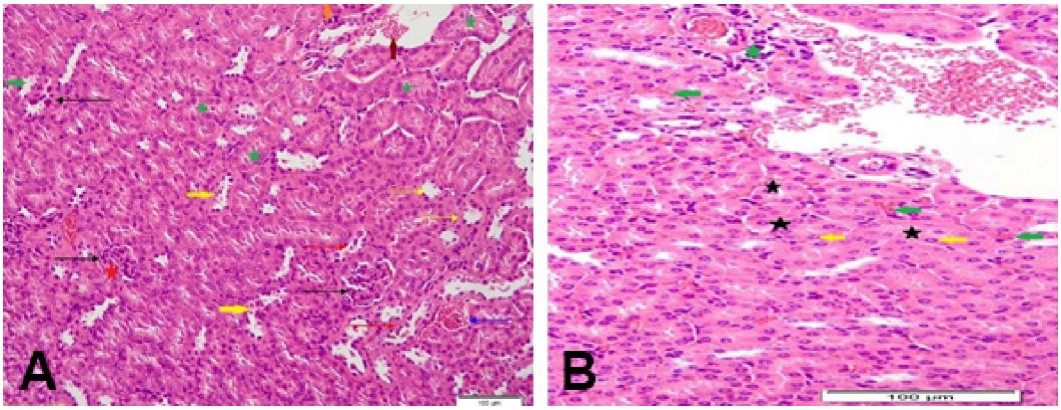

Figure 3. Transverse section of abnormal kidney's tissue of mice in dacarbazine group (Gr3) A: x100 and B: x200.

3.4. Examining the cross sections in the kidney's tissue of mice (Gr4)

When it was investigated that the mice of (category a) treated with propolis two hours prior to administrating the drug were the best, whereas the kidney's tissue appeared to be normal in its tissue structure as it is the matter in the control group. M.C has recovered its size and round shape, as the bleeding has been disappeared in capillaries that was seen in its nephrons (G) resulting from treating with the drug only. Besides, Bowman's capsule has recovered its normal lumen (Figure 4A), and a sharp decreasein degeneration of epithelium cells of the convoluted tubules (P.C.T/ D.C.T) it also recovered their normal

shape and structure compared to the control group. The bleeding that has been spread over many places in the interstitial tissue disappearance (Figure 4B).

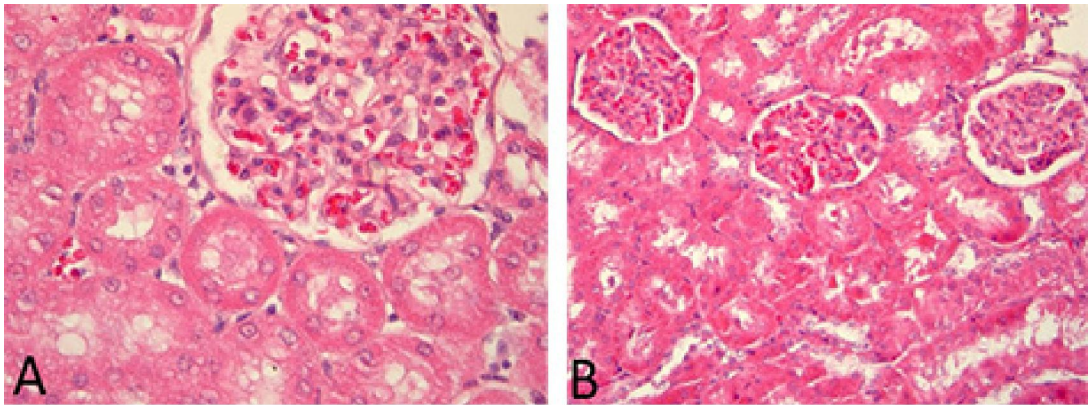

Figure 4. Transverse section of semi normal kidney's tissue of mice in dacarbazine and propolis group (Gr4 category a) A: x100 and B: x200.

Concerning the kidney's tissue of mice in category (b) treated with the drug and propolis in the same time (Figure 5A), and in mice of category (c) that have been treated with propolis after two hours of administrating drug (Figure 5B) whereas no improvement in the kidney's tissue of both category. Malpighian corpuscles andconvoluted tubules have still been infected and abnormal in their shape and structure. Bleeding is still there in many interstitial tissue places of kidney.

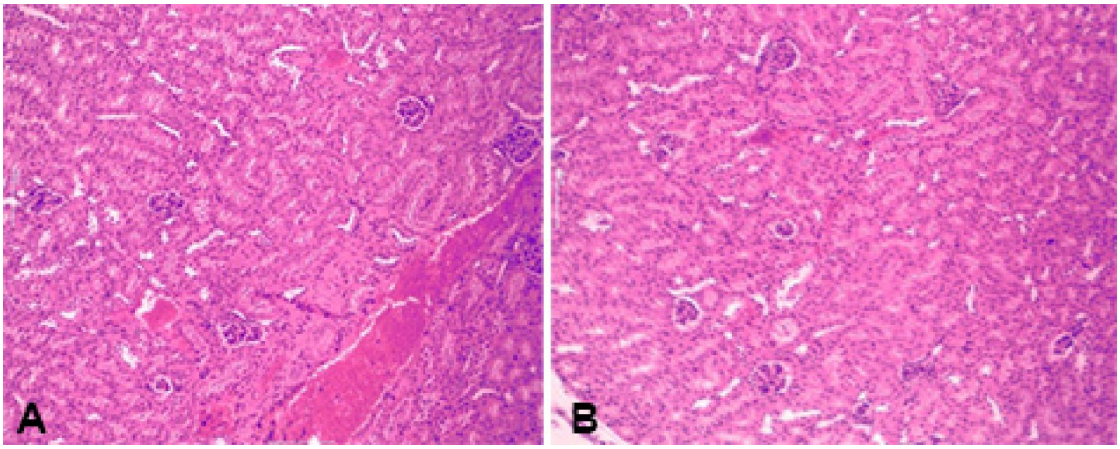

Figure 5. A) Transverse section of abnormal kidney's tissue of mice in dacarbazine and propolis group (Gr4 category b) x100; B) Transverse section of abnormal kidney's tissue of mice in dacarbazine and propolis group (Gr4 category c) x100.

4. Discussion

It was known that most of drugs used in chemotherapy for cancers may cause toxic side effects on many organ systems including the kidneys (Milijasevic et al., 2014). The most common side effects resulted from chemotherapy with dacarbazine are nausea and vomiting, impaired liver and kidney function, thrombosis of the hepatic veins, hepatic necrosis and anemia. (Sleijfer et al., 2005, Berger et al., 2008; Grimer et al., 2010). Dacarbazine is considered to be as one of alkylation agent against lymph tumors and other solid tumors (Marchesi et al., 2007). DTIC may need to a metabolic activation in order for its toxic effect to appear, and thus may take place by N-demethylation in the liver's microsomes. (Yamagata et al., 1998; Long and Dolan, 2001). However, the mechanism of dacarbazine acts is not exactly known, but it is believed that there are three hypotheses explaining how it works:1) Inhibition of DNA synthesis by acting as a purine analogue, 2) action as alkylating agent, 3) interacting with sh groups (Saunders and Schultz, 1970). By detection of aminoimidazolecarboxamide (c14) in urine and separation of N-7 and O-6 from DNA strands, its

primary mode of action appears to be alkylation of nucleic acid (Mizuno et al., 1975). Milijasevic et al. (2014) declared that the mechanism dacarbazine acts in as an alkylation factor is by methylation of DNA and inhibition of purine, RNA and protein synthesis and it is active during all phases of cell cycle. Interstrand cross-linkage between double strand of DNA is considered to be as the main factor in cytotoxicity for most medically effective alkylation agent, the matter that make DNA template strand as inactivestrand so that the DNA is no longer producing leading to the death of cells (Erikson et al., 1989). The ability of such agent to intervene with keeping DNA molecule safe in the tissues of quick-division shows the basis of their curing applications and their privileges that cause cytotoxicity. Not only that, but also these agent have harmful effects on tissues of low-average division such as kidney and liver, but they could be very toxic on tissues of quick-division such as cells of bone marrow (Padmalatha and Vijayalaxmi, 2011). Nephrotoxicity is considered to be as the most common side effect when chemotherapy with drugs against tumors. That what we observed in our previous study when treating mice with cisplatin (Quita and Kurdi, 2009) or when treating them with cyclophosphamide (Quita and Al-Amri, 2015). Sugumar et al. (2007) identified the renal damage in mice with the following histopathological signs: glomerular inflammation, the appearance of epithelial cytoplasmic vacuolization in cortical tubules, interstitial edema, and mild hemorrhagic changes in the renal cortex. That what our study's result showed. Treating mice with a dosage of dacarbazine (3,5 mg/kg) for ten days has induced many changes of tissues in the kidney's tissue which appeared in glomerular inflammation of Malpighian corpuscles, in addition to vacuoles, degeneration and necrosis of renal tubules, these changes may be attributed to increase loud of reabsorption that is more than kidney tubules so the water is trapped inside. (Huldtsfer et al., 1975; El. Naggar et., 1985). In this study, it was also showed that there is clear bleeding in the kidney's tissues, especially glomeruliand interstitial tissues. That may be attributed to that dacarbazine may cause an increase in vascular permeability of the blood vessels walls. Odema has been witnessed in the kidney's interstitial tissue. Scientist explained that Odema may refer to the first phase of inflammation and a reaction of such chemical drugs against cells membranes as well as an increase in permeability (Chabner, 2006). Such changes highlight the truth of previous reports mentioning that anti-cancer drugs can cause Nephrotoxicity (Jiany et al., 2006; Abrahan et al., 2007; Hamsa and Kuttan, 2011; Deviand Mazumdar, 2016).

The other matter that should be discussed is that the alkylatingagent release free radicals. That what Mazmudar et al. (2011) and Iranshahi et al. (2015) said that treating with chemotherapy drugs may release free radical such as reactive oxygen species (ROS) and oxidative stress, and it is known that such agent able to damage tissue and cells through different mechanism; whereas dacarbazine was proved that it is able to produce free OH radicals which are known to be able to damage DNA molecule and other vital molecules in the cell such as: cell membrane and suborganel membranes (pourahmad et al., 2009). That what may explain the reason behind damaging and death of cells in kidney's tissue which has been observed in the current study as a result for treating with dacarbazine. Therefore, natural compounds that possess antioxidant properties may contribute to the protection of cells and tissues against the deleterious effects of ROS and other free radicals induced by antineoplastic drugs (Weijl et al., 1997; Iranshahi et al., 2015). That what may have been observed in the study. The co-administration treatment of male mice with propolis two hours before treating with therapeutic dose of dacarbazine and for ten consecutive days showed that there has been a good improvement in the kidney's tissue (category a) whereas Malpighian Capsules have recovered their size, shape or normal structure. Besides, all bleeding has disappeared whether in glomeruli (G) or in interstitial tissue, as the sharpness of deterioration and necrosis in lining cells for nephrons. The results of our study meet with what El-Kenawy et al. (2002) observed when treating male mice with a dose of 34mg/kg of aluminum chloride and propolis with a dose of 50 mg/kg, that treatment using (ALCLs) has caused a heavy damage in the kidney such as: Shrunken glomeruli, interaglomerular congestion and degeneration of epithelial cells of the renal tubules. While in the kidney of rat treated with both propolis and ALCL□, there were decreases in the vasculature of the renal glomeruli. Both of the proximal and the distal convoluted tubules were near to similar from the control group. In fact, in recent studies, propolis has been demonstrated to play an important role in preventing the oxidative stress, apoptosis, and necrosis induced by lead (El-Masry et al., 2011).The improvement in the kidney's tissue, proved in this study, came as a result of treating with propolis which has an antioxidant protective effect on the kidney's cells and the biological importance of compounds of its components, such as: Flavonoids and phenolic acids (Sforcin, 2007; Kedzia, 2009; Forkt et al, 2010).

Propolis is a natural composite balsam that is produced by honey bees from the gum of various plants. Recently, this compound has gained popularity both as a medicine with anti-bacterial, anti-viral, anti-

inflammatory, anti-oxidant properties and as a food that improves health and prevents disease (Marcucci, 1995; Matsuno et al., 1997; Szliszka et al., 2009; Pessolato et al., 2011; Szliszka and Krol, 2013). Propolis has been proven to have various bioactivities that are anti-pathogenic, immunoregulatory, anti-tumor and hepatoprotective (Bankova, 2005; Sforcin, 2007), anticarcinogenic (El-Khawaga et al., 2003), neuroprotective (Nakajima et al., 2007), antimicrobial (Papova et al., 2005). Beneficial biological effect of propolis has been widely used in dermatology for injuries healing, thermal damage and external ulcers therapy, healing time reduction, wound contraction increase, and tissue repair acceleration (Ramos and De Miranda, 2007). It has also been used as a health drink in Asian, European and American countries (Banskota et al., 2001). More than 300 components have been found in propolis responsible for the biological activity, mainly composed are flavonoids (chrysine, galangine, pinocembrine and pinobanxine), phenolic acids (caffeic acid, p-cumaric acid and ferulic acid) and their esters (phenylethyl and 1.1- dimethylallyl) (Sforcin, 2007; Kedzia, 2009; Forkt et al., 2010). Many of these phenolic compounds have been shown to be cytoprotective by scavenging superoxide anion, hydroxyl radical, hydrogen peroxides and reducing lipid peroxidation (Yousef et al., 2004;Mani etal.,2006).Propolis is also reported to inhibit the generation of superoxide anion. Furthermore, propolis has been determined to reverse the consumption of glutathione. Which is synthesized in the liver and has radical scavenging activity (Castaldo and Capasso, 2002). On the other hand, co-administration of the dacarbazine and propolis in this study in category b and c, no improvement was detected, where the histopathological changes ofkidney were still very obvious. In conclusion, the finding of our study indicate that dacarbazine can adversely damage the kidney tissue, while propolis co-administration could effectively prevent these adverse effects and protect the kidney.

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