Effects of bioflavonoid ternatin on liver regeneration and oxidative stress in rats[1]

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ABSTRACT

PURPOSE: To evaluate the effect of bioflavonoid ternatin (TRT) on rat liver regeneration and oxidative stress after 70% partial hepatectomy (PH).

METHODS: Thirty six young male Wistar rats were randomly assigned to two groups of 18 animals each – control (G1) and experimental (G2) – and were submitted to PH under inhalatory diethylether anesthesia. G1 rats received daily intraperitoneal (ip) injections of saline (NaCl 0.9% solution) 0.1 mL/kg for 14 days; G2 animals received daily ip injections of TRT 0.1% 1.0mg/kg for 14 days. At 36h (T1), 168h (T2) and 336h (T3) post-PH timepoints, a subgroup of six rats in each group was chosen in a randomized way to complementary hepatectomy (CH) and blood samples haversting. Collected material was saved for laboratory analysis (total bilirubin (TB), D-Glucose, glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) and assessment of liver regeneration.

RESULTS: TRT induced a significant decrease in liver and plasma GSH concentrations; liver regeneration process was not affected. TRT promoted a significant decrease in blood glucose levels 168h after partial hepatectomy compared with controls. TB levels remained unchanged.

CONCLUSION: Intraperitoneal bioflavonoid ternatin injection in partially hepatectomized rats induces a decrease in oxidative stress and a significant hypoglycemic state, but does not promote any change in the evolution of liver regeneration.

Key words: Hepatectomy. Oxidative Stress. Liver Regeneration. Flavonoids. Rats.

Introduction

The liver exhibits a remarkable regenerative capacity after tissue damage, including partial hepatectomy (PH)[1]. Following removal of the two major lobes of the rat liver (70% partial hepatectomy, Higgins-Anderson partial hepatectomy), the remaining minor lobes rapidly undergo an essentially hyperplastic response to repopulate loss of tissue and cells[2] attaining its original size by three to 14 days[2-4].

Reactive oxygen species (ROS), antioxidant substances and lipid peroxidation have been implicated in control mechanisms of cellular growth and proliferation[5-7]. The administration of exogenous antioxidants such as α-tocopherol (vitamin E) and reduced gluthatione (GSH) retards liver regeneration[7,8]. Moreover, several studies have reported the genesis and/or formation of free radicals as an important factor on the liver regeneration phenomenon, necessary to its natural outcome[6,9].

Flavonoids are claimed to have protective effects against free radicals induced lipid peroxidation of living cell membranes[10]. Ternatin (TRT), 4'.5-dihydroxy-3.3',7.8-tetramethoxyflavone, a natural antioxidant, was evaluated under the classical experimental model of partial hepatectomy due to Higgins and Anderson[1] to assess its effects on rat liver regeneration and oxidative stress.

TRT was isolated from the flowering tops of *Egletes viscosa* L. (Asteraceae)[11], popularly known as "macela da terra", which is a small empirical medicinal herb that grows abundantly in northeast of Brazil and others areas of South America. Previous studies have demonstrated anti-inflammatory, antianaphylactic, antihrombotic and antihepatotoxic properties of TRT[12-14]; these pharmacological effects have been related to its free radical scavenging and antioxidant properties[15].

Methods

All procedures were approved by the Commission of Ethics on Animal Research (now Committee of Ethics on the Use of Animals (CEUA), Federal University of Ceara, protocol 14/06 on August 11, 2006. Surgical procedures and animal handling were conducted in accordance with the Brazilian Federal Law No. 11794 of October 8, 2008 (http://www.planalto.gov.br/ccivil_03/_Ato2007-2010/2008/Lei/L11794.htm). Rats were raised under controlled conditions, housed in polypropylene cages at temperature of 23±4ºC on a light schedule of 12h light/dark cycle and fed regular rat chow and potable water *ad libitum*. Thirty six young (70±10 days) male Wistar rats weighting 100-235g were included in the study and randomly assigned to two groups (n=18) – control (G1) and experiment (G2). All rats were submitted to the classical Higgins-Anderson 70% partial hepatectomy[1] (PH). G1 rats received daily intraperitoneal (ip) injections of saline (NaCl 0.9% solution) 0.1 mL/kg for 14 days; G2 animals received daily ip injections of TRT 0.1% 1.0mg/kg for 14 days. At 36h (T1), 168h (T2) and 336h (T3) post-PH, a subgroup of six rats in each group was chosen in a randomized way to complementary hepatectomy (CH) and blood samples haversting. All surgical procedures were performed under inhalatory diethylether anesthesia. Collected material was stored for laboratory analysis

Thiobarbituric acid reactive substances (TBARS), glutathione (GSH), glycemia and total bilirubin (TB) were assayed. Tissue samples were snap-frozen in liquid nitrogen and stored in glass tubes at -70ºC until subsequent preparation and analysis of liver tissue homogenate. Plasma samples were obtained from heparinized blood after 10 minutes of refrigerated centrifugation (4.000 rotations/minute) and were stored as well.

Chemicals and drugs

Saline (NaCl 0.9%) was obtained from Química Farmacêutica Gaspar Viana, Brazil. TRT was isolated from dried flower buds of *E. viscosa* and its chemical identification was confirmed by Prof. E. R .Silveira of the UFC. The voucher specimen (#16327) is deposited in the *Herbarium* Prisco Viana (UFC). TRT 100mg was dissolved in 3% dimethylsulfoxide (DMSO) 100mL in order to achieve a 0.1% TRT solution. DMSO was purchased from Cromato Produtos Químicos Ltda, Brazil. All other chemicals were of analytical grade.

Biochemical determinations

The amount of GSH was determined from a standard curve simultaneously obtained under the same conditions with various concentrations of GSH. Liver regeneration was estimated by weighting the rat liver residual lobes. TB content was valuated following Meites modification of Malloy and Evelyn procedure[16]. D-glucose was determined according to the Slein´s method[17].

Lipid peroxidation, a measure of free radical damage, was assayed by measuring malondialdehyde (MDA) as thiobarbituric acid-reactive substances (TBARS) levels using the thiobarbituric acid method[18]. In brief, H_3PO_4 (1%, 3ml) and aqueous TBA solution (0.6%, 3 mL) were added to the 10% homogenate (0.5 ml). The assay medium was shaken and heated on a boiling-water bath for 45 min. After cooling, 4 ml of *n*-butanol was added and the mixture shaken. After separation of the *n*-butanol layer by centrifugation

at 1200 g for 15 min its optical density was determined in a spectrophotometer (Beckman DU 640 B) with 535 and 520nm as absorption wavelengths, respectively. The difference between the results of the two optical density determinations was taken as the TBA value and the amount of malondialdehyde (MDA) in the testis was calculated, comparing with MDA standards and expressed as µmol MDA per gram of wet tissue. GSH levels were estimated by the method of Sedlak and Lindsay[19] which is based on the reaction between thiol groups and 5-5'-dithiobis-(2-nitrobenzoic acid) to give a compound that absorbs light at 412 nm. The amount of GSH was determined from a standard curve simultaneously obtained under the same conditions with various concentrations of GSH.

Statistical analysis

GraphPad Prism 4.0 (GraphPad Software, San Diego, California, USA, www.graphpad.com) was used for computation and statistical analysis. To ensure the appropriateness of parametric testing, all data were examined for normality, using Kolmorogov-Smirnov test (with Dalal-Wilkinson-Lilliefor P Value). Non-parametric data were analyzed using ANOVA (Kurskal-Wallis/ Dunn) tests. Values of $p<0.05$ were accepted as statistically significant.

Results

No animal died during the experiment.

Liver regeneration

Figure 1 depicts the evolution of residual liver lobes in each subgroup throughout the experiment. There was a significant increase in liver weight at T3 timepoint in both groups.

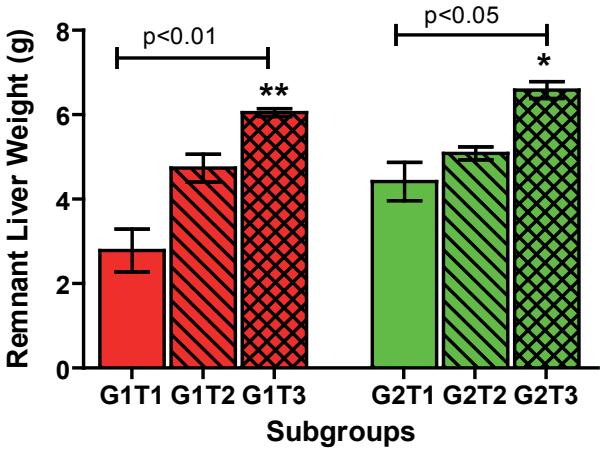

FIGURE 1 - Liver regeneration in G1 (control) and G2 (experiment) groups. Bars represent mean ± SD of G1 (red bars) and G2 (green bars) subgroups at 36h (T1), 168h (T2) and 336h (T3) timepoints post-PH. Values were significantly different (G1:$p<0.01$, G2:$p<0.05$) at T3 timepoint compared with T1 timepoint, in both groups, by ANOVA (Kruskal-Wallis/Dunn) test.

Total billirubin

Although there was an apparent decrease of TB concentrations in both groups (G1 / G2), no significant differences were demonstrated, as illustrated in Figure 2.

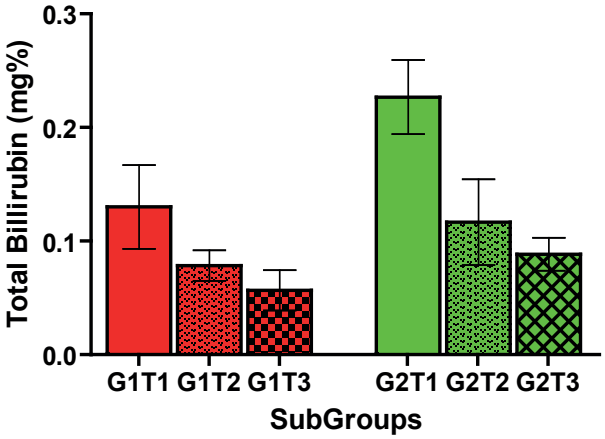

FIGURE 2 - Blood total billirubin concentrations in G1 (control) and G2 (experiment) groups. Bars represent mean ± SD of G1 (red bars) and G2 (green bars) subgroups at 36h (T1), 168h (T2) and 336h (T3) timepoints post-PH. Values were not significantly different by ANOVA (Kruskal-Wallis/Dunn) test.

D-Glucose

D-Glucose levels decreased significantly ($p<0.01$) in TRT-treated rats in T2 timepoint compared with control T2 (Figure 3).

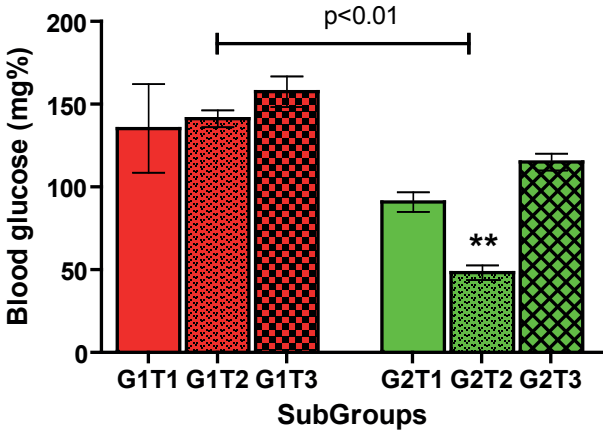

FIGURE 3 - D-Glucose levels in G1 (control) and G2 (experiment) groups. Bars represent mean ± SD of G1 (red bars) and G2 (green bars) subgroups at 36h (T1), 168h (T2) and 336h (T3) timepoints post-PH. Values were significantly different (G1 vs. G2, $p<0.01$) at T2 timepoint by ANOVA (Kruskal-Wallis/Dunn) test.

Plasma GSH

Plasma GSH concentrations decreased significantly in TRT treated rats, 336h post-PH (T3) compared with T1 ($p<0.001$) and T2 ($p<0.05$) (Figure 4).

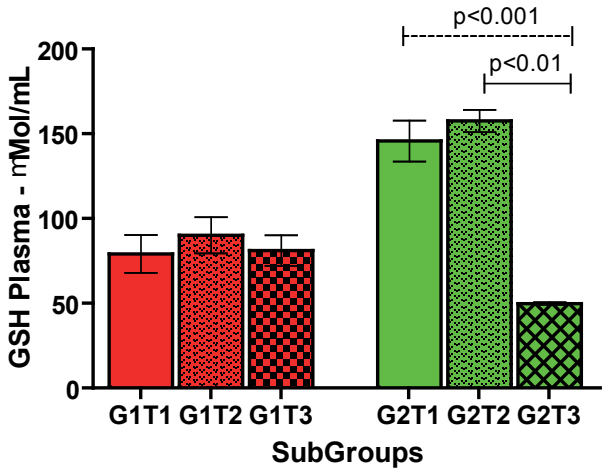

FIGURE 4 - Plasma GSH concentrations in G1 (control) and G2 (experiment) groups. Bars represent mean ± SD of G1 (red bars) and G2 - TRT subgroups at 36h (T1), 168h (T2) and 336h (T3) timepoints post-PH. Values were significantly different at T2 and T3 compared with T1 timepoint in TRT-treated rats by ANOVA (Kruskal-Wallis/Dunn) test.

Liver GSH

Liver GSH concentrations decreased significantly ($p<0.01$) in TRT treated rats, 168h post-PH (Figure 5).

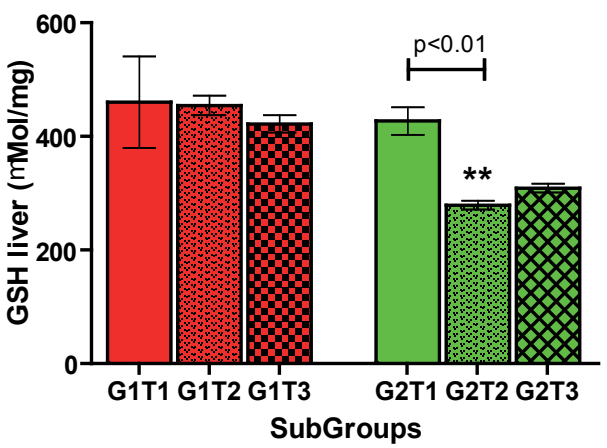

FIGURE 5 - Liver GSH concentrations in G1 (control) and G2 (TNT) groups. Bars represent mean ± SD of G1 (red bars) and G2 (green bars) subgroups at 36h (T1), 168h (T2) and 336h (T3) timepoints post-PH. Values were significantly different ($p<0.01$) at T2 compared with T1 timepoint in TRT-treated rats by ANOVA (Kruskal-Wallis/Dunn) test.

Plasma TBARS

Plasma TBARS values were not different in G1 and G2 rats (Figure 6).

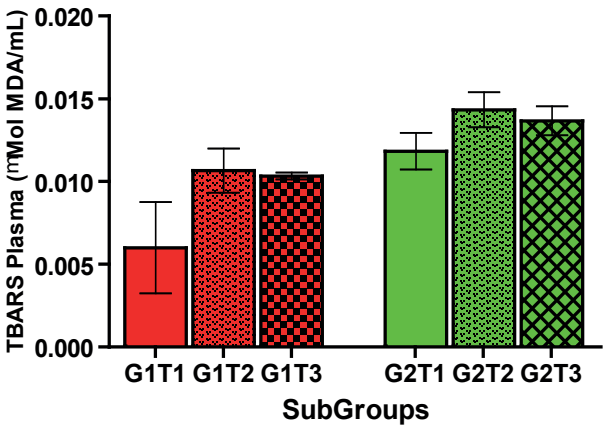

FIGURE 6 - Plasma TBARS concentrations in G1 (control) and G2 (experiment) groups. Bars represent mean ± SD of G1 - control (red bars) and G2 - TRT (green bars) subgroups at 36h (T1), 168h (T2) and 336h (T3) timepoints post-PH. Values were not significantly different in control and TRT-treated rats by ANOVA (Kruskal-Wallis/Dunn) test.

Liver TBARS

Liver TBARS values decreased in T2 and T3 subgroups compared with T1 in control rats. No differences were found in TRT-treated rats (Figure7).

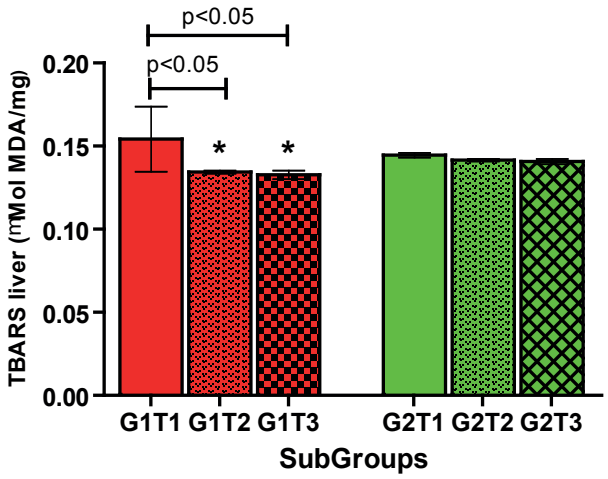

FIGURE 7 - Liver TBARS concentrations in G1 (control) and G2 (experiment) groups. Bars represent mean ± SD of G1 - control (red bars) and G2 - TRT (green bars) subgroups at 36h (T1), 168h (T2) and 336h (T3) timepoints post-PH. TBARS concentrations decreased significantly different in control rats in T2 and T3 timepoints compared with T1 by ANOVA (Kruskal-Wallis/Dunn) test.

Discussion

The rat was chosen for this study because it is the most studied animal in hepatic regeneration research[20]. Male young animals were used whereas older rats may compromise the regenerative process[21]. Besides, steroids present in greater quantity in females may influence hepatic regeneration[22]. The maximum period of sample collecting (14 days) was chosen based on previous studies that show that liver regeneration in rats subjected to HP is completed in two weeks[1,20].

Considering that TB concentrations did not show any significant change at any time we can perceive that TRT administration had no effects on TB levels. The absence of significant differences in the weight of the remnant liver in G1 and G2 groups suggests that TRT administration does not interfere in a significant way with the natural post-HP rat liver regeneration.

Some antioxidants have different effects on liver regeneration. In fact, the administration of omega-3 polyunsaturated fatty acids (PUFA), inhibits the liver regeneration[23] and the administration of α-tocopherol (vitamin E) and GSH retards liver regeneration evolution[7,8].

Partial hepatectomy is related to the formation of free radicals[24-26]. Indeed, many studies have shown an increased production of free radicals production measured by malonyldialdehyde in liver mitochondria following partial hepatectomy[24-26]. Decreased liver and plasma GSH observed in G1 and G2 rats could be related to the utilization of GSH in order to attenuate the oxidative stress generated by the partial hepatectomy.

The lipid peroxidation inhibitory effects of several flavonoids such as luteolin, apigenin, galangin, gardenin D, (+) catechin[27], quercitin[28], rutin (quercetin-3-rhamnosyl glucoside)[29] ant TRT[30] have been previously reported. In the present study, liver TBARS values decreased in T2 and T3 subgroups compared with T1 in control rats. This could be explained by the reduction of GSH concentration in G1 rats. The absence of reduction of TBARS concentration in rats subjected to HP and treated with TRT suggests that the antiperoxidative effect of TRT was not observed here.

In the present study, blood glucose concentrations decreased significantly in TRT-treated group. Studies have demonstrated that insulin levels decrease[31,32] while glucagon levels increase[33] in partially hepatectomized rats. These changes may reflect part of homeostatic mechanism to maintain glucose levels within normal limits. Moreover, antioxidants improve insulin action, due in part by the protection of β-cells from free radicals injuries[32].

Conclusions

Bioflavonoid ternatin administration induces an inhibitory effect on oxidative stress but does not change the liver regeneration evolution. Moreover, TRT promotes a significant hypoglycemic state in TRT-treated rats.

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