ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Hepatoprotective Effect Of (-)-a-bisabolol In An Experimental Model Of Paracetamol-Induced Injury.

Valéria do Amaral[1]*, Gabriel Fernando Esteves Cardia[1], Edvalkia Magna Rocha[1], Rita de Cássia Lima Ribeiro[2], Jurandir Fernando Comar[3], Juliana Nunes de Lima Martins[3], Francielli Maria de Souza Silva-Comar[1], Ciomar Aparecida Bersani-Amado[1], and Roberto Kenji Nakamura Cuman[1].

[1]Department of Pharmacology and Therapeutics, State University of Maringá, Maringá, Paraná, Brazil.
[2]Department of Veterinary Medicine, State University of Maringá, Maringá, Paraná, Brazil.
[3]Department of Biochemistry, State University of Maringá, Maringá, Paraná, Brazil.

ABSTRACT

The increase in morbidity and mortality due to acute liver failure worldwide has sparked research to identify the etiopathogenesis involved. Among the causes is the use of non-steroidal anti-inflammatory drugs (AINEs). Paracetamol (APAP) is the AINEs responsible for more than 40% of cases of drug-induced liver disease (DILI). Its active metabolite causes lipid peroxidation, inflammation and damage to hepatocytes. Alpha-bisabolol (BISA), a component of chamomile essential oil, has anti-inflammatory and antioxidant activities, among others. The work intended to investigate a hepatoprotection of BISA in mice treated with doses of 50, 100 and 200 mg/kg of BISA (via gavage), for 7 days before induction of DILI by APAP. The release of AST, ALT, FA, GGT, the production of TBARS, NO, MPO activity and histopathological examination were analyzed. Our results reduced the reduction of liver injury markers, reduced NO and improved TBARS, in MPO activity in all used doses of BISA, when compared to APAP. The data were confirmed by histopathological analysis, which showed less cell damage and inflammatory infiltrate in animals treated with BISA. Our results obtained that the effect of BISA as hepatoprotective is promising and can be used as a useful tool in DILI.

Keywords: (-)-α-BISA; Hepatoprotective; Acetaminophen; Antioxidant activities; Silimarin

https://doi.org/10.33887/rjpbcs/2021.12.3.3

Corresponding author

ISSN: 0975-8585

INTRODUCTION

The study of liver diseases due to non-viral causes has been gaining prominence in the scientific scenario. Recent research shows that there has been an increase in the number of cases of illness due to chronic diseases, among them, non-viral liver diseases were highlighted worldwide [1].

Global data show that the number of early deaths, due to liver disease, reaches 2 million/year. Non-alcoholic liver disease (DHNA) and alcohol-related liver disease (DHRA) account for more than half a million deaths/year [2]. Another common cause of severe liver failure is drug intoxication, with the class of non-steroidal anti-inflammatory drugs (NSAIDs) being the most representative. Paracetamol (APAP) is the NSAID responsible for the largest number of cases of drug-induced liver disease (DILI) [3-4]. It is estimated that 40% - 50% of hospitalizations of patients with acute liver failure are due to overdose of paracetamol, characterizing the main cause of DILI [4-5].

Liver damage caused by APAP is associated with the accumulation of its hepatic metabolite, N-acetyl-p-benzoquinone - imine (NAPQI) and lactic acidosis, which lead to cell collapse. The increase in NAPQI levels occurs by stimulating the activity of the cytochrome P450 pathway, particularly the isoenzyme CYP2E1 [6]. The excess of NAPQI promotes loss of mitochondrial function, reduction of ATP, increased production of ROS (reactive oxygen species) and peroxynitrites, in addition to DNA damage.

High doses of paracetamol produce saturation of phase II reactions, depletion of glutathione reserves, damage to hepatocytes by oxidative stress and local inflammation [7-8].

Stine and Lewis (2014) [9] report that therapeutic alternatives for cases of non-viral hepatitis are scarce. They mention that the administration of antioxidants from 4 to 16 hours after APAP intoxication, may help in the prevention of DILI hepatotoxicity. The same authors state that the use of vegetable drugs found on the market with hepatoprotective activity, such as silymarin, does not offer prophylactic action and guaranteed effectiveness when it comes to DILI. In addition, it has been reported that the use of NAC in APAP overdose leads to serious side effects, such as kidney failure, thrombocytopenia and death.

The effectiveness of NAC administration in DILI and other liver disease etiologies is limited or inconclusive [10-11]. Chamomile is a popular plant, widely used for therapeutic purposes. Some studies prove that several pharmacological properties of the plant, including its action: antispasmodic, antisecretory, antibacterial, antifungal, anti-allergic, antidepressant, anti-inflammatory, antioxidant, healing, neuroprotective and gastroprotective [12-16]. Among the constituents of chamomile essential oil, the most representative is α-bisabolol (BISA). BISA is an example of a terpene with anti-inflammatory, healing, anti-mutagenic, antioxidant activity, inhibitor of cytochrome P450 pathway, anti-platelet, among others [17-21].

Terpenoid compounds have important and proven antioxidant activity. For this reason, they become compounds of great interest in research aimed at discovering plant drugs that inhibit or prevent the appearance of tissue damage, caused by the excess and/or accumulation of free radicals [22-23]. Thus, the study of the hepatoprotective activity of BISA becomes important, since its pharmacological properties can serve as an aid in the development of alternative therapy in the treatment of DILI.

MATERIALS AND METHODS

Chemicals

(-)- α- bisabolol and all reagents used were purchased from Sigma-Aldrich (St Louis, MO, USA).

Animals

After approval by the Ethics Committee on the Use of Animals of UEM (CEUA 4286240219), male mice of the Swiss lineage from the Central Vivarium of the State University of Maringá - UEM, with an initial weight of 20-30 g, were used for the study. The animals were kept in containment boxes, under controlled environmental conditions (T 22 ± 2ºC); 12/12 h light / dark cycle and received standard food and water ad libitum until the beginning of the experiments.

***In Vivo* Assays**

Animal treatment

The doses of essential oils used in this study were similar to those in our previous studies of anti-inflammatory activity and antioxidant activity. The animals were divided into the following groups and treated as indicated: (1) vehicle - filtered water (n = 6); (2) Silymarin (SLM) (200 mg/Kg) plus APAP (250 mg/Kg) (n = 6); (3) APAP (250 mg / kg) (n = 6); (4) BISA 50 mg/kg (n = 8); (5) BISA 100 mg / kg (n = 8); (6) BISA 200 mg / kg (n = 8). The animals were fasted for 12 hours before the beginning of the experiments.

The animals of the BISA group received alpha-bisabolol for 7 days, in the indicated dosages. On the seventh day of treatment, the animals were fasted for 8 h and received, via gavage, a single dose of paracetamol at a concentration of 250 mg/kg. Group 4 received the standard hepatoprotective drug (silymarin 200 mg/kg) orally for 7 days and underwent a single dose of paracetamol 250 mg/kg for comparative purposes. After 12 hours of APAP administration, the animals were euthanized at random, by inhalation of isoflurane.

Biological Samples Collection

Blood and liver samples were collected from the animals. Blood was collected by puncture of the inferior vena cava and then centrifuged at 3,000g for 15 minutes at 4ºC to obtain the serum. The liver was extracted and immediately weighed. Serological samples were used to obtain the concentration of hepatobiliary enzymes ALT (alanine aminotransferase), AST (aspartate aminotransferase), ALP (alkaline phosphatase) and γGT (glutamyl transferase range) using commercial kits Analyze ® Gold Kits. The liver tissue samples were segmented into 4 portions for histopathological analysis, cell viability, biochemical parameters of liver function, oxidative stress markers and oxidative damage markers.

***In Vitro* Assays**

Histopathological Analysis

The livers of rats were collected and inspected macroscopically. The largest right lobe of each liver was excised and fixed in a 10% formalin solution for histopathologic analyses of all animals. Subsequently, the livers were dehydrated in increasing concentrations of alcohol (80 - 100%, v/v) and embedded in paraffin blocks which were sectioned in 6 µm thickness on a Leica Rotary Microtome (Leica Microsystems, Gladesville, NSW, Australia). The organ sections were stained with hematoxylin/eosin (H&E) for evaluation of tissue morphology using light microscopy. The changes in tissue morphology were assessed for nuclear variations, cytoplasmic eosinophilia, swelling and vacuolation in both periportal and central areas.

Histopathologic scoring of liver samples

Liver samples were prepared to facilitate comparisons of the same liver lobes from all animals. Each treatment was evaluated on the following items: hepatocyte vacuolar degeneration, pycnotic nucleus, hepatocyte necrosis, neutrophilic, lymphocytic and mixed inflammatory infiltrate, vessel congestion and cholangiohepatitis. Each assessment was scored between 0-3 (0: no change, 1: mild, 2: moderate, 3: severe). The maximum score for each sample was 17 whereas the minimum score was 0.

Cell Viability Analysis (MTT Assay)

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl-2H-tetrazolium bromide] assay is based on the mitochondrial enzyme reduction of the tetrazolium dye to detect and determine cell viability. The neutrophils were obtained from the peritoneal cavity of mice 4 h after injection of 200 µl zymosan solutions (1 mg/cavity, i.p.). Briefly, the cells were plated at a density of 5 × 105 cells/ well in a volume of 100 µl RPMI medium (supplemented with 10% of fetal bovine serum (FBS) and penicillin 100 U/ml + streptomycin 100 µg/ml) into 96-well plates. After 90-min exposure to BISA (3, 10, 30, or 90 µg/ml) or vehicle (0.1% Tween 80 solution, used as control), 10 µl of MTT (5 mg/ml) stock solution was added to each well.

After 2 h of incubation at 37 °C, the supernatant was removed and 100 µl of dimethyl sulfoxide (DMSO) was added to each well. Cells were incubated at 25 °C for a further 10 min and the absorbance was measured using a Biochrom Asys Expert plus microplate reader (Asys®) at a wavelength of 540 nm. The values of the blank wells were subtracted from each well of treated and control cells. The percentage of viability was determined by the following formula:

%Viable cells = (Absorbance of the treated cells - Absorbance of the blank) / (Absorbance of the control - Absorbance of the control) x 100.

Data were presented as values of three independent experiments performed in triplicate.

Determination of serum AST, ALT, GLU, GGT and ALP levels

After 12 hours of hepatotoxicity induced by APAP, all rats were anesthetized with halothane 3% and blood was collected from inferior vena cava for determination of plasmatic ALT (alanine aminotransferase), AST (aspartate aminotransferase), GGT 25 (Gamma-glutamyl transferase) and ALP (alkaline phosphatase) using the Analyze ® Gold Kits.

Analysis of oxidative stress markers

Determination of MPO (myeloperoxidase) activity

The MPO enzyme activity was measured in the supernatant of a homogenate of liver tissue sections. Briefly, the liver sections were put in phosphate-buffered saline (PBS) in a Potter homogenizer and the homogenate was stirred in a vortex and centrifuged. Ten microliters of the supernatant were added to each well in triplicate in a 96-well microplate. The PBS solution (200 µl) contained 4.21 mg o-dianisidine dihydrochloride (Sigma), 22.5 ml doubledistilled water, 2.5 ml potassium phosphate buffer (pH=6), and 10 µl of 1% H_2O_2 was added. The enzyme reaction was stopped by 30 µl the addition of sodium acetate (2.23 g in 20 ml of double-distilled water). Enzyme activity was determined by the absorbance measured at 450 nm using a microplate spectrophotometer (Asys Expert Plus®).

Determination of NO (oxid nitric) production

The NO production was determined by the Griess method in the supernatant of liver tissue sections, which determines the nitrite production [24]. Two hundred microliters of the supernatant were added to each well in triplicate in a 96-well microplate. Sequentially, solution (50 µl) was added to Griess (1g sulfanilamide in 2.5ml fosforic acid and 0.1g dihydrochloride of N-1-naftiletilonodiamina milli-Q water) at room temperature. The reading was taken using an ELISA plate reader at a wavelength of 550 nm, as described. ON production was calculated from a standard curve of sodium nitrite. The results were expressed as µM.

Determination of APAP-induced lipid peroxidation inhibitory activity

For the evaluation of anti-thiobarbituric acid reactive substances (TBARS), the technique was performed according to the method described by Buege and Aust (1978) [25].

Statistical analysis

Data were expressed as the mean ± SEM for each group. Results were statistically analyzed by using one-way variance analysis (ANOVA) followed by Tukey's test. Differences were considered significant when $p < 0.05$. Statistical analyzes were performed using GraphPad Prism® (Version 5.0 GraphPad Software, Inc). Results are expressed and representative in separate experiments.

ISSN: 0975-8585

RESULTS

BISA not present cytotoxicity

For cell viability analysis, BISA was tested in different concentrations (3, 10, 30, and 90 µg / ml). The results obtained showed that BISA does not present cytotoxicity in any of the concentrations used. The cell viability found was 97, 78, 87, and 83%, in increasing order of concentration.

BISA reduces serum ALT and AST levels

The analysis of the protective effect of BISA on hepatocyte damage, induced by APAP 250 mg / Kg, through the serum measurement of hepatobiliary enzymes, is shown in Figures 1 to 4. Our results show that treatment with BISA, at concentrations of 100 and 200 mg / Kg, significantly reduced serum AST levels, by 80.61% and 80.05% respectively (Figure 1), when compared to the APAP group. Thus, showing a protective effect on tissue injury imposed by the administration of the hepatotoxic agent. However, the dose of 50 mg / Kg was not able to prevent serious injury to the liver tissue imposed by the hepatotoxic agent. It was also possible to observe that the results obtained with the highest concentrations of BISA are comparable to those seen with the association of SLM 200mg / Kg plus APAP 250 mg / Kg (89.29%).

All groups exposed to pretreatment with BISA showed a significant reduction in the serum concentration of the liver enzyme ALT, compared to animals treated with the hepatotoxic agent (APAP 250 mg / Kg). We were able to observe that the BISA hepatoprotection pattern was comparable to the SLM plus APAP control (reduction of 73.11 %) and there was no significant difference between the concentrations of α-bisabolol used (Figure 2). The percentage of reduction in enzyme activity was 77.56% at a concentration of 50 mg / kg, 92.09% at 100 mg / kg and 96.50% at 200 mg / kg of BISA.

BISA promotes a reduction in GGT and ALP

Our results showed that the plasma levels of GGT and ALP also suffered a significant reduction with pretreatment of BISA, regardless of the dosage administered, when compared to the control groups (Figures 3 and 4). The SLM plus APAP control group reduced plasma ALP levels by 54.15%. However, the percentage reduction rate for GGT was 46.24 %, 52.43 % and 52.61 %, at concentrations of 50 mg/kg, 100 mg/kg and 200 mg/kg of BISA, respectively. The values of ALP, in the animals treated with BISA were 73.88% for 50 mg / Kg, 75.83 for 100 mg/Kg and 77.61 mg/Kg for 200 mg/Kg. The percentage of ALP reduction in the SLM plus APAP group was 54.15 %, compared to the group intoxicated with APAP.

BISA reduces the NO production and MPO activity

Nitrite levels were analyzed as a parameter to assess NO production in liver tissue, after inducing cell damage by APAP intoxication. It was observed that treatment with BISA significantly reduced nitrate levels in all previously treated groups with the terpenoid when compared with the APAP group (Figure 5). The reduction in the dose of 50 mg/kg of BISA was 38.77%, 24.22% (BISA 100mg/kg) and 36.61% (BISA 200mg/kg). Similar to the other results, treatment with BISA was comparable to the SLM plus APAP control.

MPO levels (Figure 6) in the liver tissue decreased in animals treated with BISA plus APAP when compared to the APAP group. The dosage of 50 mg/kg of BISA reduced the enzyme activity by 52.20% and the doses of 100 mg/kg and 200 mg/Kg inhibited MPO activity by 43.42% and 54.59%, respectively. Our results showed that there is no significant difference between the protective effect of BISA and the SLM plus APAP group (reduction of 61.35%).

We can see in figure 7 that there was a significant increase in TBARS in the APAP group, when compared to the control group and the SLM plus APAP group (200 mg/kg and 250 mg/kg). All groups treated with BISA, regardless of concentration, reduced this parameter, equaling the group treated with SLM (200 mg/Kg). The reductions were 31.42% with the administration of BISA 50 mg/kg, 40.91% in the dose of 100 mg/kg and 57.87% in 200 mg/kg of BISA.

ISSN: 0975-8585

Table 1. Histopathological analysis of experimental groups

Treatment	Vehicle	(SLM) 200 mg/Kg	APAP 250 mg/Kg	BISA 50 mg/kg	BISA 100 mg/kg	BISA 200 mg/Kg
Number of animals per experiment	3	4	4	4	4	4
Morphological changes in the liver						
Vacuolar degeneration of hepatocytes	0/3 **n°aa**	2/4 **n°aa**	4/4 **n°aa**	3/4 **n°aa**	4/4 **n°aa**	2/4 **n°aa**
Picnotic core	0/3 **n°aa**	0/4 **n°aa**	4/4 **n°aa**	1/3 **n°aa**.	2/3 **n°aa**	2/4 **n°aa**
Hepatocyte necrosis	0/3 **n°aa**	0/4 **n°aa**	4/4 **n°aa**	1/3 **n°aa**	2/3 **n°aa**	2/4 **n°aa**
Inflammatory infiltrate N= neutrophils L= lymphocytes M= mixed	0/3 **n°aa** -	0/4 **n°aa** N	4/4 **n°aa**, N	1/3 **n°aa** N	2/3 **n°aa** M	2/4 **n°aa** N

Abbreviations: SLM: Silymarin; APAP: paracetamol; BISA: (-)- α- bisabolol. */ number of animals evaluated

BISA improvement histopathological changes in the liver

Histopathological analysis of the hepatic segment of the control and treated with silymarin alone animals revealed normal cell architecture with different sinusoidal spaces of preserved liver cells and central vein (Figure 8 A and B).

However, the administration of APAP (250 mg/kg) caused a breakdown in the hepatic architecture. Important changes in the hepatic parenchyma were observed, such as edematous degeneration, areas of necrosis in zones I, II and III (black arrow), in addition to sinusoid congestion, hemorrhage (blue arrow) and dilation of the centrilobular vein (yellow arrow). The previous administration of BISA in concentrations of 50 mg/kg; 100 mg/kg and 200 mg / kg, for 7 consecutive days, caused hepatocellular ballooning in the treated animals.

However, all doses of BISA used were able to minimize changes in the animals' liver parenchyma, as well as inhibiting congestion, hemorrhage and necrosis in zones I, II and III. The administration of BISA decreased leukocyte infiltration (blue arrow in 5) in relation to APAP. In addition, they ensured the preservation of cellular areas (black arrow in 6) and the architecture of the hepatic portal space (*) (Figure 9). Such results are comparable to the hepatoprotective effect observed with the previous administration of SLM 200 mg/kg plus APAP 250 mg/kg, which can be seen in 2.

As shown in Table 1, the histological scores of the liver tissue were maximum (16) for treatment with APAP and the minimum was (0) for treatment with a vehicle - water. The Silymarin group (SLM) 200 mg/kg plus APAP 250 mg/kg totaled 2 points. The BISA 50 mg/kg treatment totaled 6 points, the BISA 100 mg/kg totaled 10 points and the treatment with BISA 200 mg/kg totaled 8 points.

ISSN: 0975-8585

Taking all these results together, we can suggest that BISA showed, hepatoprotective activity against the challenge of inducing acute hepatitis by administering paracetamol.

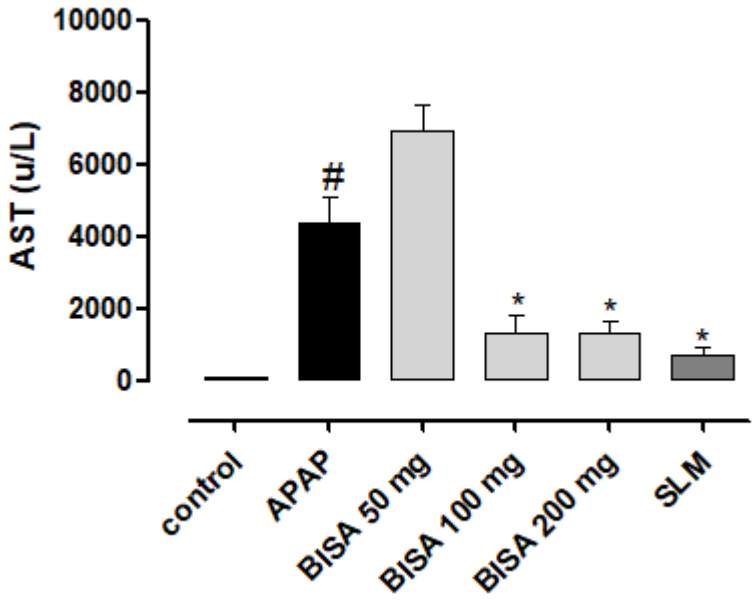

Figure 1. Represents serum AST levels in all untreated groups, treated with APAP (250 mg / kg, orally), pretreated with SLM (200 mg / kg) *plus* APAP (250 mg/Kg) and pretreated with BISA at concentrations of 50, 100 and 200 mg / kg, after 12 hours of APAP poisoning. The results represent the mean ± SEM of 8 animals per group.

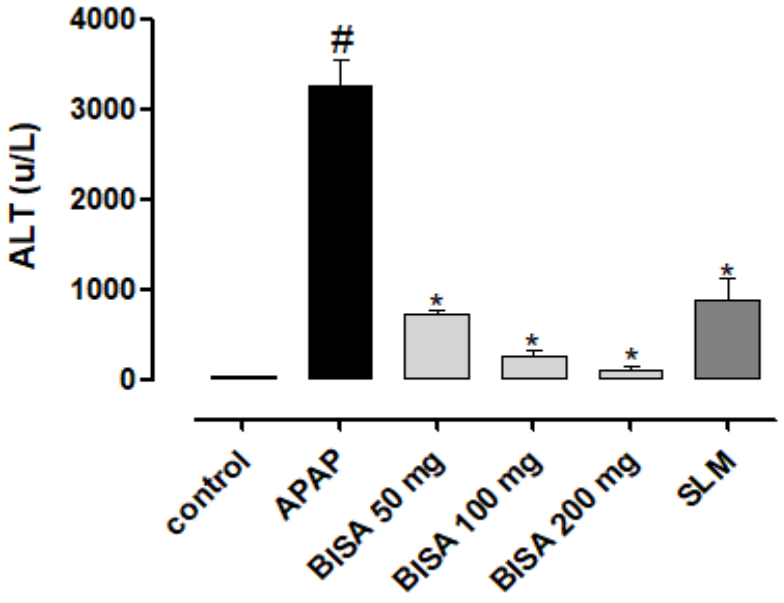

Figure 2. Represents serum ALT levels in all untreated groups, treated with APAP (250 mg / kg), pretreated with SLM (200 mg / kg) plus APAP (250 mg/Kg) and pretreated with BISA at concentrations of 50, 100 and 200 mg / kg, orogastric, 12 h after APAP poisoning. The results represent the mean ± SEM of 8 animals per group.

ISSN: 0975-8585

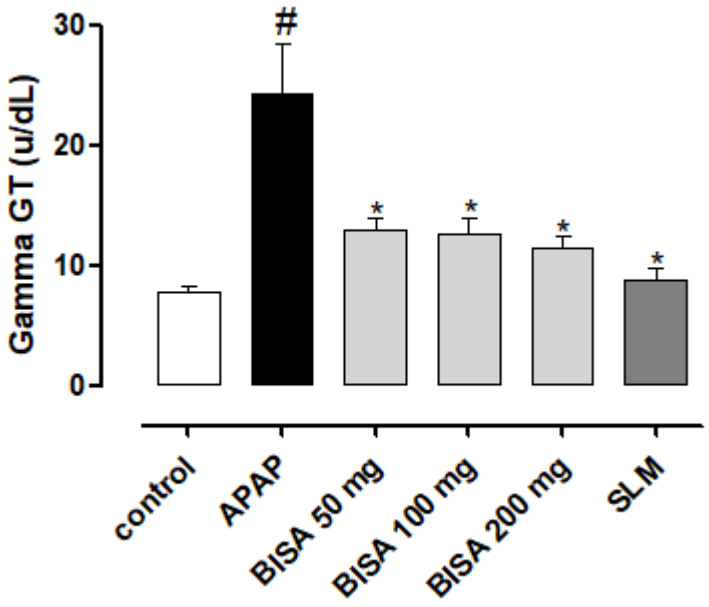

Figure 3. Represents serum γGT levels in all untreated groups, treated with APAP (250 mg / kg), pretreated with SLM (200 mg / kg) *plus* APAP (250 mg/Kg) and pretreated with BISA at concentrations of 50, 100 and 200 mg / kg, orogastric, 12 h after APAP poisoning. The results represent the mean ± SEM of 8 animals per group.

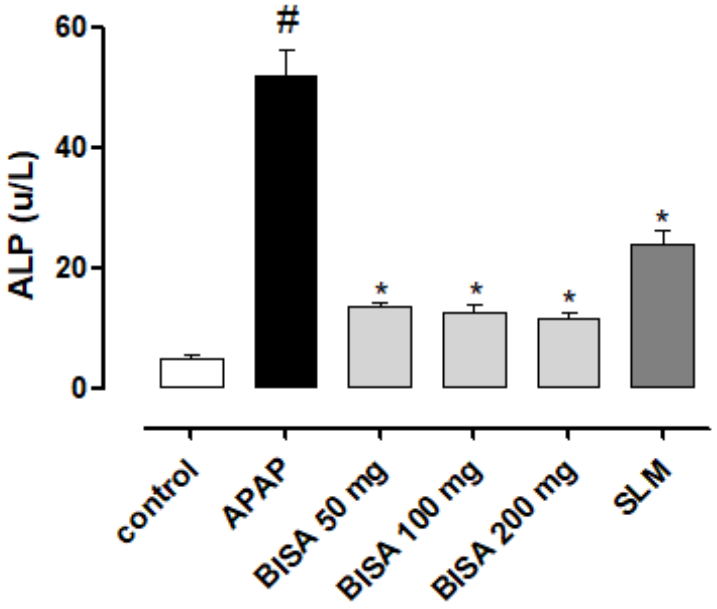

Figure 4. Represents serum ALP levels in all untreated groups, treated with APAP (250 mg / kg), pretreated with SLM (200 mg / kg) and pretreated with BISA at concentrations of 50, 100 and 200 mg / kg, orogastric, 12 h after APAP poisoning. The results represent the mean ± SEM of 8 animals per group.

ISSN: 0975-8585

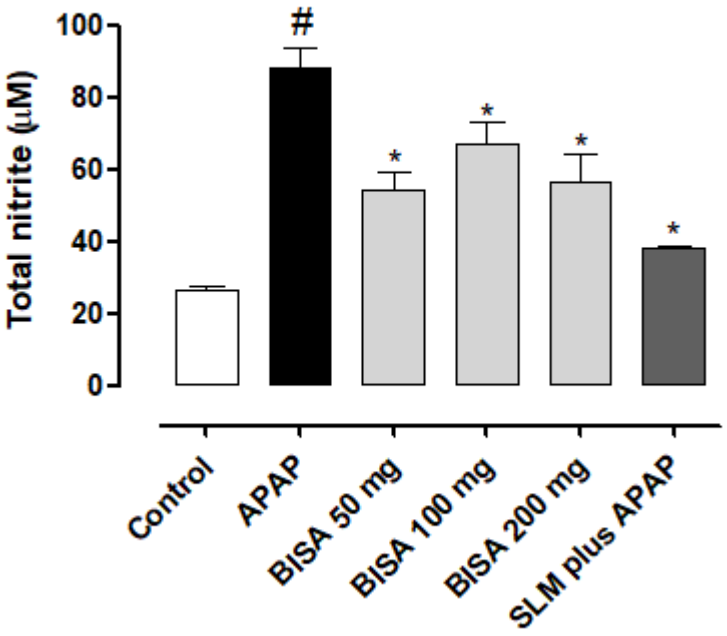

Figure 5. Represents the influence of pre-treatment with BISA on the production of nitrites in liver tissue of mice in DILI model and in untreated groups, treated with APAP (250 mg / kg), pre-treated with SLM (200 mg / kg) and pretreated with BISA at concentrations of 50, 100 and 200 mg / kg, orogastrically, 12 h after APAP intoxication. The results represent the mean ± SEM of 8 animals per group.

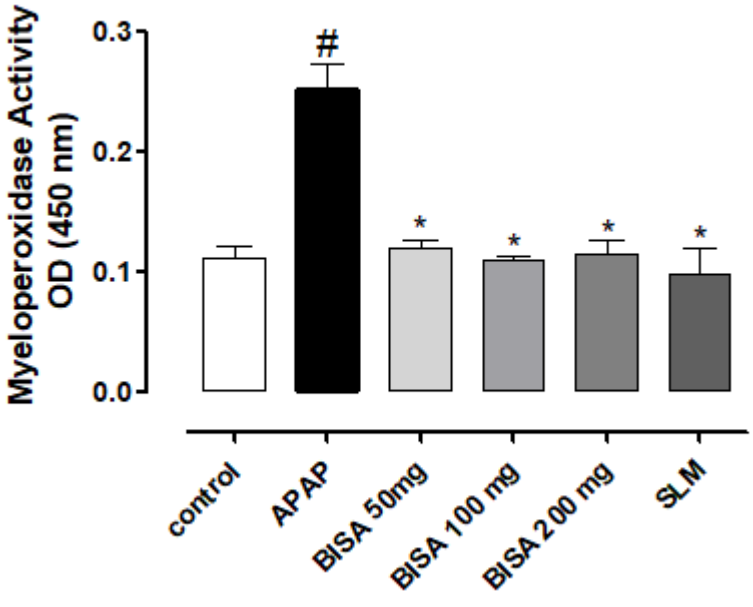

Figure 6. Represents the influence of pre-treatment with BISA on the production of MPO in liver tissue of mice in DILI model and in untreated groups, treated with APAP (250 mg / kg), pre-treated with SLM (200 mg / kg) *plus* APAP (250 mg/Kg) and pretreated with BISA at concentrations of 50, 100 and 200 mg / kg, orogastrically, 12 h after APAP intoxication. The results represent the mean ± SEM of 8 animals per group.

ISSN: 0975-8585

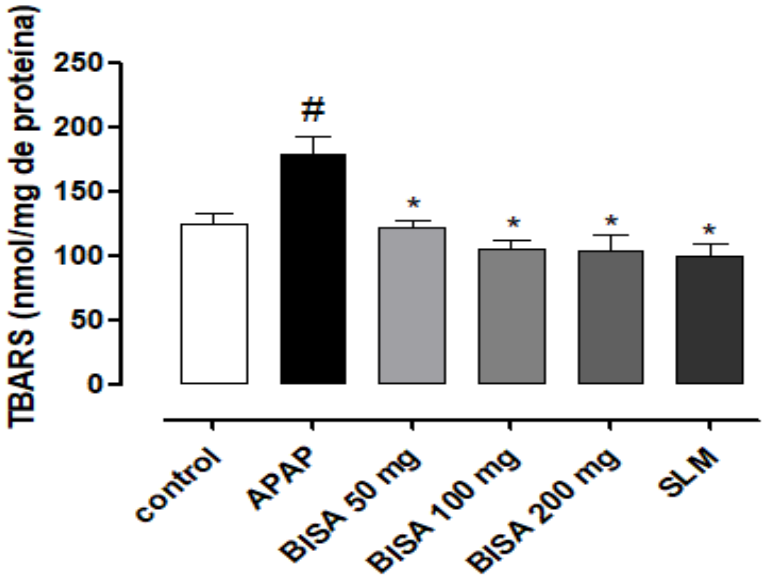

Figure 7. Concentration of TBARS in mouse liver homogenate in a DILI model and in untreated groups, treated with APAP (250 mg / kg), pretreated with SLM (200 mg / kg) and pretreated with BISA in the concentrations of 50, 100 and 200 mg / kg, by orogastric route, after 12 h of APAP poisoning. The results represent the mean ± SEM of 8 animals per group.

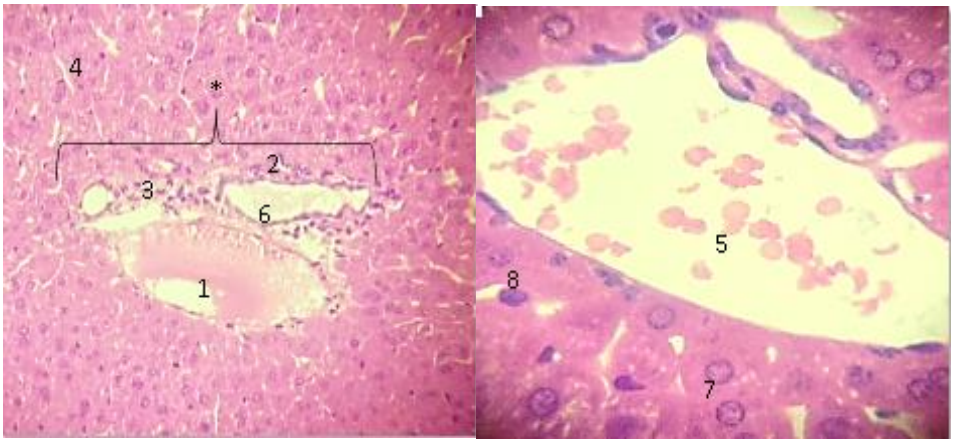

Figure 8. Microphotography of liver histological section of animals in the control group (vehicle - filtered water and silymarin alone) in 10x / 0.2 objective and HE staining. In A, the integrity of the hepatic portal space (*) and hepatic cell can be observed, as seen in: 1. Hepatic portal vein. 2. Terminal branch of the Hepatic Artery; 3. Bile duct; 4. Hepatocyte; 6. Lymphatic vessel. In B, the microphotography of histological sections of the liver of control animals (vehicle - filtered water) in a 40x / 0.2 objective and HE stain is observed. Being 5. Centrilobular vein; 7. Hepatocyte cords and 8. Sinusoidal capillaries, all with preserved cell architecture and without histological abnormalities.

ISSN: 0975-8585

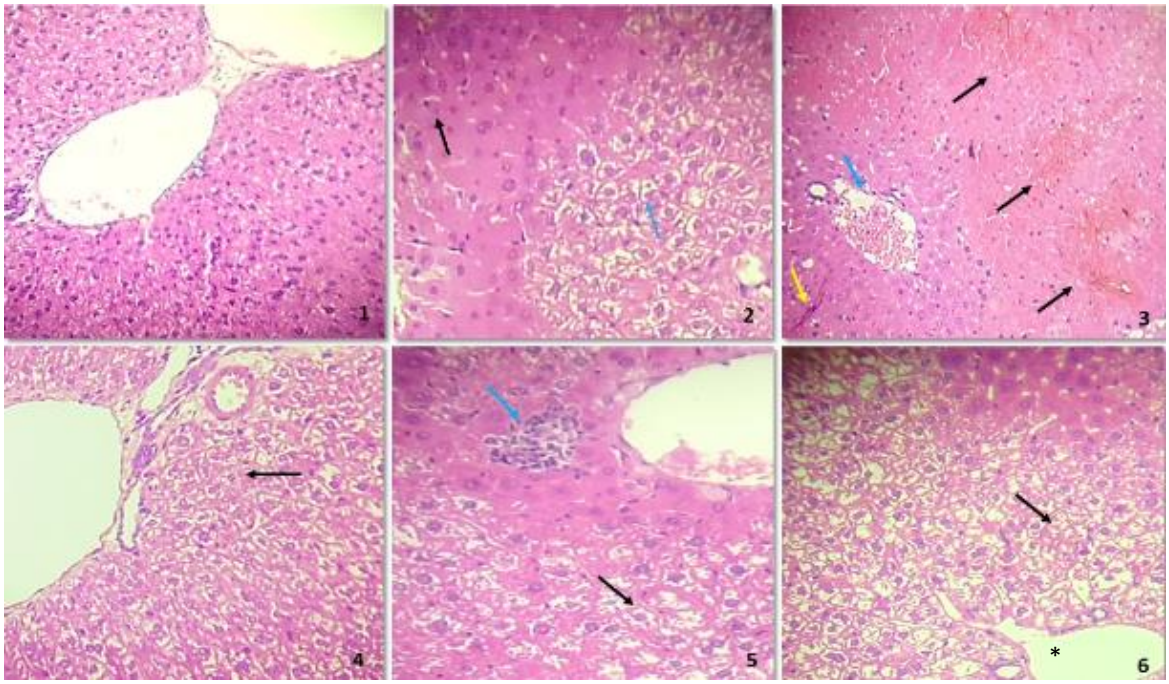

Figure 9. Morphological analysis of liver tissue in a 10x / 0.25: 1-objective filtered water: Liver tissue showing normal architecture, formed of hepatocyte cords with eosinophilic prismatic cytoplasm, central nucleus, sinusoid capillaries without changes and the portal triad with appearance containing venule, bile duct and artery without presenting any morphological alteration. 2-Silymarin (SLM) (200 mg / Kg) plus APAP (250 mg / Kg): Focally observed hepatocytes with pycnotic nucleus (black arrow) and hydropic degeneration (blue arrow). 3-APAP (250 mg / kg): Loss of normal liver parenchyma architecture. Dilatation of the centrilubular vein and sinusoid congestion and hemorrhage (blue arrow). Areas of hydropic degeneration and necrosis of hepatocytes in zone I, II, III (black arrow). 4- BISA 50 mg / kg: Hepatocytes showing vacuolar cytoplasm, focal hydropic degeneration (black arrow). 5- BISA 100 mg / kg: Hepatocytes showing vacuolar cytoplasm, multifocal hydropic degeneration (black arrow), focal inflammatory infiltrate (blue arrow) is observed. 6- BISA 200 mg / Kg: Hepatocytes showing vacuolar cytoplasm, multifocal hydropic degeneration (black arrow).

DISCUSSION

Acute drug-induced hepatitis has been, over time, a major clinical challenge, since it generates severe lesions to the liver tissue which have as a consequence, liver failure and disastrous clinical outcomes [26, 27].

The literature reports that the most frequent cases of acute hepatitis seen at the clinic are related to the intentional or unintentional ingestion of excessive doses of AINES and other medications [28-31]. Among the drugs that belong to the class of anti-inflammatory drugs, the one with the greatest hepatotoxic potential referred to in the literature is paracetamol (APAP) [32-33]. Considering these facts, the APAP-induced hepatotoxicity model is the most used for the investigation of plant drugs, which have a hepatoprotective possibility. Besides, the use of APAP to assess the hepatoprotective action of natural products is the most recommended, since the pathogenicity involved in liver injury is well established and has clinical relevance [34].

The main mechanisms involved in liver damage from APAP ingestion are related to the increase in reactive oxygen species (ROS), which stimulate lipid peroxidation and the acute inflammatory response, which together end up causing tissue necrosis [34-36]. The reactive metabolite of APAP - N-acetyl-p-benzoquinone imine (NAPQI), is the protagonist of the blockade of metabolic pathways that generate mitochondrial dysfunction and oxidative stress [37]. It is known that antioxidant defense systems, whether enzymatic or not, play a crucial role in inhibiting the harmful mechanisms caused by NAPQI, ROS and lipid peroxidation. The glutathione system (GSH) is the main responsible for the antioxidant activity in these conditions [38-39].

The therapeutic arsenal, approved by the FDA, used in hepatic inflammatory emergencies caused by medications, is extremely restricted. Being silymarin (SLM) and N-acetylcysteine (NAC), the most used antidotes in primary treatment, for APAP poisoning [40-41]. Asadi-Samani et al. (2015) [42] state that the use of conventional and synthetic drugs for the treatment of liver diseases is clinically unsatisfactory and causes serious side effects. Therefore, interest in studies with natural hepatoprotective therapies has been encouraged and widely disseminated.

BISA is a terpenoid, derived from chamomile and which has been studied for presenting pharmacological activities of interest. Among the attributions to its actions are: anti-inflammatory, anti-irritant, antioxidant, antibacterial, antinociceptive activity, among others [12-15, 43]. However, there are few studies on its hepatoprotective potential. Thus, the objective of this research was to evaluate the antioxidant potential of BISA, in different concentrations, in a DILI model induced by APAP.

In the present study, we observed that the previous administration of BISA, at concentrations of 100 mg/kg and 200 mg/kg, was able to drastically reduce serum levels of ALT and AST, GGT, and ALP after the induction of liver damage by paracetamol. However, the 50 mg / kg dose of BISA was able to reduce only the ALT, GGT and ALP enzymes. Thus, we can suggest that the results may be related to the concentration and bioavailability of BISA and a pharmacological profile of the dose-dependent type of the terpenoid [44].

A study conducted by Vinholes et al. (2013) [45] collaborate with our findings since the authors show that sesquiterpenoids, including BISA, have hepatoprotective activity, which is justified by their structural characteristics and the dose used. The authors attribute to BISA the capacity for hepatoprotection by two different mechanisms. One of them is related to high liposolubility and the other to the polarity of terpenoids. These properties promote membrane stabilization and can inhibit lipid peroxidation, induced by oxidative stress, as well as reduce cell death due to free radicals. Complementarily, the study data reveal that terpenoids have the ability to increase the activity of DOS (superoxide dismutase). The increase in DOS activity has a protective action against the production of free radicals and improves their elimination [46,47].

Liver damage induced by medications may follow a hepatocellular morphological pattern, which will be observed by an increase in serum transaminases (AST and ALT), as well as in ALP and GGT [48]. In our studies, we can observe that, also, BISA (50mg/kg, 100mg/kg and 200mg/kg) has been shown to reduce the levels of ALP and GGT, when compared to the APAP group and more, the results are comparable to the SLM plus APAP group. These results contribute to the finding that the terpenoid is able to minimize the damage in hepatocytes, related to APAP.

Nitrite levels were reduced in all doses of BISA administered when compared to the APAP group. Our data are contrary to those reported by Cavalcante et al (2020) [44], in this study the authors did not observe changes in the concentration of nitrites in the peritoneal exudate of animals treated with 50 mg/kg and 100 mg/kg of BISA. However, an important reduction of nitrites in the lung tissue was observed at doses of 50 mg/kg and 100 mg/kg. In addition, in the same study, the authors attribute to BISA the ability to contain the inflammatory infiltrate in the peritoneal cavity by inhibiting chemotaxis. We can argue that our results were partly discordant, due to differences in the mechanisms of injury involved in both studies, which differed by the type of experimental model and time of treatment of the animals.

However, the findings by Kim et al (2011) [49] are in line with our results. The authors report that the use of BISA, in a model of inflammation induced by LPS, was able to decrease the inflammatory activity in RAW264.7 cells, as well as, reduced the production of NO and E2 prostaglandins by inhibiting the cyclooxygenase - 2 and NOSi (inducible nitric oxide synthase).

Regarding MPO activity, our results showed that pretreatment with BISA, in all concentrations used, drastically reduced the activity of the enzyme, its effect being comparable to the negative and silymarin plus APAP controls. In this way, BISA has shown remarkable efficiency for inhibiting oxidative stress induced by APAP.

Liver injuries induced by APAP are originated from oxidative stress and elevation of reactive oxygen derivatives, which promotes increased hepatic lipid peroxidation and consequent elevation of TBARS [50]. The experimental results showed that treatment with BISA, in all doses used, was able to inhibit the levels of production of thiobarbituric acid. Such action has a hepatoprotective character, since the decrease in TBARS

levels demonstrates that there is less damage to the hepatocyte cell membrane lipids [51]. Our data are consistent with those of Rocha et al. (2011) [18] and Agatanovic-Kustrin et al. (2015) [52], who attribute to BISA the ability to increase DOS activity and act on the antioxidant defense mechanisms of cells. The authors state that the inhibition of lipid peroxidation and antioxidant activity are one of the protective mechanisms of the tested compound. Other authors [18, 46, 53] collaborate with our results, since they attribute to the terpenoid antioxidant, anti-apoptotic and anti-inflammatory activity, demonstrated by its ability to stabilize membranes and improve mitochondrial function.

In our studies, we were able to verify that the serum results of ALT, AST, GGT, ALP and those of cellular damage are consistent with the histopathological findings. Serum and tissue analyzes showed that the effects of BISA, at all doses, were comparable and even higher than those of the SLM plus APAP group.

Gathering all the findings, we can conclude that (-) - α - bisabolol was able to reduce liver damage in animals treated with APAP. It can be inferred that BISA is capable of decreasing the deleterious effects of oxidative stress on liver cells, as well as causing a protective effect on liver tissue due to its ability to decrease lipid peroxidation and inflammatory activity promoted by paracetamol.

Considering our data, BISA has an important hepatoprotective potential and can be considered a useful tool for the treatment of situations in which there is severe acute liver failure, caused by toxicity. However, further investigations into BISA's activity in these clinical conditions are necessary.

CONCLUSIONS

The (-) α- bisabolol proved to have an extremely relevant hepatoprotective characteristic, which can guarantee its clinical use as a preventive and treatment drug for cases of medicated hepatitis. Chamomile essential oil contains terpenoid compounds that have been shown to have important anti-inflammatory and antioxidant activity in doses of 50, 100, and 200 mg/kg. All concentrations used were able to significantly reduce the damage to liver tissue, caused by the reactive derivative of APAP, as well as the intense inflammatory process that NAPQI stimulates at a dose of 250mg / Kg. This statement can be confirmed by the reduction of all levels of enzyme markers, related to liver damage, as well as by the reduction of the production of TBARS, NO, and MPO. The results also demonstrated that BISA can be used safely since it does not present cytotoxicity. Besides, the antioxidant and anti-inflammatory activity of BISA is dose-dependent, which makes it very useful and versatile as an option drug in cases of therapies with hepatoprotective purposes.

ACKNOWLEDGMENT

We gratefully thank the University Cesumar, especially the Department of Medicine for the encouragement and full support for this research. We would also like to thank the members of the Pharmaceutical Sciences Program and the Inflammation Laboratory, especially the laboratory technician Jailson for their assistance in carrying out our studies.

REFERENCES

[1] Rowe IA. Lessons from Epidemiology: The Burden of Liver Disease. Dig Dis. 2017; (4), pp. 304-309.
[2] GBD 2017 DALYs and HALE Collaborators. Global, regional, and national disability-adjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study. 2017; 392, pp. 1859-1922.
[3] Reis ARM, Braga lS, Pavanelli MF. Hepatotoxicidade pelo uso de paracetamol: Uma revisão da literatura. Revista Iniciare. 2017; 2 (1), pp. 2-9.
[4] Chalasani NP, Hayashi PH, Bonkovsky HL, Navarro VJ, Lee WM, Fontana RJ. Diretriz Clínica ACG: o diagnóstico e tratamento de lesão hepática idiossincrática induzida por drogas. Am J Gastroenterol. 2014; 109 (7), pp. 950–966.
[5] Wong A, Graudins A. Risk prediction of hepatotoxicity in paracetamol poisoning. Clin Toxicol (Phila). 2017; 55(8), pp. 879-892.
[6] Ogilvie JD, Rieder MJ, Lim R. Acetaminophen overdose in children. CMAJ. 2012; 184 (13), pp. 1492–1496.

[7] Santos CCO, Moraes MO. Hepatotoxicidade por paracetamol. Monografia de Mestrado, Faculdade de Pindamonhangaba, 24p.,2014.
[8] Bajt ML, Knight TR, Lemasters JJ, Jaeschke H. Acetaminophen-induced oxidant stress and cell injury in cultured mouse hepatocytes: protection by N-acetyl cysteine. Toxicol Sci. 2004; 80 (2), pp. 343-349.
[9] Stine JG, Lewis JH. Current and future directions in the treatment and prevention of drug-induced liver injury: a systematic review. Expert Rev Gastroenterol Hepatol. 2016; 10(4), pp. 517-536.
[10] Chughlay MF, Kramer N, Spearman CW, Werfalli M, Cohen K. N-acetylcysteine for non-paracetamol drug-induced liver injury: a systematic review. Br J Clin Pharmacol. 2016; 81 (6), pp. 1021-1029.
[11] Mahmoudi, G.A., Astaraki, P., Mohtashami, A.Z., Ahadi, M. Nacetylcysteine overdose after acetaminophen poisoning. Int. Med. Case Rep. 2015; 8, pp. 65- 69.
[12] Pacífico et al. Prospecção Científica e Tecnológica de Matricaria recutita L. (Camomila). Revista GEINTEC. 2018, 8, (2), pp. 4339-4356.
[13] Mehmood M.H. et al. Antidiarrhoeal, antisecretory and antispasmodic activities of Matricaria chamomilla are mediated predominantly through K+ -channels activation. BMC Complement. Altern. Med. 2015; 15, pp. 2-9.
[14] Munir, N. et al. Evaluation of antioxidant and antimicrobial potential of two endangered plant species atropa belladonna and matricaria chamomilla. Afr. J. Tradit. Complement. Altern. Med. 2014; 11, pp. 111-117.
[15] Ranpariya V.L et al. Neuroprotective activity of Matricaria recutita against fluoride-induced stress in rats. Pharm. Biol. 2011; 49, pp. 696-701.
[16] Gupta V et al. Pharmacological Potential of Matricaria recutita-A Review. International Journal of Pharmaceutical Sciences and Drug Research. 2010; 2(1), pp. 12-16.
[17] Solovastru LG et al. Randomized, controlled study of innovative spray formulation containing ozonated oil and α-bisabolol in the topical treatment of chronic venous leg ulcers. Adv Skin Wound Care. 2015; 28 (9), pp. 406-409.
[18] Rocha NR et al. Anti-nociceptive and anti-inflammatory activities of (-)-α-bisabolol in rodents. Naunyn Schmiedebergs Arch Pharmacol. 2011; 384 (6), pp. 525-533.
[19] Moura Rocha NF et al. Gastroprotection of (-)-alpha-bisabolol on acute gastric mucosal lesions in mice: the possible involved pharmacological mechanisms. Fundam Clin Pharmacol. 2010; 24 (1), pp. 63-71.
[20] MacKay DL, Blumberg JB. A Review of the bioactivity and potential health benefits of chamomile tea (Matricaria recutita L.) Phytother Res. 2006; 20 (7), pp. 519-530.
[21] Gomes- Carneiro et al. Evaluation of mutagenic and antimutagenic activities of alpha-bisabolol in the Salmonella/microsome assay. Mutat Res. 2005; 1;585(1-2), pp. 105-112.
[22] Del Ré PV., Jorge N. Spices as natural antioxidants: their application in food and implication for health. Rev. bras. plantas med. 2012; 14 (2), pp.389-399.
[23] Toscan CM. Atividade antimicrobiana e antioxidante de terpenoides. Dissertação apresentada ao Programa de Pós- Graduação de Biotecnologia da Universidade de Caxias do Sul. 2010; 84p.
[24] Saleh, T.S.F., J.B. Calixto and Y.S. Medeiros. Effects of anti-inflammatory drugs upon nitrate and myeloperoxidase levels in the mouse pleurisy induced by carrageenan. Peptides. 1999; 20, pp. 949-956.
[25] Buege J. A., Aust S. D., "Microsomal Lipid Peroxidation," Vol. 52, ed. by Fleisher S., Parker I., Academic Press, New York, 1978, pp. 302-310.
[26] Ortega-Alonso A, Andrade RJ. Chronic liver injury induced by drugs and toxins. J Dig Dis. 2018; 19 (9), pp. 514-521.
[27] Kaplowitz N. Idiosyncratic drug hepatotoxicity. Nat Rev Drug Discov. 2005; 4 (6), pp. 489-99.
[28] WHO - World Health Organization. Regulatory situation of herbal medicines. A worldwide review, Geneva, 1998.
[29] Lunardelli MJM, Becker MW e Blatt CR. Lesão hepática induzida por medicamentos: qual o papel do farmacêutico clínico? Rev. Bras. Farm. Hosp. Serv. Saúde. 2016; 7 (4), pp. 31-35.
[30] Farias PO. Epidemiological aspects of intoxications by non-opioid analgesics and non steroidal anti-inflammatory drugs in an emergency public hospital of Brazil. Rev Med Minas Gerais. 2016; 26 (5), pp. 11-15.
[31] Tajiri K, Shimizu Y. Practical guidelines for the diagnosis and early management of drug-induced liver injury. World J Gastroenterol, 2008; 14, pp. 6774-6785.
[32] Piotrowska Natalia, Jolanta Klukowska-Ro¨tzler , Beat Lehmann , Gert Krummrey, Manuel Haschke, Aristomenis K. Exadaktylos, Evangelia Liakoni. Presentations Related to Acute Paracetamol Int. Emergency Medicine International. 2019; pp. 1-8.

[33] Toussaint K, Yang XC, Zielinski MA, Reigle KL, Sacavage SD, Nagar S, Raffa RB. What do we (not) know about how paracetamol (acetaminophen) works? J Clin Pharm Ther. 2010; 35, pp. 617–638.
[34] Du Kuo. Anup Ramachandran, Hartmut Jaeschke. Oxidative stree during acetaminophen hapatotoxicity: Sources, pathophysiological role and therapeutic potencial. Redox Biology. 2016; 10, pp. 148-156.
[35] Zhang, Yinfeng & Dai, Menghong & Yuan, Zonghui. Methods for Detection of Reactive Oxygen Species. Analytical Methods. 2018; 10 (38), pp. 1-17.
[36] Jaeschke H, Ramachandran A. Oxidant Stress and Lipid Peroxidation in Acetaminophen Hepatotoxicity. React Oxyg Species (Apex). 2018; 5 (15), pp. 145-158.
[37] McGill MR, Williams CD, Xie Y, Ramachandran A, Jaeschke H. Lesão hepática induzida por acetaminofeno em ratos e camundongos: comparação de adutos de proteína, disfunção mitocondrial e estresse oxidativo no mecanismo de toxicidade. Toxicol Appl Pharmacol. 2012; 264 (3), pp. 387-394.
[38] Sümer, Engin, Gözde Erkanli Senturkb, Özlem Unay Demirelc, Erdem Yesilada. Comparative biochemical and histopathological evaluations proved that receptacle is the most effective part of Cynara scolymus against liver and kidney damages. Journal of Ethnopharmacology. 2020; 249, pp. 1-8.
[39] El-Shafey, G.M. Abd-Allah, A.M. Mohamadin, G.I. Harisa, A.D. Mariee. Quercetin protects against acetaminophen-induced hepatorenal toxicity by reducing reactive oxygen and nitrogen species. Pathophysiology. 2015; 22, pp. 49-55
[40] Baghbahadorani FK, Miraj S. The Impact of Silymarin on Improvement of Hepatic Abnormalities in Patients with Severe Preeclampsia: A Randomized Clinical Trial. Electron Physician. 2017; 1;9 (8) pp. 5098-5106.
[41] Heard K.J. et al. Acetaminophen-cysteine adducts during therapeutic dosing and following overdose. BMC Gastroenterology. 2011; 11 (20), pp. 1-9.
[42] Asadi-Samani M, Kafash-Farkhad N, Azimi N, Fasihi A, Alinia-Ahandani E, Rafieian-Kopae M. Medicinal plants with hepatoprotective activity in Iranian folk medicine. Asian Pac J Trop Biomed 2015; 5 (2), pp. 146-157.
[43] Teixeira GFD, Vieira-Neto AE, Costa FN, Alves e Silva AR, Campos AR. Antinociceptive effect of (-)-a-bisabolol in nanocapsules. Biomedicine & Pharmacotherapy. 2017; 91, pp. 946-950.
[44] Cavalcante HAO, Silva-Filho SE, Wiirzler LAM. Efeito de (-) - α-Bisabolol na Resposta Inflamatória no Modelo Experimental de Infecção Sistêmica em Camundongos C57BL / 6. Inflammation. 2020; 43, pp. 193-203.
[45] Vinholes J, Rudnitskaya A, Gonçalves P, Martel F, Coimbra MA, Rocha SM. Hepatoprotection of sesquiterpenoide: A quantitative dtructure-activity relationship (QSAR) approuch. Food Chem. 2014; 145, pp. 78-84.
[46] Leite GO, Ecker A, Seeger RL, Krum BN, Lugokenski TH, Fachinetto R, Sudati JH, Barbosa NV, Wagner C. Protective effect of (−)-α-bisabolol on rotenone-induced toxicity in Drosophila melanogaster. Canadian Journal of Physiology and Pharmacology. 2018; 96 (4), pp. 359-365.
[47] Nunes RCA, Viana RS, Neto NBM. Atividade enzimática da superóxido dismutase em resposta aos fitorreguladores auxina e citocinina em Gerbera sp. Comunicata Scientiae. 2015; 6 (1), pp. 83-89.
[48] Bertolami MC. Mecanismos de hepatotoxicidade. Arq. Bras. Cardiol. 2005; 85 (5), pp.25-27.
[49] Kim S, Jung E, Kim JH, Park YH, Lee J, Park D. Inhibitory effects of (-)-α-bisabolol on LPS-induced inflammatory response in RAW264.7 macrophages. Food Chem Toxicol. 2011; 49 (10), pp. 2580-2585.
[50] Albano E, Rundgren M, Harvison PJ, Nelson SD, Moldeus P. Mechanisms of N-acetyl-p-benzoquinone imine cytotoxicity. Mol. Pharmacol. 1985; 28, pp. 306-311.
[51] Jiang W, Huang S, Matsuda Y, Saito H, Uramaru N, Ho H, Wu J, Huang G. Protective Effects of Tormentic Acid, a Major Component of Suspension Cultures of Eriobotrya japonica Cells, on Acetaminophen-Induced Hepatotoxicity in Mice. Molecules. 2017; 22 (830), 1-15.
[52] Agatonovic-Kustrin S, Ortakand DB, Morton DW, Yusof AP. Rapid evaluation and comparison of natural products and antioxidant activity in calendula, feverfew, and German chamomile extracts. Journal of Chromatography A. 2015; 1385, pp. 103-110.
[53] Amora-Silva BF, Ribeiro SC, Vieira CL, Mendes FR, Vieira-Neto AE, Abdon APV, Costa FN, Campos AR. Clinical efficacy of new α-bisabolol mouthwashes in postoperative complications of maxillofacial surgeries: a randomized, controlled, triple-blind clinical trial. Clin Oral Investig. 2019; 23 (2), pp. 577-584.