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Effect of *Chelidonium majus* on Metabolic Abnormalities Induced by HAART in Mice

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Authors' contributions

This work was carried out in collaboration between all authors. Author ARTP designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Authors ALPPS, NAS and RPR conducted the experimental analyses of the study. Author MSJ conducted research in the literature. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Extracts of *Chelidonium majus*, extensively used in traditional systems of medicine, have been tested for their possible anti-tumor, hepato-protective and anti-genotoxic effects, to investigate their potential in cancer therapy. Although the highly active antiretroviral therapy (HAART) has changed the natural history of HIV infection, several adverse events may limit its efficacy. Antiretroviral drugs are associated with increased risk of severe hepatotoxicity. These complications increase morbidity and mortality significantly. Current study evaluated the effect of *Chelidonium majus* on metabolic alterations induced in mice subjected to HAART.
Methodology: Four-weeks old male *Swiss Webster* mice, weighing approximately 28-30 g, provided by the Central Animal Laboratory of the State University of Maringá, were used in the experiments. The drug in the form of mother tincture was prepared with the juice of the root of *C. majus*, mixed in equal parts of grain alcohol (P.A.) and comprised 12 animals per experimental group: (I) animals treated with HAART diluted in 1.2 mL water gavage/day, (II) animals treated with HAART diluted in 1.2 mL water gavage/day + *C. majus* diluted $1x10^{12}$ in water 1.0 mL, daily added once to the drinking water (1:10 mL) available *ad libitum*, (III) non-treated animals (control group)

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received 1.2 mL water by gavage/day. The experimental groups were treated for 15 days. Overall clinical evaluation was performed and serum cholesterol, triglycerides, hepatic enzymes (AST and ALT) were assessed by specific methods. Results were analyzed with GraphPad Prism by Student´s t test.

Results: Results demonstrate lower weight gain, 30% less (p=0.004) in the group treated with HAART, whereas the group treated with HAART and *C. majus* had the same weight gain of control group. The evaluation of metabolic parameters showed a significant difference on levels of plasma triglycerides in animals treated with HAART + *C. majus* (19.6% less). ALT parameter was 23.7% lower in patients treated with *C. majus* when compared to group treated only with HAART. AST decreased by 65.2% in the group treated with *C. majus*, with the same levels of control.

Conclusion: *C. majus*, diluted $1x10^{12}$, improved metabolic changes induced by HAART in mice.

Keywords: Chelidonium; HIV / AIDS; antiretroviral; metabolic abnormalities.

1. INTRODUCTION

Highly Active Antiretroviral Therapy (HAART) is the current care standard in the treatment of patients with HIV/ AIDS. In fact, HAART has remained the only regimen which is potent enough to decrease viral infection. Although HAART has been extremely potent in the treatment of HIV it has many adverse effects. With the initiation of HAART, a few cases may present with new disease manifestations or may undergo worsening of existing manifestations caused by the virus itself [1].

HAART controls viremia and reduces mortality, albeit with significant toxic effects, such as lipodystrophy, pancreatitis, hyperlipidemia, lactic acidosis and insulin resistance. It should also be taken into account that antiretroviral drugs are metabolized in the liver and their use in long-term therapy may lead to hepatotoxic effects [2,3,4].

Chelidonium majus belongs to *Papaveraceae* family, native to Europe, and grows in damp places and on cliffs. The plant produces a yellow, viscous and bitter juice. It was initially used as a remedy for liver due to its color similar to bile. Galen and other ancient physicians treated jaundice with the plant.

Due to the plant´s popular use, some of its components were extracted, namely, acetylcholinesterase, butyrylcholinesterase, and several secondary metabolites with pharmacological activity, such as isoquinolones, flavonoids and phenolic acid [5]. Studies indicated effects on immune response [6], stimulation of apoptosis [7] antioxidant potential, antimitotic effects [8], immunomodulation, stimulation in the production of nitric oxide (NO) and tumor necrosis factor (TNF-α) in macrophages [9], inflammatory mediators and inhibition of antiretroviral activity *in vitro* and *in vivo* [10], suppressive effects on arthritis in mice [11], anti-tumor effect on liver tumors induced in rats [12], bactericidal [13] and antihelmintic effects [14].

Current study evaluated the effect of *C. majus* on metabolic alterations induced in mice to antiretroviral therapy (HAART).

2. MATERIALS AND METHODS

2.1 Animals

Four-week old male Swiss Webster mice, weighing approximately 28-30 g, provided by the Central Animal Laboratory of the State University of Maringá, were used in the experiments. The Committee for Ethics in Animal Experiments of the State University of Maringá approved the experiments (Protocol 084/2013).

The animals, kept in cages with food and water *ad libitum*, were monitored daily for 7 days, for clinical evaluation. They were kept in a vivarium of the Laboratory of Parasitology / DBS/UEM under ideal conditions: temperature 22℃±2℃, 70% humidity and photoperiod (light / dark cycle 12 h).

2.2 Preparation of *Chelidonium majus*

The drug in the form of mother tincture, prepared with the pressed juice of the root of *C. majus*, was mixed in equal parts of grain alcohol (P.A.) obtained from the laboratory HN CRISTIANO, São Paulo, Brazil (lot 5387). The mother tincture was then diluted in $1x10^{12}$ water. The method for drug preparation followed the Brazilian Homeopathic Pharmacopoeia [15]. The dilution was considered free from any toxicity [16].

2.3 Preparation of HAART

Protocol was based on a standard therapeutic regimen of patients from Brazil. The calculation of the dose used was proportional to weight of animals, as employed in humans. The animals received treatment consisting of 5 mg / kg of atazanavir sulfate, 5 mg / kg of tenofovir disoproxil fumarate, 1.67mg / kg of ritonavir and 2.5 mg / kg of lamivudine, diluted in 1.2 mL of water.

Treatment period lasted 15 days and drug was always administered at 09:00 am.

2.4 Treatment Schedule

Taking 12 animals per experimental group, we have (I) animals treated with HAART diluted in 1.2 mL water gavage/day, (II) animals treated with HAART diluted in 1.2 mL water gavage/day + *C. majus* diluted in $1x10^{12}$ in water 1.0 mL once a day, added to the drinking water (1:10 mL) available *ad libitum*, (III) non-treated animals (control group) received 1.2 mL water by gavage/ day. The experimental groups were treated for 15 days.

2.5 Evaluation

2.5.1 Assessment of body weight

Animals were weighed on a semi-analytical balance BL320H Mars Shimadzu before the start of the treatment and at the end of the experiment. Results were given in mean of group.

2.5.2 Clinical evaluation

Qualitative parameters, such as physical appearance of the animals during the treatment (hair bristling and irritability).

2.5.3 Laboratory evaluation

Performed by plasma levels of aspartate amino-transferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) were evaluated by the kinetic colorimetric method; triglycerides, total cholesterol and creatinine were evaluated by enzymatic colorimetric method, both provided by GOLD ANALISA DIAGNÓSTICA LTDA.

2.5.4 Macroscopic evaluation of organs (liver and spleen)

Liver and spleen of all animals were examined macroscopically and weighed at the end of the experiments.

2.6 Statistical Analysis

Group-comparing statistics were performed by Graph Pad Prism 6.0 (Graph Pad, San Diego, CA, USA) with Student´s *t* test; $p<0.05$ was statistically significant.

3. RESULTS AND DISCUSSION

The literature has shown that the use of antiretroviral therapy in HIV-infected individuals is capable of inducing metabolic alterations characterized by insulin resistance, diabetes mellitus and abnormal lipid metabolism in the distribution of body fat, milky acidosis, osteopenia and coronary heart disease, among others [17,18,19].

On the other hand, studies on metabolic alterations in mice undergoing antiretroviral therapy are scarce and limited to the study of antiretroviral effects alone and not on therapeutic HAART. Current study evaluated the effect of a HAART regimen commonly used for HIV / AIDS patients and the effect of *C. majus* on mice with metabolic abnormalities induced by HAART.

Analyzing clinical parameters such as body weight gain (Fig. 1) in mice treated with HAART (group I) compared with those treated with HAART + *C. majus* (Group II) and non-treated (group III) revealed that mice treated with HAART had less weight gain when compared to non-treated groups (Group III) and treated with HAART + *C. majus*. The latter showed weight gain equal to the group not treated with HAART.

Results in current experiments demonstrate a lower weight gain, 30% less ($p=0.004$) in the group treated with HAART, whereas the group treated with HAART and *C. majus* showed the same weight gain as that of the control group.

Result has shown that *C. majus* may have improved the appetite of animals or improved gastrointestinal symptoms induced by HAART. Several researchers have shown diarrhea, gastrointestinal side-effects and hepatotoxicity mainly induced by protease inhibitors [20]. Nausea, vomiting, diarrhea and low weight in pregnant woman and low birth weight have been reported [21]. Gastrointestinal disorders and complications observed in the antiretroviral period comprised acute intestinal infections, right lower quadrant pain, bleeding, bowel obstruction and perforation [22].

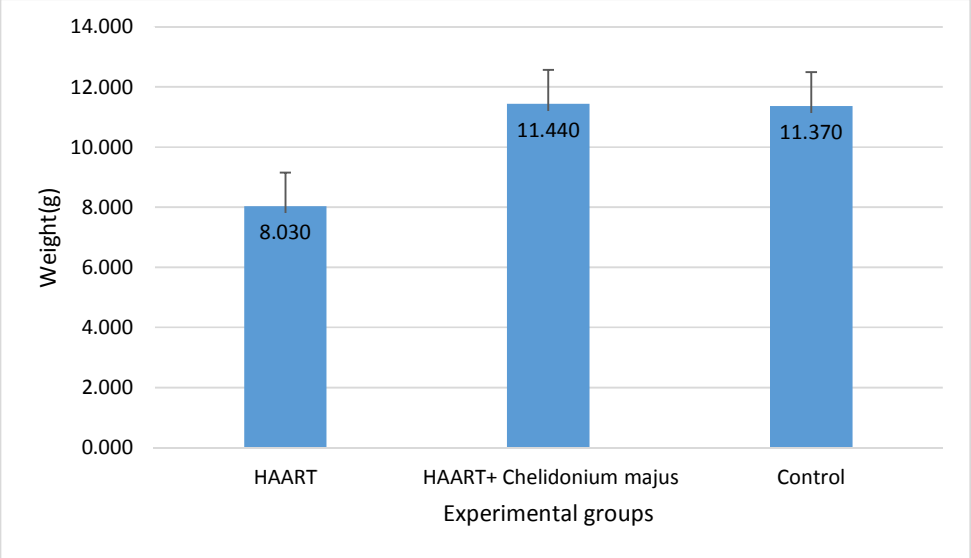

Fig. 1. Weight gain (g) of Swiss mice after 15 days

Comparison between the experimental groups group I treated with HAART (5 mg / kg of atazanavir+ 5 mg / kg of tenofovir disoproxil fumarate+ 1.67 mg / kg of ritonavir+ 2.5 mg / kg of lamivudine, diluted with 1.2 mL of water/day/15 days; Group II treated with HAART (5 mg / kg of atazanavir+ 5 mg / kg of tenofovir disoproxil fumarate + 1.67 mg / kg of ritonavir + 2.5 mg / kg of lamivudine, diluted with 1.2 mL of water/day/15 days) + Chelidonium majus (mother tincture diluted in $1x10^{12}$ in water ad libitum); Group III non-treated group (control group). Results are expressed by mean ± SD of 12 animals

In the case of the clinical examination daily performed on animals, group I (HAART) had more difficulties in postural pattern, piloerection, lethargy, emaciation and stress levels underscored in the gavage process and during the manipulation of the animals when compared to groups II (HAART + *C. majus*) and III (control group) with clinically normal animals.

Regarding to the clinical parameters, body weight gain, postural pattern, piloerection and stress manipulation, results of treated animals showed that clinical *C. majus* had similar aspects to the control group not subjected to HAART. Results may indicate that *C. majus* induces a general clinical improvement in animals treated with HAART.

Results would also suggest that the symptoms induced by HAART (Group I) in mice, such as piloerection when manipulated, stress, weight loss, hunched posture, isolation and screams, are related to pain symptoms [23]. When treated with *C. majus* (group II), the symptoms are minimal, with similar results as those of control group (group III). We would suggest that *C. majus* would be acting on the symptomatic 'pain' of the animals.

Table 1 shows the results of laboratory tests in different experimental groups with regard to triglycerides, total cholesterol, creatinine and GGT.

The evaluation of metabolic parameters showed a decrease of 19.6% in the levels of plasma triglycerides, 23.7% in the levels of ALT and 65.2% in levels of AST in the animals treated with a combination of HAART and *C. majus* when compared with the group treated with HAART alone. These findings were similar with the control group.

Fig. 2 (ALT) and Fig. 3 (AST) show results of hepatic enzymes.

HAART used in these experiments consists of lamivudine, ritonavir, tenofovir and atazanavir. Its effects and adverse reactions are well known in the literature. Atazanavir sulfate induces high liver enzymes, especially the enzyme alanine aminotransferase (ALT) [24,25]. High liver transaminase exceeding five times the upper limit of normal, clinical hepatitis and jaundice have occurred in patients receiving ritonavir alone or in combination with other antiretroviral medicines [26]. *In vitro* studies indicate that neither tenofovir disoproxil fumarate nor tenofovir

disoproxil are the substrates for the enzymes of the complex enzyme cytochrome P450 (CYP), and therefore not part of the hepatic metabolism [27]. Cases of lactic acidosis and severe hepatomegaly with steatosis (including fatalities) have been reported with the use of antiretroviral nucleoside analogs isolated or in combination, including lamivudine, in treatments for HIV infection [28].

AST (aspartate transferase) and ALT (alanine transferase) are enzymes of great clinical interest because they are indicative of the diagnosis of liver and heart damage caused by myocardial infarction, infections or toxic drugs, since these enzymes are released into the bloodstream after the establishment of the injury.

Figs. 2 and 3 show that *C. majus* protects the liver of mice from possible damage caused by antiretroviral therapy. ALT parameter showed levels which were 23.7% lower in patients treated with *C. majus* when compared to the group treated only with HAART. AST decreased by 65.2% in the group treated with *C. majus*, with the same levels of control.

Table 1. Laboratory parameters in the experimental groups

Laboratory parameters	HAART	HAART+ *Chelidonium majus*	Control	p
Total cholesterol (mg/dL)	104.0± 22,11	101.9±15,40	97.50±24,16	0.7404
Triglyceride (mg/dL)	256.1±53,88	205.9±28,55*	226.4±38,72	0.0281*
Creatinine (mg/dL)	9.96±16.3	11.99±16.4	7.85±11.8	0.9099
GGT (U/L)	11.44±3.12	12.98±18.4	9.35±3.06	0.4557

*Comparison between the experimental groups: Group I treated with HAART(5 mg / kg of atazanavir+ 5 mg / kg of tenofovir disoproxil fumarate+ 1.67 mg / kg of ritonavir+ 2.5 mg / kg of lamivudine, diluted in 1.2 mL of water/day/15 days; Group II treated with HAART (5 mg / kg of atazanavir+ 5 mg / kg of tenofovir disoproxil fumarate+ 1.67 mg / kg of ritonavir+ 2.5 mg / kg of lamivudine, diluted in 1.2 mL of water/day/15 days) + Chelidonium majus (mother tincture diluted $1x10^{12}$ in water ad libitum) and Group III non-treated (control group). Results are expressed by mean ± SD for 12 animals. * p ≤ 0:05*

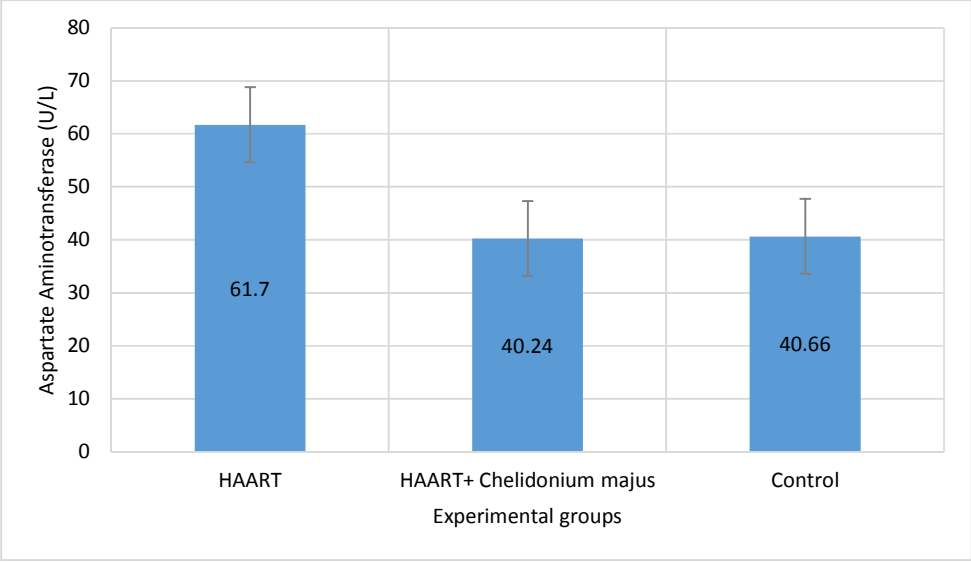

Fig. 2. Plasma levels of aspartate aminotransferase (AST) in Swiss mice of experimental and control groups after 15 days of treatment

Group I treated with HAART(5 mg / kg of atazanavir+ 5 mg / kg of tenofovir disoproxil fumarate+ 1.67 mg / kg of ritonavir+ 2.5 mg / kg of lamivudine, diluted in 1.2 mL of water/day/15 days; Group II treated with HAART (5 mg / kg of atazanavir+ 5 mg / kg of tenofovir disoproxil fumarate+ 1.67 mg / kg of ritonavir+ 2.5 mg / kg of lamivudine, diluted in 1.2 mL of water/day/15 days) + Chelidonium majus (mother tincture diluted $1x10^{12}$ in water ad libitum); Group III non-treated (control group). Results are given by mean ± SD of 12 animals

Increase in transaminases is a laboratory finding in patients using the antiretroviral therapy combination and the mechanism involved in this process is known [29,30]. Several studies suggest that liver damage associated with antiretroviral drugs may occur through four main mechanisms: hypersensitivity reactions, direct toxicity of the drug and / or its metabolism, mitochondrial toxicity and immune reconstitution inflammatory syndrome [31,32].

Table 2 demonstrates liver and spleen weight rates.

Another parameter that may be related to liver damage and an indication of this factor is the increase in the liver. However, liver weights were not significant even with changes in liver enzyme rates obtained in this study.

The spleen plays an important immunological function, acting primarily on the production of lymphocytes and monocytes in phagocytosis of bacteria, viruses and leukocytes and processing serum factors such as opsonins with a high capacity for the stimulation of phagocytosis. The spleen´s immuno-protection effect has been tested in several experimental studies. Current results showed that *C. majus* had an effect on the spleen, with a size equal to that of control. Studies show that *C. majus* increased lytic activity to YAC-1 in spleen lymphocytes in tumor cells [33].

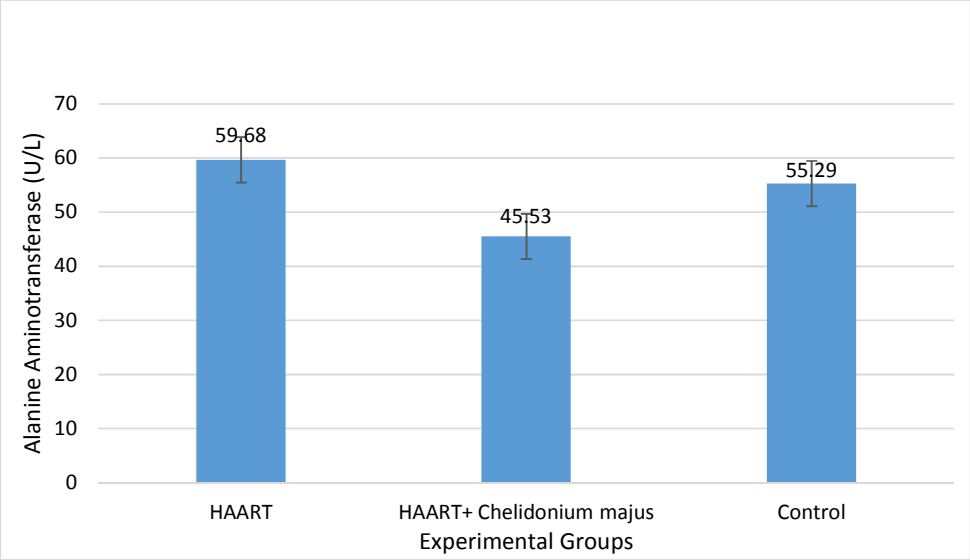

Fig. 3. Plasma levels of alanine aminotranferas (ALT) in Swiss mice of experimental and control groups after 15 days of treatment

Group I treated with HAART(5 mg / kg of atazanavir+ 5 mg / kg of tenofovir disoproxil fumarate+ 1.67 mg / kg of ritonavir+ 2.5 mg / kg of lamivudine, diluted in 1.2 mL of water/day/15 days; Group II treated with HAART (5 mg / kg of atazanavir+ 5 mg / kg of tenofovir disoproxil fumarate+ 1.67 mg / kg of ritonavir+ 2.5 mg / kg of lamivudine, diluted in 1.2 mL of water/day/15 days) + Chelidonium majus (mother tincture diluted $1x10^{12}$ in water ad libitum); Group III non-treated (control group). Results are given by mean ± SD of 12 animals

Table 2. Weight of the liver and spleen of experimental groups of mice (values represent mean ± SD)

Weight	Group			*P*
	HAART	HAART + *Chelidonium majus*	Control	
Liver	2.274±0.20	2.234±0.18	2.085±0.25	0.0894
Spleen	0.224±0.05	0.195±0.02	0.162±0.02	0.0004*

*Comparison between experimental groups: Group I treated with HAART(5mg / kg of atazanavir+ 5mg / kg of tenofovir disoproxil fumarate+ 1.67 mg / kg of ritonavir+ 2.5 mg / kg of lamivudine, diluted in 1.2 mL of water/day/15 days; Group II treated with HAART (5 mg / kg of atazanavir+ 5 mg / kg of tenofovir disoproxil fumarate+ 1.67 mg / kg of ritonavir+ 2.5 mg / kg of lamivudine, diluted in 1.2 mL of water/day/15 days) + Chelidonium majus (mother tincture diluted $1x10^{12}$ in water ad libitum); Group III non-treated (control group). Results are given by mean ± SD of 12 animals. * p ≤ 0:05*

4. CONCLUSION

Current study suggests that *C. majus,* diluted 10^{12}, reduced the toxic effects of HAART in mice. There was a decrease in triglyceride levels, higher weight gain and better AST and ALT levels. Evaluated parameters indicate that *C. majus* may be decreasing HAART-induced hepatotoxicity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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