Evaluation of antioxidant activity of propolis (bee glue) on the histopathologyof hepatocytes in mice treated with dacarbazine

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Abstract: The current study aims to assess the protective effect of propolis against hepatocyte histopathological changes during dacarbasine (DTIC) treatment in mice model. To achieve this goal a total of 30 male mice were divided into four groups: The first served as a control (Gr1). The second (Gr2) and third (Gr3) groups received propolis (50 mg/kg bw) and dacarbazine (3.5 mg/kg bw) respectively. The fourth 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. 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 livers were removed and subjected for light microscopic study. DTIC treatment induced liver damage, loss of hepatocytes architecture and vacuolar degeneration, inflammatory cellular infiltration in between hepatocellular necrosis and blood sinusoids congestion and dilation were detected. In fourth group the liver restored the normal histological structure only in the first category, there are marked reduction of cytoplasmic vacuoles, less dilation of central and portal veins and reduction of sinusoids congestion. While in the second and third categories showed no improvement in the liver damage.
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1. Introduction

Cancer chemotherapy is among the treatments that play an important role in most solid tumor treatment. Nevertheless, they are accompanied by many of the side damages and side effects. Such side effects have their impact on the vitality and activity of cancer patients, both for short-term and long-term. There are proposals by the researchers suggesting that the combined treatment of chemotherapy and chemopreventive agents that possess anti-cancer activity, may enhance the efficacy of chemotherapy and reduces the systemic toxicity induced by chemotherapy ***(Sarkar and Li, 2006).***

Dacarbazine is a cytotoxic alkylating agent, and has an effective effect on lymphoma as well as against various types of solid tumors ***(Marchesi et al., 2007),*** as it is used in the treatment of Hodgkin's disease and Malignant melanoma ***(Lev et al., 2003)*** and DTIC needs metabolic activation in order to its toxic effect may show up, which is done by N-demethylation process in liver 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).

Besides, dacarbazine has cytotoxicity due to its generation of many types of reactive oxygen species (ROS), H_2O_2, O_2^- ***(Pourahmad et al., 2009).*** However dacarbazine-induced hepatic failure (Frosch*et al.,* 1979) and sever liver damage and eosinophilia in a patient treated with the drug for malignant melanoma (Czarnetzki and Macher, 1981). Also,DTIC - induced hepatocytes cytotoxicity in rat (Pourahmad*et al.,*2009) and hepatotoxicity in mice (Horiguchi*et al.,* 2010).

Given what the dacarbazine has shown in previous studies of its development of hepatotoxicity, it has become in general trend, the use of natural materials or compounds possess anti-oxidant properties and so safe that they limit the toxic effects resulting from treatment with such drugs.

Since Propolis is a natural balm compound produced by honey bees from various plants gum, studies have confirmed that Propolis contains more than 300 compounds, responsible for its biological activities. The most important compounds thereof 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 studies have shown that these compounds possess cytoprotective effects **(Bahorun *et al., 1994;* Rice-Evans, *2004).***

Based on above, the current study, will evaluate if propolis, with what it possesses of anti-oxidant properties, may be used as a protective agent against the histopathological changes that may be induced by dacarbazinein mice liver and find out what is the treatment most appropriate and most effective when using propolis with the drug.

2. Materials and Methods

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.

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.

Methods used

1. Experiment design

Thirty male mice were divided into four groups:

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

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

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

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

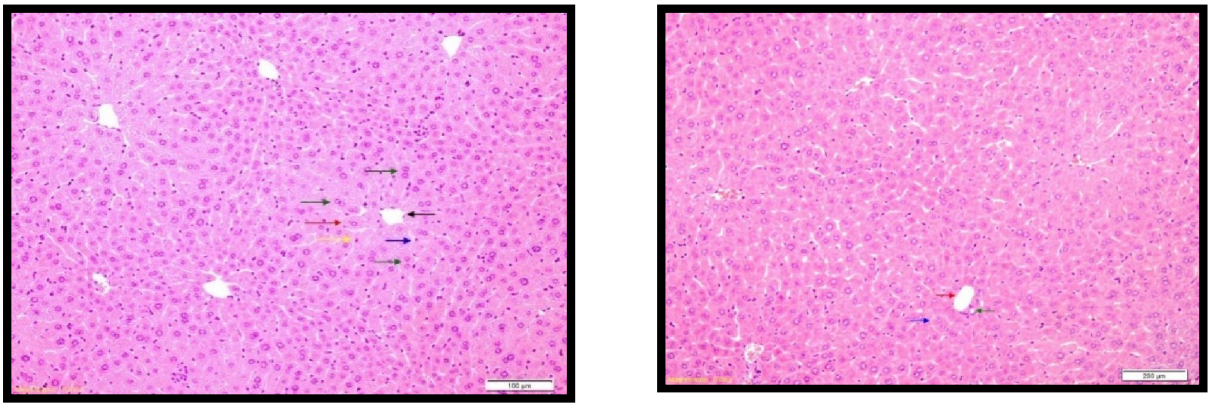
2. Method of treatment

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 liver were taken out to be prepared for histological study.

3. 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

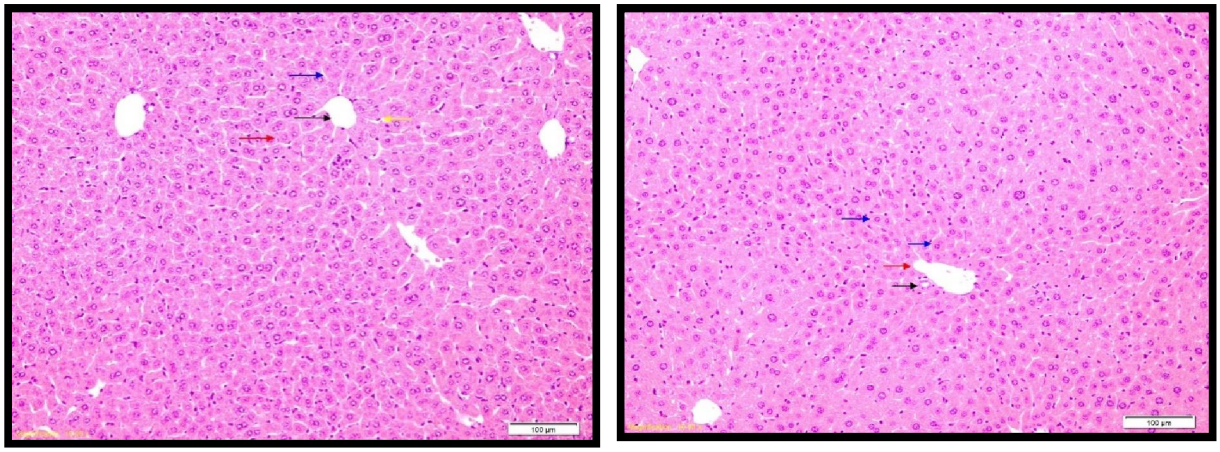


a **b**

Fig-(1) – control group (Gr1): a: Transverse section in the liver of a mouse showing normal structure, normal central vein (black arrow), normal hepatocyte with polygonal shape (red arrow) and normal sinusoidal space (yellow arrow), kupffer cells (blue arrow) and binucleated hepatocytes (green arrow). H+E (x200) b: Transverse section in the liver of a mouse showing normal portal vein (red arrow), normal hepatocyte (blue arrow) and bile ductile (green arrow). H+E (x200).

The light microscopic examination of control liver group (Gr1) showed normal arrangement of hepatocytes architecture, hepatic lobes containing cords of hepatocytes with sinusoids between there cords (Fig1a). The central vein and portal treads appeared normal (Fig1b).

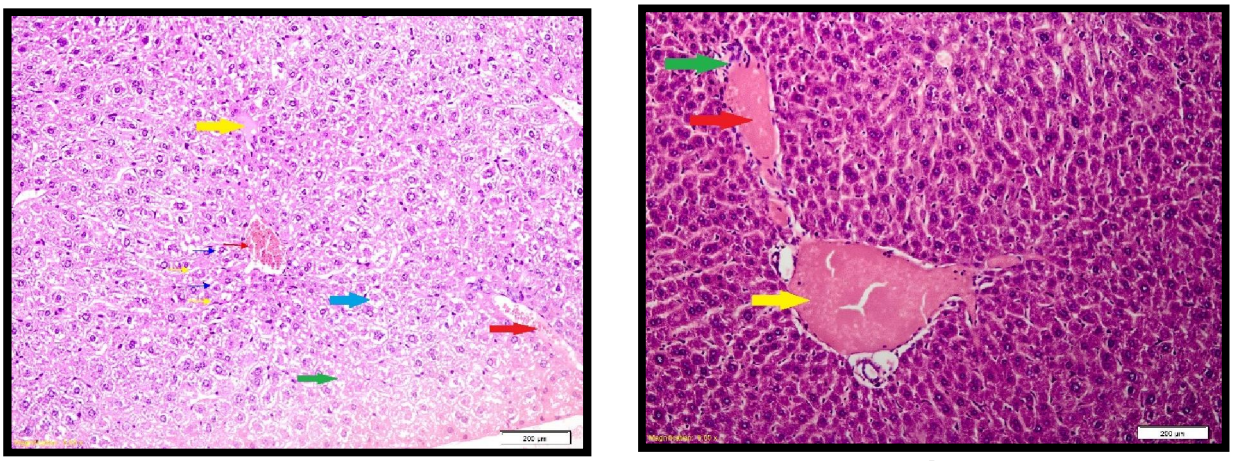
Examination of livers of mice of the second group (Gr2) revealed that propolis treatment showed no effect, the liver's tissue kept its normal structures as same as control (Fig 2 a & b).



a **b**

Fig-(2) – propolis 50mg/kg (Gr2): a: Transverse section in the liver of a mouse showing normal hepatocytes structure, normal central vein (black arrow) as same as control group. H+E (x200). b: Transverse section in the liver of a mouse showing normal portal space as same as control group. H+E (x200).

While the histological examination of livers treated with dacarbazine (Gr3) showed severe damage in the liver tissues. Where the liver lost its hepatic architecture, dilation and congestion of central hepatic vein, severe congestion in blood sinusoids and increasing in the number of Kupffer cells indicated to occur of sever inflammation , a lot of cytoplasmic vacuoles in hepatocytes , vacuolar degeneration, and necrosis with pyknotic nuclei and odema in blood sinusoids were detected (Fig 3a). The same alterations were observed in portal space, in addition to odema in blood sinusoids and portal vein (Fig 3b).



a **b**

Fig-(3) – dacarbazine 3.5mg/kg group (Gr3): a: Transverse section in the liver of a mouse showing central vein congestion and dilation (red arrow), dilated and odema in blood sinusoid (yellow arrow), vacuolar degeneration (blue arrow) and necrotic hepatocytes (green arrow). H+E (x200) b: Transverse section in the liver of a mouse showing portal vein dilation and odema (yellow arrow) and odema in blood sinusoid (red arrow). Many kupffer cells (green arrow). H+E (x200).

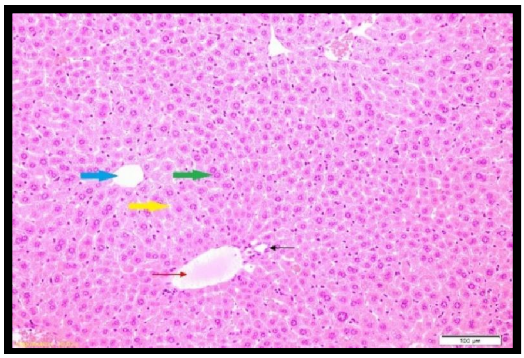
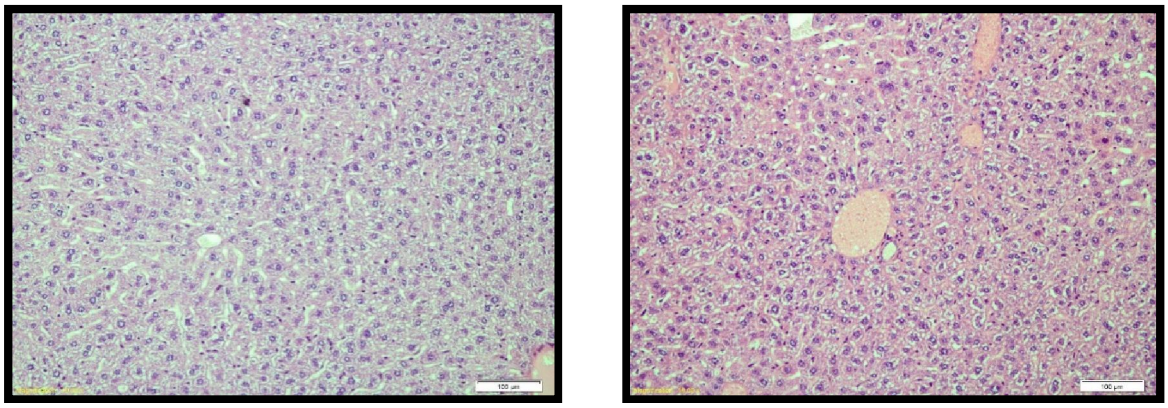


Fig-(4) – propolis (50mg/kg) + dacarbazine (3.5mg/kg) group (Gr4A): Transverse section in the liver of a mouse showing resorted of hepatocytes architecture, normal binucleated hepatocyte (green arrow), disappeared congest sinusoidal space (yellow arrow), normal central vein (blue arrow), a normal portal space showing portal vein (red arrow) and bile ductile (black arrow). H+E (x200).

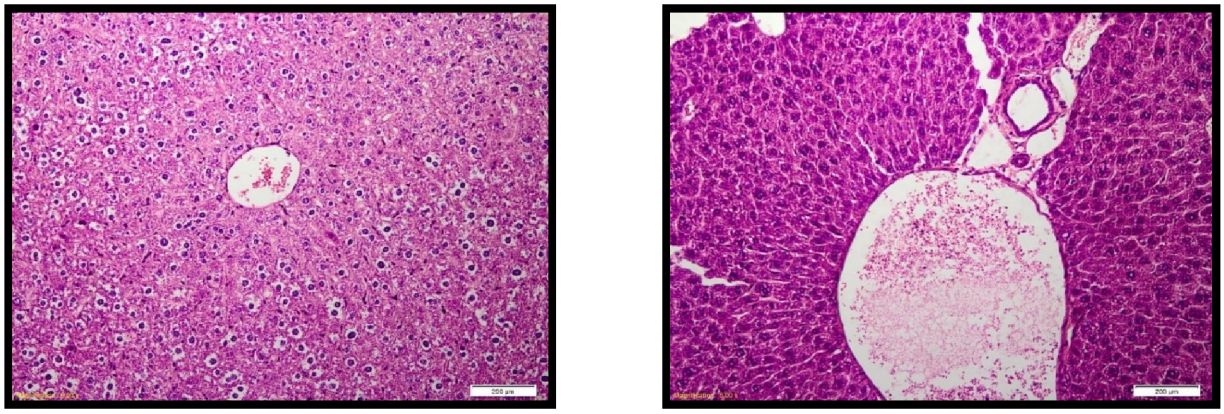
On the other hand, the co-administration with dacarbazine and propolis (Gr4 category a) showed obvious improvement for the liver. Thus, the liver's tissue almost restored its normal structures, the congestion and dilation decreased in both central and portal hepatic vein and blood sinusoids and are overall improvement in the tissue with vacuolar degeneration was detected. These improvement led to reduction in the spreading of inflammation cells (Fig4).

On the other hand, co-administration of the dacarbazine and propolis in category b and c, no improvement was detected, where the histopathological changes of hepatocytes were still very obvious (Fig 5 a & b) (Fig 6 a & b) respectively.



a **b**

Fig-(5) – propolis (50mg/kg) + dacarbazine (3.5mg/kg) group (Gr4B) a: Transverse section in the liver of a mouse showing abnormal hepatocytes and congested central vein. b: Transverse section in the liver of a mouse showing vacuolar degeneration and odema with dilated portal vein. H+E (x200).



a **b**

Fig-(6) – propolis (50mg/kg) + dacarbazine (3.5mg/kg) group (Gr4c) a: Transverse section in the liver of a mouse showing abnormal hepatocytes structure, dilated and congested central vein and vacuolar degeneration. b: Transverse section in the liver of a mouse showing abnormal congested and dilated portal vein. H+E (x200).

4. Discussion

The results that have been reached in this research showed that the dacarbazine induced histolopathological changes in the liver of male mice treated compared to the control group, where the liver has lost its normal radial composition, so that the hepatic cells showed vacuolar degeneration with pyknotic nuclei, and some areas were necrosis of hepatocytes, so that its cytoplasm has degenerated, and its nuclei chromatin had fallen in fragments (karyorrhexis) or (karyolysis), and became of unclear features.

In addition, dilation and congestion of blood vessels and sinusoids occurred as well as in some areas, there is rupture of epithelial cells layer, lining such blood vessels, resulting in occurrence of hemorrhage.

Ariens et al. (1976) explained that the tissue toxicity appears in the histological section in the form of cytolysis accompanied by the formation of vacuoles and fatty accumulation and necrosis in the tissue.

This is observed by ***Frosh et al. (1979)*** when treating a 55 years old woman suffering from Malignant melanoma, with dacarbazin,where the woman died because of what brought about by the drug of hepatic failure due to necrosis of the liver cells.

Also, treatment with dacarbazin-induced hepatotoxicity in rats and mice ***(Pourahmad et al., 2009; Horiguchi et al., 2010)***.

Where dacarbazin belongs to the group of alkylating agents, (***Horiguchi et al., 2010),***namelyultra-interacting agent that transform alkyl group to important cell parts through its combination with amino, phosphate, sulfhydryl, carboxylgroups, and it is also believed that these agents acting as alkylate DNA (***Marchesi et al., 2007)***.

This is what it was referred to by ***Vijayalaxmi and D'souza (2004),*** that the important strategy in cancer treatment based on the use of anti-cancer drugs with its alkylating characteristics, as the interaction of a molecule of DNA with alkylating agent result in a covalently modified bases, and such alkylating agents working either as topoisomerase inhibitors or free radical generation agents, and in all cases they are eventually attack the DNA molecules resulting in chromosomal changes.

The interstrand cross-linkage between double strands of DNA is the main factor in the cytotoxicity of most of the medically effective alkylating agents, which leads to make the template strand of DNA inactive, so that the production of DNA stops, leading eventually to cell death ***(Erikson et al., 1989)*** and the ability of those alkylating agents on interfering with the safety and performance of a molecule of DNA in the fast-dividing tissue, showing the principle for its therapeutic applications and its characteristics that lead to cytotoxicity, not only that, but some alkylating agents are characterized by influencing adverse effect on the low division rate of tissues such as the liver and kidneys, but It may be highly toxic to rapidly dividing tissues such as bone marrow cells ***(Padmalatha and Vijayalaxmi, 2001).***

The other aspect that has to be discussed, is the fact that alkylating agents generate free radicals.As it was stated by ***Mazmudar et al. (2011)*** that the treatment with chemotherapeutic drugs result in launching of the free radicals such as reactive oxygen species represented in hydrogen peroxide (H_2O_2), superoxide, and this is what is referred to by ***Pourahmad et al. (2009)***, that the treatment with dacarbazin result in launching of free hydroxyl radicals [OH] known by its ability to destroy the DNA, and other vital molecules in the cell, such as the cell membranes and the suborganel membranes.

It is suggested that H_2O_2 could cross the Lysosomal membrane, react with lysosomal Fe2+ to form hydroxyl radical (Haber – Weiss reaction) which is the major cause of lysosomal membrane leakiness, proteases, and other digestive enzymes, release and finally the cell death (**Pourahmad*et al.*, 2009.)**

These free radicals have the ability to oxidation of surrounding molecules, such as molecules of DNA, lipids and proteins, and may result in the destruction of these vital molecules results in many ailments such as aging and cancer ***(Feig et al., 1994).***

Recent study noted that dacarbazine damage DNA by forming adducts with bases, the most common of which occur at the N7 position of guanine and the N3 position of adenine, which are repaired by the base excision repair mechanism. On the other hand, another important lesion formed, in terms of mutagenesis and cytotoxicity, is O^6-alkylguanine **(Marchesi *et al.*, 2007).**

Some studies show that biological compounds with antioxidant properties intake can help control reactions to chemotherapy and my 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) .**

This is what has been observed in this study, that the treatment of male mice with propolis two hours prior to treatment with therapeutic dose of dacarbazin for a period of 10 days (category a) has brought about a marked improvement in liver tissue where the liver cells restored their normal composition, and there has been a decrease in the number of inflammatory cells, the congestion and dilation decreased in both central and portal vein and blood sinusoids. Perhaps it can be explained by the effectiveness of propolis antioxidant and the importance of biological activities of its

components compounds, such as: flavonoids and phenolic acids **(Sforcin, 2007; Kedzia, 2009; Fork*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-iflammatory, 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, 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; Fork*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 (Bahorun*et al.,* 1994; Rice-Evans, 2004).

On the other hand, co-administration of the dacarbazine and propolis in category b and c, no improvement was detected, where the histopathological changes of hepatocytes were still very obvious.

Hence it is clear that the dacarbazine have had adversely affect the liver and that dual treatment with propolis two hours before the drug treatment has provided a protective effect to the liver tissue.

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