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ORIGINAL ARTICLE

Effectiveness of resveratrol as a hepatoprotector in a rat model of paracetamol-induced liver injury

Eficácia do resveratrol como hepatoprotetor em modelo de rato com lesão hepática induzida por paracetamol

Renan Marcel Bonilha Dezena[1], Sthéfani Schiavon oberta da Rocha Braga[1], Matheus Arnosti Landi[1], Gustavo Henrique da Silva[1], Gisele Mara Silva Gonçalves[1]

1. Department of Pharmaceutical Sciences, Pontifical Catholic University of Campinas (PUC-CAMPINAS), Campinas, SP, Brazil.

Abstract

Objective: Evaluate the effectiveness of resveratrol as a hepatoprotector in a rat model of paracetamol-induced liver injury and its biodistribution to understand its pharmacokinetics. **Methodology:** As an experimental approach, animals were divided into the test group with 4 subgroups and the control group with 4 subgroups. Animals of the "treated" group were subjected to resveratrol pre-treatment for eight days, followed by intoxication with a high dose of paracetamol on the 8th day. Animals were euthanized to collect the blood and liver tissue samples 24 and 72 h after the last administration. Hepatoprotective activity was evaluated through serum levels of glycogen and hepatic enzymes, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), histological and morphometric analysis of the liver tissue. For biodistribution analysis, different organs (organs, kidneys, heart and lungs) were collected and macerated, and resveratrol was quantified using high-performance liquid chromatography. Statistical analyses of morphometry, transaminases and alkaline phosphatase measurements, and biodistribution results were performed using GraphPad Prism® 3.0. Differences between groups were compared using ANOVA, followed by the Bonferroni test. Statistical significance was set at $p < 0.05$. **Results**: Resveratrol has a hepatoprotective action against acute intoxication by paracetamol, as evidenced by the histological decrease in necrosis and inflammatory foci, preservation of glycogen and other 1,2-glycols in zone 3, and reduction of serum ALT and AST levels. An increased presence of collagen was observed in acinar zones 1 and 3 with picrosirius red staining; therefore, quantification was performed in these regions showing smaller collagen areas in the R and RP groups than in the PC and NC groups Paracetamol caused a significant reduction in the resveratrol concentration in serum and the organs studied, indicating that the antioxidant activity of resveratrol is related to its hepatoprotective action. **Conclusion:** Resveratrol has hepatoprotective properties and can mitigate some of the liver damage caused by high doses of paracetamol, as indicated by changes in tissue characteristics and liver enzyme levels.

Keywords: Biodistribution; pharmacokinetic; high-performance liquid chromatograph (HPLC).

Resumo

Objetivo: Avaliar a eficácia do resveratrol como hepatoprotetor em modelo de rato com lesão hepática induzida por paracetamol e sua biodistribuição para compreender sua farmacocinética. **Metodologia**: Como abordagem experimental, os animais foram divididos em grupo teste com 4 subgrupos e grupo controle com 4 subgrupos. Os animais do grupo "tratado" foram submetidos ao pré-tratamento com resveratrol durante oito dias, seguido de intoxicação com alta dose de paracetamol no oitavo dia. Os animais foram eutanasiados para coleta de amostras de sangue e tecido hepático 24 e 72 horas após a última administração. A atividade hepatoprotetora foi avaliada através dos níveis séricos de glicogênio e de enzimas hepáticas, como aspartato aminotransferase (AST), alanina aminotransferase (ALT) e fosfatase alcalina (ALP), análise histológica e morfométrica do tecido hepático. Para análise de biodistribuição, diferentes órgãos (órgãos, rins, coração e pulmões) foram coletados e macerados, e o resveratrol foi quantificado por cromatografia líquida de alta eficiência. Análises estatísticas de morfometria, medidas de transaminases e fosfatase alcalina e resultados de biodistribuição foram realizadas utilizando GraphPad Prism® 3.0. As diferenças entre os grupos foram comparadas por meio de ANOVA, seguida do teste de Bonferroni. A significância estatística foi estabelecida em $p < 0,05$. **Resultados**: O resveratrol tem ação hepatoprotetora contra a intoxicação aguda por paracetamol, evidenciada pela diminuição histológica da necrose e dos focos inflamatórios, preservação do glicogênio e outros 1,2-glicóis na zona 3 e redução dos níveis séricos de ALT e AST. Foi observada presença aumentada de colágeno nas zonas acinares 1 e 3 com coloração picrosirius red; portanto, foi realizada quantificação nessas regiões mostrando menores áreas de colágeno nos grupos tratados com resveratrol e resveratrol associado com paracetamol do que nos grupos controles positivo e negativo. O paracetamol causou redução significativa na concentração de resveratrol no soro e nos órgãos estudados, indicando que a atividade antioxidante do resveratrol está relacionada à sua ação hepatoprotetora. **Conclusão**: O resveratrol possui propriedades hepatoprotetoras e pode mitigar alguns dos danos hepáticos causados por altas doses de paracetamol, conforme indicado por alterações nas características dos tecidos e nos níveis de enzimas hepáticas.

Palavras-Chave: Biodistribuição; farmacocinética; cromatógrafo líquido de alta eficiência (CLAE).

INTRODUCTION

The liver is responsible for bile secretion, glucose storage in the form of glycogen, cholesterol synthesis, and biotransformation of medicines, nutrients, bioactive, and toxic substances. Hepatocyte injury is very common because of the intense metabolism of a wide variety of substances[1]. Hepatitis is caused by toxicity arising from the inflammatory process caused by the

Correspondence: Renan Marcel Bonilha Dezena. Adrees:Av. John Boyde Dunlop, s/n, Jd. Ipaussurama, Campinas-SP. CEP: 13060-904. E-mail: renan_marcel@hotmail.com

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action of drugs, chemicals, viruses, and microorganisms[2].

Studies in the USA showed that liver injury induced by active pharmaceutical ingredients (API) is a relevant issue, representing approximately 5% of hospitalizations for adverse drug reactions[3].

Among the several factors that participate in cellular aggression, chemical bond formation between cellular components, oxidation, and lipid peroxidation are the most determinant, causing disturbances in mitochondrial metabolism and cytoskeleton disorganization, and, in more extreme cases, stimulating apoptosis signaling and cell death[4].

Paracetamol was first synthesized in 1878 by the American scientist Harmon Northrop Morse5. However, only after the 1950s was it introduced in the market as a pharmaceutical product[5]. Paracetamol is currently one of the most prescribed and administered analgesics in most countries; however, it is highly hepatotoxic when used indiscriminately without medical advice or even in cases of attempted suicide[6].

The trigger for this liver toxicity is related to the biotransformation of API into N-acetyl-p-benzoquinone imine (NAPQI), a reactive compound that eliminates glutathione, resulting in glutathione depletion, and impairs the proper functioning of the entire cellular respiratory system via the production of superoxide due to the formation of adducts with mitochondrial proteins. In sequence, the nitration of proteins inside the mitochondria and, consequently, oxidative stress causes the alteration in the cell membrane permeability, mitochondria rupture, DNA fragmentation, and apoptosis[7].

Resveratrol (C14H12O3) is a polyphenol naturally found in over 70 plant species, including the vine (Vitis vinifera)[8]. Recently, resveratrol has been shown to prolong life and confer vasoprotection in animal models of diabetes mellitus, improving endothelial function and attenuating vascular inflammation[9-10]. Similar protective effects were observed in aged rats treated with resveratrol[11]. Studies in humans showed that a resveratrol-predominant diet, such as the one practiced in the Mediterranean, is associated with reduced incidence of cardiovascular mortality[12]. Resveratrol occurs in two isoforms (cis and trans), and trans-resveratrol is more biologically active[13].

In recent decades, resveratrol has gained the attention of scientists for its anticancer, anti-inflammatory, hypoglycemic, and cardiovascular benefits[14]. Resveratrol has been the focus of in vitro and in vivo studies, investigating its biological activities, mainly its antioxidant, anti-inflammatory, anti-platelet, antiatherogenic, estrogen-like, growth-promoting, growth inhibition, immunomodulation, and chemoprevention properties[15]. Resveratrol is a free radical scavenger and potent antioxidant, enhancing the activities of a variety of antioxidant enzymes[16]. The antioxidant activity of polyphenolic compounds depends on the redox properties of their phenolic hydroxyl groups and the electron displacement potential throughout their chemical structure[17].

Resveratrol was found to have hepatoprotective and antifibrogenic activities in liver injury induced by dimethylnitrosamine, suggesting that it may be useful in preventing liver fibrosis development[18]. Similar results were also observed in fishes with hydrogen peroxide-induced liver injury. Thus, resveratrol may be a therapeutic option to minimize oxidative stress owing to its antioxidant activity against free radicals, in addition to its anti-inflammatory action according to the literature[19].

This study aimed to evaluate the effectiveness of resveratrol as a hepatoprotector in a rat model of paracetamol-induced liver injury and its biodistribution to understand its pharmacokinetics.

MATERIAL AND METHODS

Animals and experimental design

Forty nonisogenic male Albino Wistar rats (Rattus norvegicus) were used in this study at 50 days of age. Animals were provided by the Campus II, Animal House of Pontifical Catholic University (PUC) of Campinas.

Rats were maintained in the Animal House of the Laboratory of Surgical Technique and Experimental Surgery at the Life Sciences Center of Pontifical Catholic University of Campinas, with controlled illumination and ventilation, and provided solid Nuvilab ration and ad libitum water until the age of 60 days. The study was approved by the Animal Ethics Committee of Pontifical Catholic University of Campinas, according to CI CEUA No. 003/2012 (protocol number 2012070332).

Animals were divided into two groups (20 animals in each group): the treated and control groups. The treated group was further divided into four subgroups: the resveratrol 24 h (R24), resveratrol 72 h (R72), resveratrol + paracetamol 24 h (RP24), and resveratrol + paracetamol 72 h (RP72) groups, composed of five animals each. Animals in the resveratrol 24 and 72 h groups received resveratrol suspension at a dose of 10 mg/kg/day by oral route (o.r.) for a period of eight days and were sacrificed 24 h and 72 h, respectively, after the last administration. Animals in the resveratrol + paracetamol 24 and 72 h groups received resveratrol suspension at a dose of 10 mg/kg/day by o.r. for a period of eight days and a combined last administration of resveratrol and paracetamol (Pharma Nostra lot 09124425G) at a dose of 3 g/kg by o.r. These rats were euthanized after 24 and 72 h, respectively.

The control group was also divided into four subgroups: the negative control 24 h (NC24), negative control 72 h (NC72), positive control 24 h (PC24), and positive control 72 h (PC72), composed of five animals in each group. Animals in the negative control 24 and 72 h groups received only water for a period of eight days by o.r. and were euthanized 24 and 72 h after the last water administration, respectively. Animals in the positive

control 24 and 72 h groups received water by o.r. for a period of eight days and a paracetamol solution at a dose of 3 g/kg on the last day. These rats were euthanized after 24 and 72 h, respectively. The paracetamol dose was established according to a previously published protocol [20], and the resveratrol dose was determined according to the method described by literature [21]. The entire treatment was administered via the oral route, that is, intragastric gavage. Resveratrol for administration was prepared daily and diluted with water. The gavage was performed at 8 am and no anesthetic was used in its needle.

For the sacrifice procedure, animals were first anesthetized with a ketamine solution (100 mg/kg intraperitoneal). Ketamine was chosen as the anesthetic agent because it is non-hepatotoxic and does not interfere with experimental results. An incision was made in the thoracic region to access the left heart ventricle, and blood was drawn to measure liver transaminase and alkaline phosphatase levels. The anesthetic plane was deepened until the animal was sacrificed, and the liver tissue was removed. The liver was fragmented and fixed in a 10% buffered formalin solution (for histological analysis). The kidneys, heart, and lungs were excised. Fragments weighing approximately 200 mg were frozen for subsequent maceration.

Histological examination.

Liver tissue underwent standard histological processing, namely tissue paraffin inclusion (Synth®), followed by the obtention of 5 µm thick sections using a Leica RM2245 Rotatory Microtome. The slides (with sections) were stained with hematoxylin-eosin, picrosirius red, and periodic acid Schiff, and images were obtained using a photomicroscope (Nikon Eclipse E200) coupled to a camera (Nikon Colpix 4500). For each group, the type and extension of the hepatic lesions were evaluated by the presence of steatosis, inflammatory infiltrate, fibrosis, and necrosis.

Measurement of liver transaminases and alkaline phosphatase

After blood collection, the samples (n=4 per group) were centrifuged at 3000 rpm for 5 min for serum separation. The levels of enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), indicators of hepatocellular damage, were evaluated using LaborLab® enzyme test kits (kinetic-colorimetric method), and results were read in a UV Varian spectrophotometer, according to manufacturer's specifications.

Reference values for rats, AST: 50–150 U/L and ALT: 10–40 U/L (Hasan, Tamanna, Haque, 2018).

Biodistribution analysis of resveratrol

After collection, organs were macerated at a ratio of 200 mg of tissue to 0.2 ml of ethanol P.A. (Merck®), and the suspension was centrifuged at 3000 rpm for 5 min. This process was repeated five times to maximize resveratrol extraction. The supernatant was concentrated in an incubator at 50°C under a continuous flow of nitrogen. Samples were resuspended in ethanol (0.5 ml) and filtered through a 45 µm filter membrane. 50 µL of each sample were directly injected into a high-performance liquid chromatograph (HPLC) Varian Prostar 320/210. The column used was a Microsorb-MV 100-S C18 250x4.6. Ethanol P.A. was used as a mobile-phase solvent at an elution flow rate of 1 ml/min. Chromatographic peaks were quantified using wavelengths of 370 nm. The calibration curve for quantification was constructed using the following standards of resveratrol: 0.002, 0.006, 0.01, and 0.02 mg/ml.

Analysis results

Liver histological analysis was performed using a specific methodology for each parameter. For necrosis evaluation, in HE-stained slides (a section of 2 liver fragments from different lobules), the number of centrilobular veins (clv) affected by the lesion was determined (vcl), and the results were expressed in vcl/cm2. The TPS Dig® 1.30 software was used. For fibrosis evaluation in Picrosirius Red-stained slides, five micrographs of the centrilobular area and five micrographs of the portal area randomly obtained at 480x magnification were analyzed (a total of 50 micrographs per group) using the AreaMed® software to measure the fibrosis-compromised area (collagen fibers). To evaluate the distribution of glycogen and other 1,2-glycols, a qualitative assessment of PAS-stained slides was performed, comparing treated animals to negative and positive control groups.

Statistical analyses of morphometry, transaminases and alkaline phosphatase measurements, and biodistribution results were performed using GraphPad Prism® 3.0. Differences between groups were compared using ANOVA, followed by the Bonferroni test. Statistical significance was set at $p < 0.05$.

RESULTS

During the energy production process, free radicals (reactive oxygen/nitrogen species) are formed naturally and continuously, and most organisms have protective mechanisms against these oxidants. Maintaining a balance between oxidant and antioxidant species within intracellular and extracellular environments is essential for optimal metabolism. Free radicals can damage lipids, proteins, and DNA. Under normal conditions, we present mechanisms to neutralize ROS excess, thus protecting from an imbalance of excess oxidants, often referred to as oxidative stress[22].

Oxidative stress is an important contributor to various disorders and chronic diseases, such as cancer, cardiovascular disease, osteoporosis, diabetes, and cataracts. Antioxidants eliminate free radicals and prevent their deleterious effects; however, these mechanisms become ineffective in situations of exacerbated production and activity, leading to cellular and tissue damage[23]. The search for new molecules or substances with antioxidant capacities becomes important to combat ROS-

triggered injuries and diseases. In this study, we examined the protective effects of resveratrol against paracetamol-induced liver injury.

Histological analysis was performed with three main focuses: (a) observation and quantification of necrosis and inflammatory infiltration (hematoxylin-eosin staining), (b) quantification of collagen fibers for fibrosis determination (picrosirius red staining), and (c) qualitative analysis of glycogen and other 1,2-glycols (PAS staining).

Administration of resveratrol in an experimental model of methotrexate-induced liver injury showed reduced histological changes, such as focal necrosis, sinusoidal dilatation and congestion, Kupffer cell hyperplasia, and inflammatory infiltrate, in the portal and central lobular regions[21].

In the PC group, no steatosis but fibrosis and necrosis with infiltration of polymorphonucleates were observed. Necrosis was observed predominantly in the central-lobular region, especially in 72-h animals. This finding guided the quantification of inflammatory/necrotic foci in this region, while fibrosis was quantified in acinar zones 1 and 3 in picrosirius red-stained slides.

Figure 1 shows liver tissue preservation in NC groups, while foci of inflammatory infiltrate, accompanied by necrosis, can be observed in the PC and RP groups. In the PC24 and PC72 groups, there is a greater predominance of this type of lesion in the central-lobular regions affected (Figure 2).

Figure 1. Light micrographs showing the presence and/or absence of necrosis and inflammatory foci (arrows) using hematoxylin and eosin staining. A: NC72; B: RP72; C: PC24; D: PC72. (Magnification: 150x).

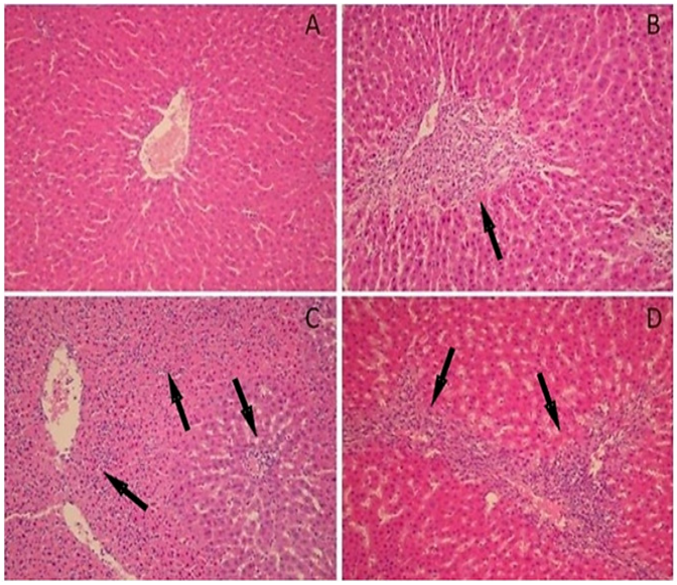
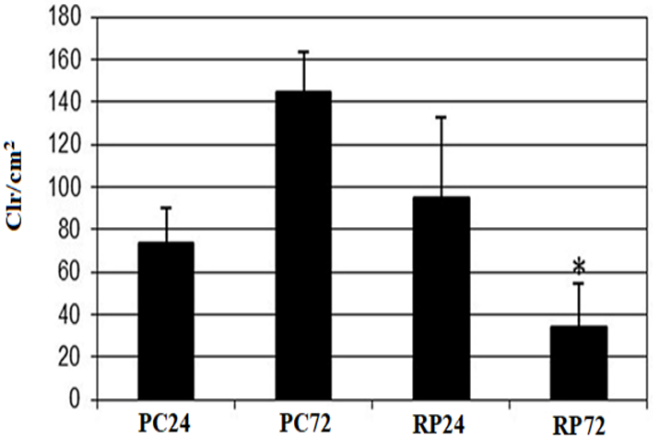


Figure 2. Qualitative analysis of inflammatory/necrotic foci in the central-lobular region of the treated and control groups. Note the decrease in injury incidence in the RP72 group when compared with PC72. $p < 0.05$ significantly different, as determined by ANOVA, followed by the Bonferroni post-hoc test.



An increased presence of collagen was observed in acinar zones 1 and 3 with picrosirius red staining; therefore, quantification was performed in these regions showing smaller collagen areas in the R and RP groups than in the PC and NC groups (Figure 3 and Figure 4).

Picrosirius red staining results demonstrated no significant differences between the groups in zone 1 or 3 acinar, uncovering low intensity of collagen fiber staining in the positive control group. This is corroborated by several reports in the literature, which could not detect fibrosis in paracetamol-induced toxic hepatitis [24-26].

Figure 3. Quantification of collagen fibers in the portal region (zone 1 acinar). $p < 0.05$ significantly different, as determined by ANOVA, followed by the Bonferroni post-hoc test.

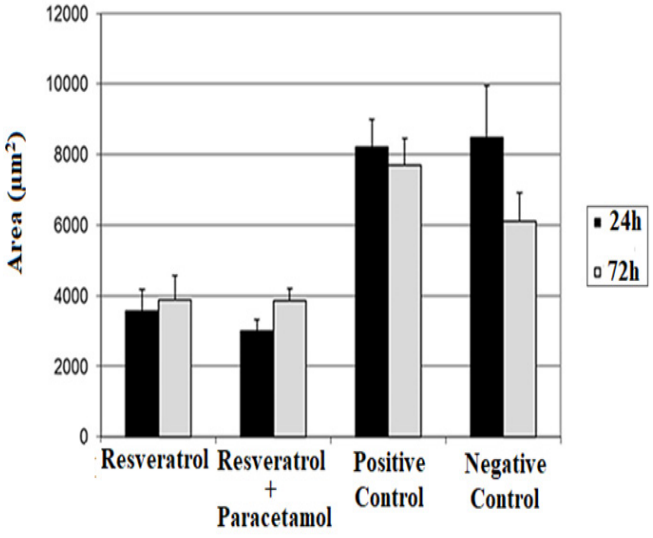
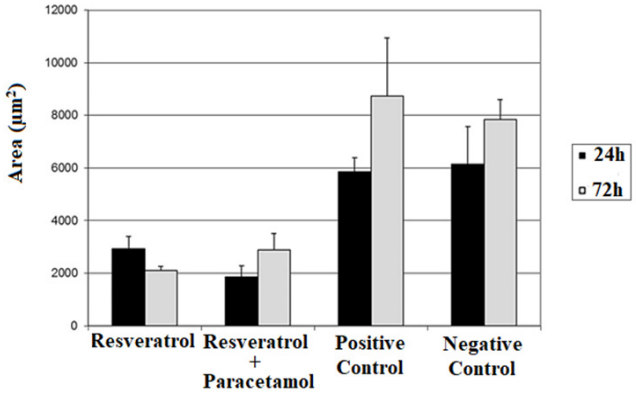


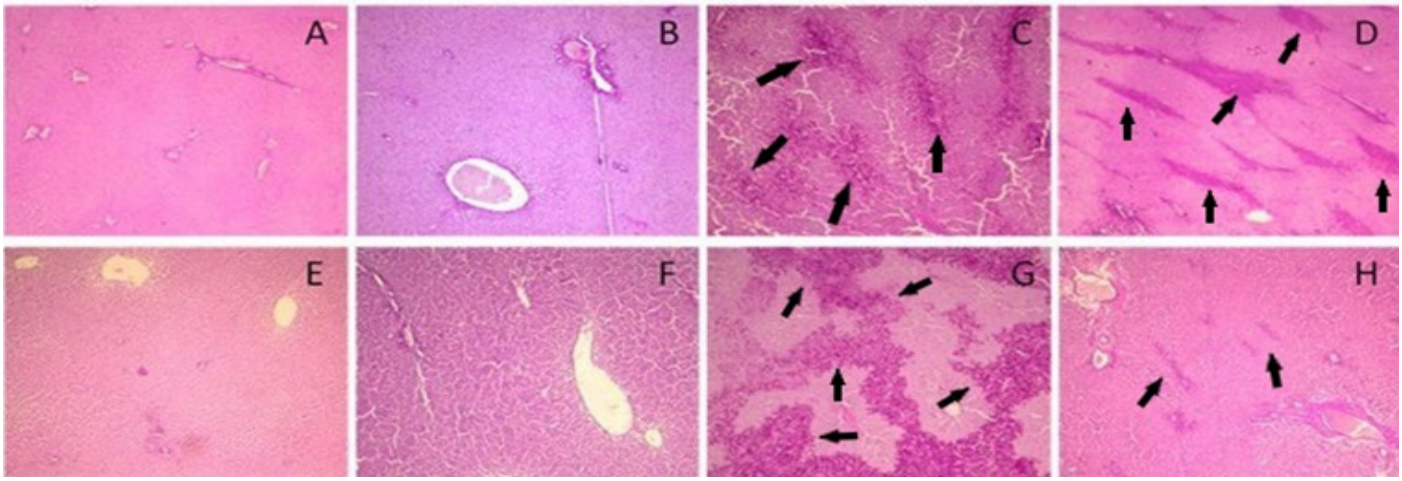
Figure 4. Quantification of collagen fibers in the central-lobular region (zone 3 acinar). $p < 0.05$ significantly different, as determined by ANOVA, followed by the Bonferroni post-hoc test.



Animals treated with a high dose (3g/Kg) of paracetamol showed a significant decrease in PAS-positive inclusions, as evidenced by the decrease in the pink color of the cells (Figure 5). This observation is clear when comparing micrographs C and D (PC) to micrographs A and B (NC), as shown in Figure 5.

The third histological parameter showed that the animals treated with a high dose of paracetamol showed an increase in positive PAS inclusions in the portal region and a decrease in those in the central-lobular region when compared to the negative control group. A decrease in liver glycogen by paracetamol has been reported in the literature as being related to its irreversible binding to a reactive metabolite or inhibition of mitochondrial energy metabolism. This parameter is considered an indicator of drug hepatotoxicity[27]. Although a quantitative analysis was not performed, it was possible to observe that pre-treatment with resveratrol promoted the homogeneous distribution of glycogen. The reduction of glycogen in zone 1 acinar was expected because this region has greater amounts of P450 complex enzymes and paracetamol-induced histopathological lesions[27].

Figure 5. Micrographs showing the distribution of glycogen (and other 1,2-glycols) using PAS staining. The intense pink color represents the accumulation of glycogen. A: NC24; B: NC72; C: PC24; D: PC72; E: R24; F: R72; G: RP24; H: RP72. (Magnification: 150x).



Comparison of micrographs G and H (RP) to those of the PC group showed apparent preservation of PAS-positive inclusions distribution, indicating the hepatoprotective activity of resveratrol. In the resveratrol + paracetamol groups, there was a preservation of positive PAS inclusion distribution, thus obtaining a 60% lesion reduction in the RP72 group, indicating the hepatoprotective activity of resveratrol (Table I).

Table 1 - Semi-quantitative analysis of PAS-positive inclusions deposition in acinar zone 1. Note the significant decrease in inclusions in the RP 72 h group compared to the PC 72 h group.

	Number of animals			
GROUP	Positive PAS Deposition in Zone 01			Reduction in relation to PC
	No deposition (-)	Discrete deposition (+)	Intense deposition (++)	
PC 24 h	-	2	3	-
PC 72 h	1	2	2	-
RP 24 h	-	1	3	No reduction
RP 72 h	4	1	-	60%

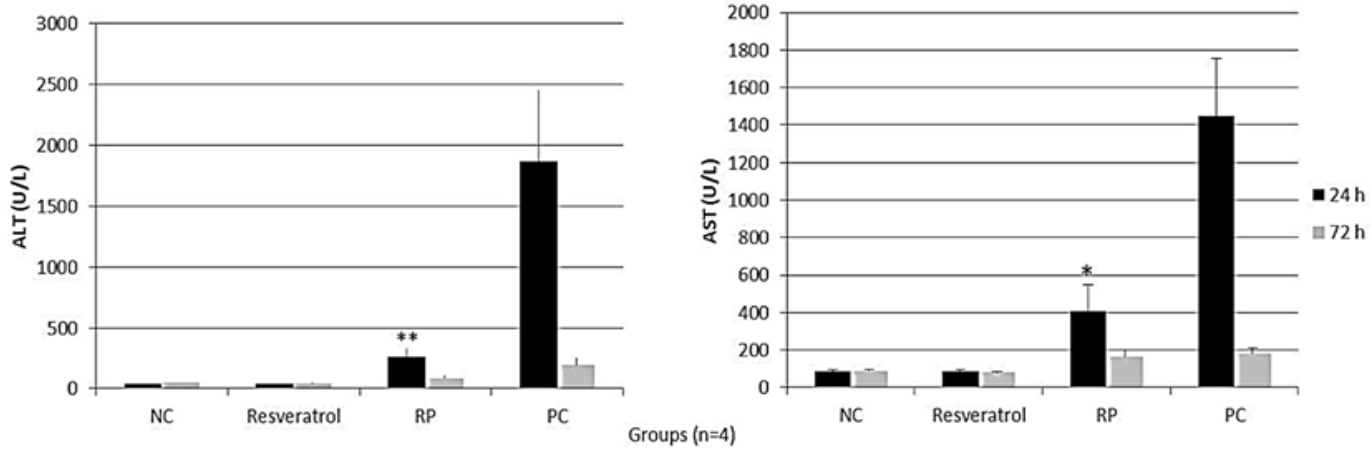
High doses (3g/kg) of paracetamol administered to rats in the PC group increased hepatic transaminases AST and ALT, as expected. Pretreatment with resveratrol prevented this increase at 24 h (Figure 6) after the administration of paracetamol, protecting the liver of these animals. No significant changes in ALP levels were observed between the groups.

AST is found in high concentrations in cardiac muscle, liver, and skeletal muscle, and more discreetly, in the kidneys and pancreas. In liver cells, AST is located in the cytoplasm (40%) and mitochondria (60%). ALT or glutamic-pyruvic transaminase (TGP) is predominantly found in the liver, at moderate concentrations in the kidneys, and in smaller amounts in the heart and skeletal muscles. In liver cells, ALT is located in the cytoplasm (90%) and mitochondria (10%). Tissue injury or disease affecting the liver parenchyma releases a greater amount of these enzymes into the bloodstream, raising the serum levels of AST and ALT, which indicates hepatocellular disease. AST and ALT levels were evaluated in patients with acute and chronic liver diseases. In these patients, the AST/ALT ratio provided useful clinical information regarding the cause and severity of liver disease. The advantage of using these parameters is that they are easily available and easy to interpret, with a low cost and wide application[28].

The increase in ALT and AST serum levels has been attributed to damage to the liver structure, as they are located in the cytoplasm and released into the bloodstream after cell damage [28]. In our study, we observed that AST and ALT serum levels of the resveratrol + paracetamol 24 h group were lower than the group treated only with paracetamol (PC). The hepatoprotective action of resveratrol and its ability to decrease serum AST and ALT in toxic hepatitis was also observed in other studies, including an experimental design of liver injury by ischemia/reperfusion and methotrexate-induced injury[29-31].

Alkaline phosphatase reflects pathological alterations of the bile duct[32]. Therefore, the increase in serum alkaline phosphatase in rats with hepatitis is related to disturbances in secretory activity, metabolite transport, or synthesis of certain altered enzymes in other hepatotoxic conditions[33]. The results showed no change in the levels of this enzyme even in the positive control group. The absence of ALP increase in the positive control group (and in the others) is in agreement with the histological findings because no changes were observed in the bile ducts under light microscopy, thus indicating that the hepatotoxicity by paracetamol is cytolytic and not cholestatic[30].

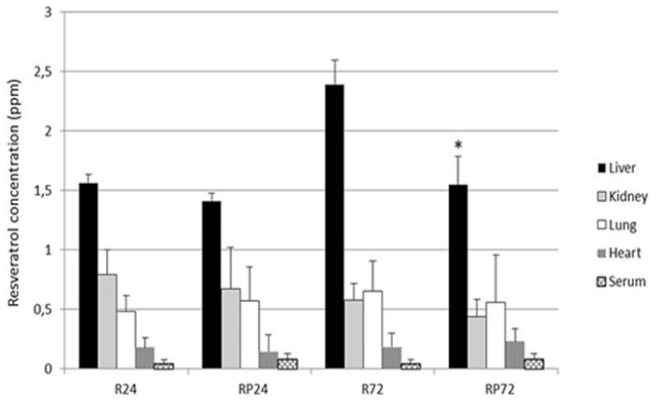
Figure 6 - Aspartate-amino transferase and Alanine-amino transferase dosage. NC: Negative Control Group; A: Resveratrol; RP: Resveratrol + Paracetamol; PC: Positive Control Group. ANOVA followed by Bonferroni's post-hoc test, * $p < 0.05$ and ** $p < 0.001$ in relation to PC 24 h.



Biodistribution of resveratrol

It was possible to observe different resveratrol concentrations in the studied tissues (liver, kidney, lung, heart, and serum), with differences between the 24 and 72 h groups (Figure 7). The difference occurred in the liver, with an increase in the drug concentration at 72 h (R72A high resveratrol consumption in the group previously intoxicated with acetaminophen (RP72), with a significant difference between these two groups

Figure 7. Resveratrol biodistribution studies. Note the significant difference (*p < 0.05), in relation to the R72 group.



Based on the hepatotoxicity mechanisms and the known antioxidant action of resveratrol, the hepatoprotective action of this polyphenol was confirmed by histological, morphometric, and biochemical findings. Notably, the lesions are related to increased free radical production due to saturation of the preferential metabolic pathway of paracetamol (glucuronidation and sulfation pathways) and activation of the secondary pathway by cytochrome P450. Therefore, it is suggested that the hepatoprotective action observed in the present study might also be related to the antioxidant action of resveratrol, as described before [13,34].

There are scarce reports in the literature on resveratrol biodistribution; however, the use of radiomarkers has made it possible to study its biodistribution after oral administration, being detected at higher concentrations in organs related to its absorption and elimination, such as the stomach, intestine, liver, and kidneys, and in smaller amounts in the colon, duodenum, lung, heart, and brain [35]. This is mainly due to resveratrol metabolization, since although its absorption is high, its oral bioavailability is very low, given its rapid metabolism in the liver[36]. In the present study, the distribution pattern in the organs of the rats followed the same pattern obtained in the liver, all of which had significantly lower concentrations than those found in the liver at 24 and 72 h[37]. Kidneys exhibited decreasing resveratrol concentrations, and in other rodent studies, the decreased resveratrol levels in the kidneys and the low concentrations found in the colon were suggested to result from excretion preferentially via the urine and in smaller amounts via the fecal route [35, 37]. The heart, lungs, and serum showed constant concentrations, with no significant differences between them.

As previously described, toxic doses of paracetamol saturate the hepatic sulfation and glucuronidation pathways, forcing drug metabolism through the cytochrome P-450 complex, where the greater formation of the toxic metabolite leads to alteration of mitochondrial metabolism with the production of reactive species of oxygen and nitrogen and peroxidation products[38]. Based on this mechanism of hepatotoxicity, the known antioxidant action of resveratrol, and the biodistribution results obtained in this study, it is possible to justify the significant decrease in resveratrol in the liver in the RP72 group. The decrease in liver resveratrol concentration is certainly due to its ability to react with the products formed in acute hepatocyte intoxication, neutralizing them and, consequently, decreasing hepatotoxicity, being excreted soon afterward.

DISCUSSION

Liver Tissue Changes

In the positive control group, there was no evidence of steatosis (fatty liver), but fibrosis and necrosis were observed. Necrosis was most prominent in the central-lobular region, particularly in animals observed at 72 hours after treatment. Inflammatory/necrotic foci were quantified in the central-lobular region, while fibrosis was quantified in specific acinar zones (1 and 3) using picrosirius red staining.

Liver Tissue Comparison

The results describe that in the negative control group, liver tissue appeared to be well-preserved. In contrast, the positive control and resveratrol + paracetamol groups showed signs of inflammatory infiltrate and necrosis. The central-lobular regions were more affected in the positive control 24 h and positive control 72 h groups.

Collagen Presence

Collagen presence was assessed using picrosirius red staining, and it was found that the resveratrol and resveratrol + paracetamol groups had smaller collagen areas compared to the positive control and negative control groups.

The results suggests that collagen fiber staining was of low intensity in the positive control group, consistent with literature reports of fibrosis not being detected in paracetamol-induced toxic hepatitis.

PAS-Positive Inclusions

High doses of paracetamol (3g/kg) resulted in a significant decrease in PAS-positive inclusions, which was observed as a decrease in the pink color of the cells.

Resveratrol treatment, especially in the resveratrol + paracetamol 72 h group, seemed to preserve the distribution of PAS-positive inclusions, indicating potential hepatoprotective activity.

Hepatic Transaminases

The study measured hepatic transaminases AST and ALT, which are markers of liver damage.

Administration of high doses of paracetamol led to an increase in AST and ALT levels in the positive control group.

Pretreatment with resveratrol appeared to prevent this increase in AST and ALT levels at 24 hours after paracetamol administration.

Resveratrol Concentration

Resveratrol concentrations were measured in various tissues (liver, kidney, lung, heart) and serum at 24 and 72 hours.

There was a significant increase in resveratrol concentration in the liver at 72 hours, particularly in the resveratrol + paracetamol 72 h group compared to the resveratrol 72 h group.

Overall, the experimental findings related to the effects of paracetamol and resveratrol on liver tissue in animal models. It suggests that resveratrol has hepatoprotective properties and can mitigate some of the liver damage caused by high doses of paracetamol, as indicated by changes in tissue characteristics and liver enzyme levels.

CONCLUSION

The resveratrol has a hepatoprotective action in acute intoxication by paracetamol, as evidenced by the histological decrease in necrosis and inflammatory foci, preservation of glycogen and other 1,2-glycols in zone 3, and reduction in serum levels of ALT and AST. Resveratrol was present in higher amounts in the liver than in other organs and fluids. This distribution pattern is explained by the physiology of these organs/fluids and agrees with reports found in the scientific literature.

Paracetamol caused a significant reduction in the resveratrol concentration in serum and the organs studied, indicating that the antioxidant activity of resveratrol is related to its hepatoprotective action.

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