Original Research Article

Effect of homeopathic drug on metabolic abnormalities induced by HAART in mice

ABSTRACT

Aims: Highly Active Antiretroviral Therapy (HAART) increased survival of AIDS patients. HAART-associated major toxic effects comprised: neuropathy, myopathy, pancreatitis, hepatic steatosis, lactic acidosis and lipoatrophy, metabolic complications (fat redistribution, insulin resistance and hyperlipidemia). *Chelidonium majus* has a long history in the treatment of several diseases exhibit apoptotic activity, antioxidant and hepatic-protective effects

Methodology: 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. Five experimental groups with 10 animals each were distributed as follows: (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* 6CH diluted in water 1.0 mL once a day, added to the drinking water (1:10 mL) available *ad libitum*, (III) animals treated with HAART diluted in 1.2 mL water gavage/day + *C. majus* 12CH diluted in water 1.0 mL once a day, added to drinking water (1:10 mL) available *ad libitum*, (IV) animals treated with HAART diluted in 1.2 mL water gavage/day + *C. majus* 30CH diluted in water 1.0 mL once a day, added to drinking water (1:10 mL) available *ad libitum, (V)* non-treated animals (control group) received 1.2 mL water by gavage/day. The experimental groups were treated for 15 days. The drug in the form of mother tincture, prepared with the presses juice of the root of C. majus was mixed in equal parts of grain alcohol (PA) obtained from the laboratory HN CRISTIANO, São Paulo, Brazil (lot 5387). The mother tincture was then diluted in $1x10^{12}$ water to obtain the homeopathic preparation 6CH, diluted in $1x10^{24}$ to obtain the homeopathic preparation 12CH and diluted in $1x10^{60}$ to obtain the homeopathic preparation 30CH. The method for drug preparation followed the Brazilian Homeopathic Pharmacopoeia. The dilution was considered free from any toxicity. 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: Showed that the HAART group presented a weight gain lower (50%) than the control group. Small little weight gain of animals using HAART may be related to the already known adverse effects of the antiretroviral. On the other hand, animals treated with *C. majus* regardless of concentration used (6CH, 12CH or 30CH) presented similar weight gain when compared to control. Clinical parameters such as, 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. *C. majus* protects the liver of mice from possible damage caused by antiretroviral therapy. ALT parameter showed levels which were 37.4% lower in mices treated with *C. majus* 6CH and 41% lower in mices treated with *C. majus* 30CH when compared to the group treated only with HAART. AST decreased in the group treated with *C. majus* 6Ch and 30CH demonstrate same levels of control.

Conclusion: Homeopathic preparations of *Chelidonium majus,* reduced the toxic effects of HAART in mice. Decrease in cholesterol and triglyceride levels, higher weight gain and better AST and ALT levels were reported. Evaluated parameters indicate that *C. majus* may be decreasing HAART-induced hepatotoxicity.

Keywords: Chelidonium, HIV/AIDS, antiretroviral, metabolic abnormalities

1. INTRODUCTION

Severe hepatotoxicity in HIV-infected patients receiving Highly Active Antirretroviral Therapy (HAART) occurs in 5-10% of cases. High liver transaminases are frequently observed in HIV-positive individuals submitted to HAART [1].

Data analyses from Amsterdam, CHORUS, ICONA and Target studies (5133 patients) demonstrate that elevated baseline alanine aminotransferase levels predicted subsequent hepatotoxicity [2]. Overall, there was a low incidence of long-term hepatotoxicity in these cohorts and no consistent association to a specific drug or drug class.

Heil *et al* [3] registered severe hepatotoxicity in 10.7% (6/56) of patients, severe hepatotoxicity occurred with efavirenz (n=2), nevirapine (n=1), indinavir (n=1), nelfinavir (n=1) and saquinavir/ritonavir (n=1).

On the other hand the underlying mechanism of hepatotoxicity is poorly understood, McRae *et al* tested the hypothesis that antiretroviral drugs modulated hepatic bile acid transport and concluded that ritonavir, saquinavir and efavirenz but not nevirapine inhibited both the hepatic uptake and biliary excretion of taurocholate [4].

Despite the studies the monitoring of patients over the years shows that there is a good virological response to antiretroviral but a high toxicity rate [5][6][7][8][9].

Homeopathy is a popular form of complementary and alternative medicine. Homeopathy is an over 200-year-old system of complementary and alternative medicine (CAM) developed by the German physician Samuel Hahnemann, MD. The classical process of manufacturing homeopathic medicines involves trituration in lactose and/or serial dilution in ethanol-water solutions and sucussion (vigorous repeated cycles of shaking via hand or standardizedmechnical arm pounding on a hard surface in glass vials containing ethanol-water solutions. Commom dilution factors are 1 part source to 99 parts diluent (1/100, centesimal or CH potencies)

Chelidonium majus (*C. majus*) is a homeopathic drug routinely used against various liver disorders. *Chelidonium majus* L (Family Papaveraceae), or greater celandine is an important plant in western phytotherapy and in traditional Chinese medicine. Crude extracts of *C.majus* as well as purified compounds derived from it exhibit a broad spectrum of biological activities (anti-inflammatory, antimicrobial, antitumoral, analgesic, hepatoprotective) that support some of the traditional uses of *C.majus* [10].

Animal experimentation in scientific research has contributed to the scientific and technological development promoting the discovery of treatment of several diseases that affect the human. The mice currently employed in experiments come from those domesticists who for a long time have been sharing with the man their home, their food and their infirmities. Its introduction as a laboratory animal is mainly due to the fact that it is small, very prolific, has a short gestation period, is easy to domesticate and maintain. The mouse is characterized by being a cosmopolitan species adapted to a wide variety of environmental conditions. It is an animal of nocturnal habits that is accommodated in any place. Mice are well developed when they receive standardized diets for laboratory animals. Mice are small animals, then agile and active. The symptoms of pain usually present in these animals as weight loss, piloerection and bent posture, isolation of the rest of the group and cries when being touched.

Current study assessed the capacity of homeopathic drug *C.majus* 6CH, 12CH and 30CH in improvement of experimentally induced antiretroviral toxicity in mice.

2. MATERIAL 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°C ± 2°C, 70% humidity and photoperiod (light / dark cycle 12 h).

2.2 Preparation of *Chelidonium majus*

The drug in the form of mother tincture and prepared from the pressed juice of the root of *C. majus*, was mixed in equal parts of grain alcohol (P.A.) obtained from laboratory HN CRISTIANO, São Paulo, Brazil (lot 5387). The mother tincture was diluted in $1x10^{12}$ water to obtain the homeopathic preparation 6CH, in $1x10^{24}$ to obtain the homeopathic preparation 12CH and in $1x10^{60}$ to obtain the homeopathic preparation 30CH. The method for drug preparation followed the Brazilian Homeopathic Pharmacopoeia [11]. (CH= centesinal dilution according to the Hahnemann method). The dilution was considered free from any toxicity [12].

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 167mg / kg/day of lopinavir+ritonavir(LPV/r) and zidovudine/lamivudine (AZT/3TC)15mg / kg/day diluted in 1.2mL of water.
Treatment period lasted 15 days and drug was administered at 09:00 h.

2.4 Treatment Schedule

Five experimental groups with 10 animals each were distributed as follows: (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* 6CH diluted in water 1.0 mL once a day, added to the drinking water (1:10 mL) available *ad libitum*, (III) animals treated with HAART diluted in 1.2 mL water gavage/day + *C. majus* 12CH diluted in water 1.0 mL once a day, added to the drinking water (1:10 mL) available *ad libitum*, (IV) animals treated with HAART diluted in 1.2 mL water gavage/day + *C. majus* 30CH diluted in water 1.0 mL once a day, added to the drinking water (1:10 mL) available *ad libitum, (V)* 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 aminotransferase (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 (AF MS n.800222-3-Reg. MS – n.80022230064, Belo Horizonte MG Brazil).

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

The antiretroviral lopinavir / ritonavir and zidovudine / lamivudine were used in the assays, following protocol routinely used with patients.

Assays revealed that the HAART group presented a weight gain lower (50%) than that of all the other groups. Animals treated with *C. majus* regardless of concentration (6CH, 12CH or 30CH) presented similar weight gain when compared to control (table 1).

Table 1. Weight gain (g) weight gain in the different experimental groups after 15 days of treatment

	HAART	Control	HAART+ *Chelidonium majus* 6CH	P	HAART+ *Chelidonium majus* 12CH	p	HAART+ *Chelidonium majus* 30CH	p
Weight gain(g)	5.724±2.104	11.30±2.077	11.4±3.972*	0.050	12.21±2.902*	0.044	13.16±3.843*	0.033

Comparison between experimental groups: Group I treated with HAART (167mg / kg/day of lopinavir+ritonavir(LPV/r) and zidovudine/lamivudine (AZT/3TC)15mg / kg/day diluted in 1.2mL of water; Group II treated with HAART (167mg / kg/day of lopinavir+ritonavir(LPV/r) and zidovudine/lamivudine (AZT/3TC)15mg / kg/day diluted in 1.2mL of water + *C. majus* 6CH 1.0 mL once a day, added to drinking water (1:10 mL) available *ad libitum*; Group III treated with HAART (167mg / kg/day of lopinavir+ritonavir(LPV/r) and zidovudine/lamivudine (AZT/3TC)15mg / kg/day diluted in 1.2mL of water + *C. majus* 12CH 1.0 mL once a day, added to drinking water (1:10 mL) available *ad libitum* Group IV treated with HAART (167mg / kg/day of lopinavir+ritonavir(LPV/r) and zidovudine/lamivudine (AZT/3TC)15mg / kg/day diluted in 1.2mL of water + *C. majus* 30CH 1.0 mL once a day, added to drinking water (1:10 mL) available *ad libitum* and Group V non-treated group (control group). Results are given as mean ± SD of 10 animals. Statistical analyzes were performed comparing HAART with HAART + *C. majus* groups.* $p \leq 0:05$.

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

The evaluation of metabolic parameters showed a significant difference in levels of plasma triglycerides and total cholesterol in animals treated with HAART (Table 2).

Table 2. Laboratory parameters in the experimental group *Chelidonium majus*

laboratory parameters	HAART	Control	HAART+ *Chelidonium majus* 6CH	P	HAART+ *Chelidonium majus* 12CH	p	HAART+ *Chelidonium majus* 30CH	p
Total cholesterol (mg/dL)	157.9± 22.37	94.39±33.	101.9±15,40*	0.004	151.9±15.40*	0.051	111.9±29.81*	0.004
Triglyceride (mg/dL)	294.1±68.5	164.6.4±45.1	205.9.1.9±68,5*	0.001	257.3±70.95	0.1207	198.2±54.6*	0.01
Creatinine (mg/dL)	9.96±16.3	7.85±11.	11.99±16.4	0.099	12.99±10.4	0.4557	10.5±2.6	0.455
GGT (U/L)	11.44±3.12	6.63±3.06	3.5±6.4	0.1207	11.4±3.4	0.1207	7.3±3.4	0.120
AST(U/L)	61.7±4.94	40±8.08	40.2±30*	0.029	44.8±30.8	0.290	37.36±6.34*	0.004
ALT(U/L)	72.73±21.23	55.29±19.09	45.52±14.6*	0.006	72.31±16.64	0.374	43.47±19.41*	0.0002

Comparison between experimental groups: Group I treated with HAART (167mg / kg/day of lopinavir+ritonavir(LPV/r) and zidovudine/lamivudine (AZT/3TC)15mg / kg/day diluted in 1.2mL of water; Group II treated with HAART (167mg / kg/day of lopinavir+ritonavir(LPV/r) and zidovudine/lamivudine (AZT/3TC)15mg / kg/day diluted in 1.2mL of water + *C. majus* 6CH 1.0 mL once a day, added to drinking water (1:10 mL) available *ad libitum*; Group III treated with HAART (167mg / kg/day of lopinavir+ritonavir(LPV/r) and zidovudine/lamivudine (AZT/3TC)15mg / kg/day diluted in 1.2mL of water + *C. majus* 12CH 1.0 mL once a day, added to drinking water (1:10 mL) available *ad libitum* Group IV treated with HAART (167mg / kg/day of lopinavir+ritonavir(LPV/r) and zidovudine/lamivudine (AZT/3TC)15mg / kg/day diluted in 1.2mL of water + *C. majus* 30CH 1.0 mL once a day, added to drinking water (1:10 mL) available *ad libitum* and Group V non-treated group (control group). Results are given as mean ± SD of 10 animals. Statistical analyzes were performed comparing HAART with HAART + *C. majus* groups.* p ≤ 0:05.

Figure 1 (ALT) and Figure 2 (AST) show results of hepatic enzymes. AST (aspartate aminotransferase) and ALT (alanine aminotransferase) are enzymes of great clinical interest because they diagnose 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.

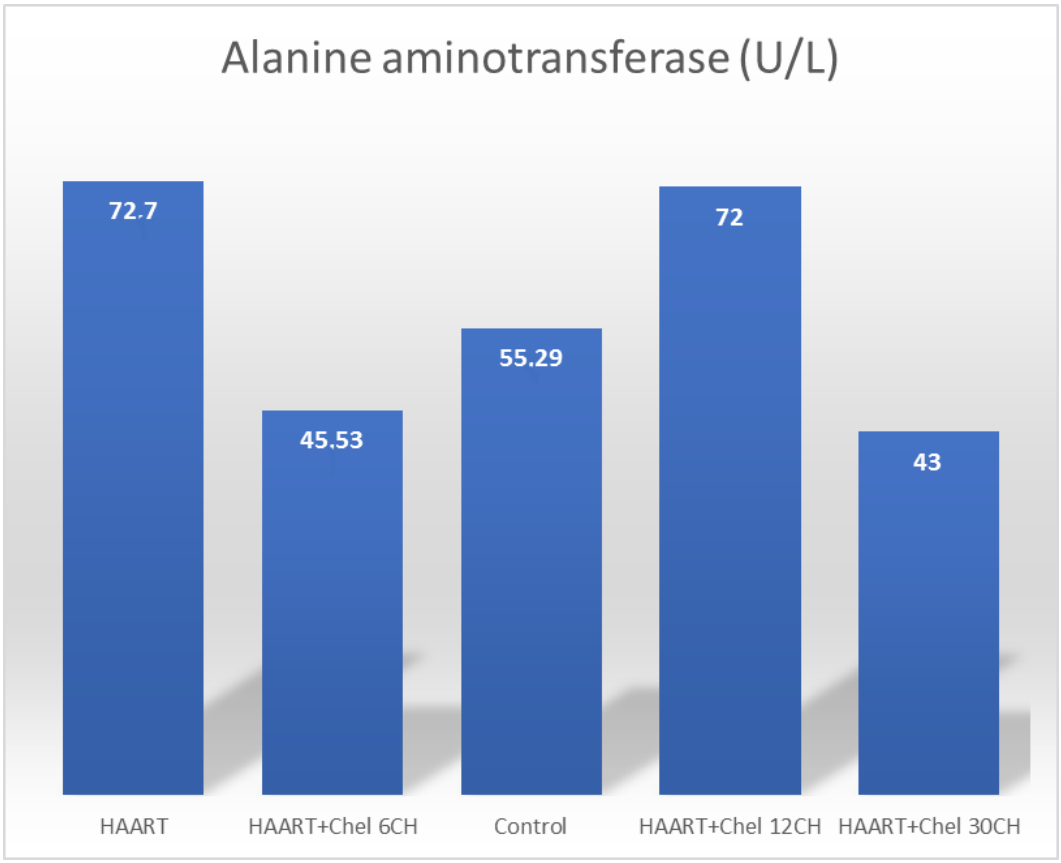

Figure 1. Alanine aminotransferase levels (U/L) of Swiss mice after 15 days. Comparison between experimental groups: Group I treated with HAART (167mg / kg/day of lopinavir+ritonavir(LPV/r) and zidovudine/lamivudine (AZT/3TC)15mg / kg/day diluted in 1.2mL of water; Group II treated with HAART (167mg / kg/day of lopinavir+ritonavir(LPV/r) and zidovudine/lamivudine (AZT/3TC)15mg / kg/day diluted in 1.2mL of water + *C. majus* 6CH 1.0 mL once a day, added to drinking water (1:10 mL) available *ad libitum*; Group III treated with HAART (167mg / kg/day of lopinavir+ritonavir(LPV/r) and zidovudine/lamivudine (AZT/3TC)15mg / kg/day diluted in 1.2mL of water + *C. majus* 12CH 1.0 mL once a day, added to drinking water (1:10 mL) available *ad libitum* Group IV treated with HAART (167mg / kg/day of lopinavir+ritonavir(LPV/r) and zidovudine/lamivudine (AZT/3TC)15mg / kg/day diluted in 1.2mL of water + *C. majus* 30CH 1.0 mL once a day, added to drinking water (1:10 mL) available *ad libitum*; Group V non-treated group (control group). Results are given as mean ± SD of 10 animals

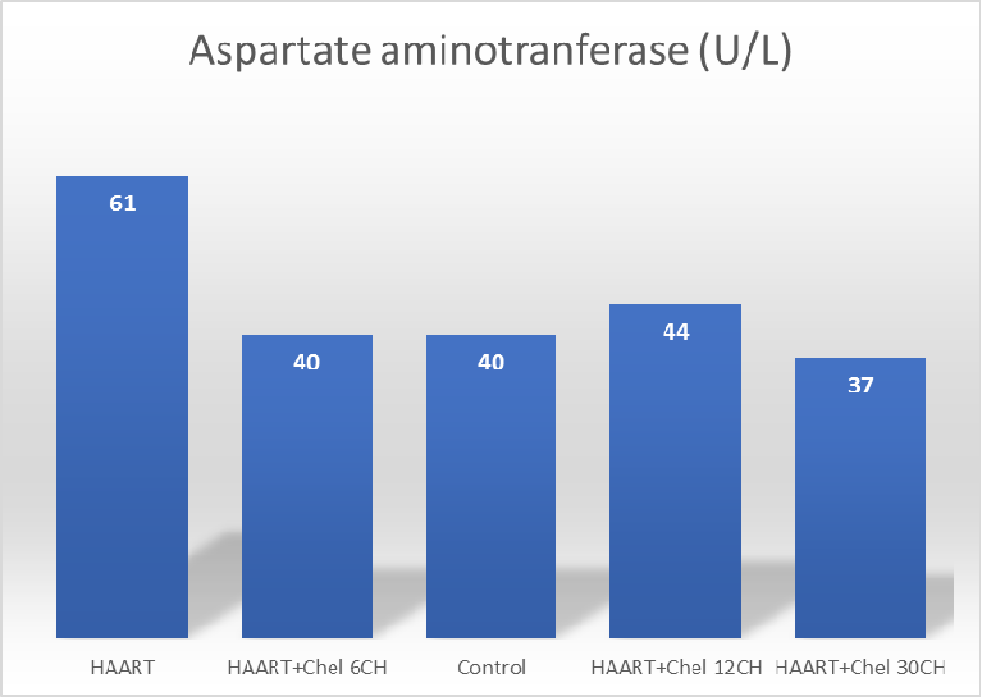

Figure 2. HAART (167mg / kg/day of lopinavir+ritonavir(LPV/r) and zidovudine/lamivudine (AZT/3TC)15mg / kg/day diluted in 1.2mL of water; Group II treated with HAART (167mg / kg/day of lopinavir+ritonavir(LPV/r) and zidovudine/lamivudine (AZT/3TC)15mg / kg/day diluted in 1.2mL of water + *C. majus* 6CH 1.0 mL once a day, added to drinking water (1:10 mL) available *ad libitum*; Group III treated with HAART (167mg / kg/day of lopinavir+ritonavir(LPV/r) and zidovudine/lamivudine (AZT/3TC)15mg / kg/day diluted in 1.2mL of water + *C. majus* 12CH 1.0 mL once a day, added to drinking water (1:10 mL) available *ad libitum*; Group IV treated with HAART (167mg / kg/day of lopinavir+ritonavir(LPV/r) and zidovudine/lamivudine (AZT/3TC)15mg / kg/day diluted in 1.2mL of water + *C. majus* 30CH 1.0 mL once a day, added to drinking water (1:10 mL) available *ad libitum*; Group V non-treated group (control group). Results are given as mean ± SD of 10 animals

4. DISCUSSION

Besides providing health benefits, HAART may have a negative impact on patient's quality of life. Identifying and treating these complications has important implications for patient survival

Slight weight gain in animals may be related to the already known adverse effects of the antiretroviral therapy.

Lopinavir, an HIV protease inhibitor, is active against HIV-1 and HIV-2. The medicinal product is only available together with low dose ritonavir formulation, to increase lopinavir concentrations and inhibit CYP3A4 metabolism [13]. According to Tavares [14], the drug is poorly tolerated at the beginning of treatment since it causes high serum triglycerides in more than 20% of patients.

The most common adverse reactions are nausea, vomiting, diarrhea, tingling or numbness in the hands, feet, around the lips, headache, feeling weak or tired, or unpleasant taste in the mouth, loss of appetite, loss of appetite. Allergic reactions including mild skin rashes, bronchospasm, angioedema, and rarely anaphylaxis and allergic rhinitis, have been reported. High hepatic transaminases, exceeding five times the upper limit of normality, clinical hepatitis and jaundice occurred in patients who received ritonavir alone or combined to other antiretroviral medicinal products [15].

The literature reports several reactions caused by lamivudine found in the literature are nausea, vomiting, stomach pain, diarrhea, pancreatic inflammation, headache, numbness, tingling sensation or weakness in the legs, fever, respiratory, nasal, cough and Pharyngitis, tiredness, generalized feeling of discomfort,

rash (red spots and plaques from the body, itching), hair loss. Joint pains, muscle disorders including rare reports of muscle tissue rupture, anemia, neutropenia, and platelet reduction have been reported in addition to the frequent increase of liver enzymes. A case of lactic acidosis and severe hepatomegaly with steatosis (including fatal cases) have been reported with the use of lamivudine in the treatment of HIV infection[16].

Results show that *C. majus* may have improved the appetite of animals or improved gastrointestinal symptoms induced by HAART. Kim et al. [17] demonstrate the therapeutic effect of chelidonic acid on ulcerative colitis.

Antiretroviral therapy is significantly associated with increase in serum lipid levels and increased dyslipidemia risk. Since antiretroviral therapy increases biosynthesis and reduces hepatic clearance of serum cholesterol, the impact of antiretroviral treatment on serum lipoprotein levels should be evaluated [18]. Current results demonstrate a beneficial effect of *C. majus* for the above parameters, although their normality has not been established. Homeopathic preparations of *C. majus* 30CH and 6CH revealed better responses in analyzed parameters.

Figure 1 and 2 show that *C. majus* protects mice liver from possible damage caused by antiretroviral therapy. ALT parameter showed levels 37.4% lower in mice treated with *C. majus* 6CH and 41% lower in mice treated with *C. majus* 30CH when compared to group treated only with HAART. AST decreased in the group treated with *C. majus* 6Ch and 30CH demonstrated same levels as control.

Gamma glutamyl transferase enzyme showed levels close to control group in groups treated with *C. majus* 6CH and 30CH (table 1). In relation to the gammaglutamyltransferase enzyme, it showed levels close to the control group in the groups treated with *C. majus* 6CH and 30CH.

Several studies investigated the effect of *C majus* on hepatic metabolism. Total ethanolic extract and the phenolic and alkaloidal fractions of the herb *C. majus* were tested for their choleretic activity with isolated perfused rat liver. Total extract significantly caused chloresis by increasing the independent flow of bile acid [19]. Evaluation of protective potentials of *C. majus*, during azo dye-induced hepatocarcinogenesis in mice demonstrated that *C. majus* 30CH and 200CH also modulated favorably some toxicity marker enzymes, such as acid and alkaline phosphatases, peroxidases, glutamate oxaloacetate and glutamate pyruvate transaminases in liver, kidney and spleen tissues of the carcinogen-fed mice [20] [21].

Due to the liver´s unique function and anatomical location and since it is exposed to several toxins and xenobiotics, including medications and alcohol; it is highly susceptible to tissue injury. Cell death in the liver occurs mainly by apoptosis or necrosis. Apoptosis is also the physiologic route to eliminate damaged or infected cells and to maintain tissue homeostasis [22].

Liver regeneration is perhaps the most studied example of compensatory growth aimed to replace loss of tissue in an organ. Hepatocytes, the main functional cells of the liver, proliferate to restore mass and to simultaneously deliver all hepatic functions necessary to maintain body homeostasis. Several hormones and xenobiotics directly alter the hepatostat and induce an increase in liver [23].

Each hepatocyte participates in multiple, narrow lumina, the bile canaliculi, and has multiple basal surfaces that face the endothelial lining [24].

Several cytogenetical and enzymatic protocols were used to test if two microdoses of *Chelidonium majus*, namely 30CH and 200CH used as homeopathic drugs, showed antitumor activity and also favorably modulated genotoxic damages produced by azo dye in mice at several intervals of fixation [25].

Current research evidenced a beneficial effect of homeopathic medicine. Best effects were obtained with dilutions 6 CH and 30CH. Components of homeopathic remedies are nanoparticles of source substance in water-based colloidal solution, not bulk-form drugs. Trituration and/or liquid succussions during classical remedy preparation establish “top-down” nanostructures. Nanoparticles stimulate hormesis, a beneficial low-dose adaptive response. Low dose homeopathic remedies are biological signals that

stimulate the organism's allostatic biological stress response network, evoking nonlinear modulatory, self-organizing change [26].

In the context of medicine and complementary and alternative medicine (CAM) researchers have previously detailed the evidence that living organisms are complex adaptative systems or networks of interconnected, interregulated components. Other investigators have extensively addressed the role of the allostatic stress response network within the organism in adaptation, maladaptation, and the development of disease [27]. The evidences demonstrated in this study corroborate with these affirmations being that there were good results for dilutions 6CH and 30 CH but not for 12 CH demonstrating an accurate adaptive mechanism coming from the use of homeopathic medicine that in its essence is a plant with high chemical pharmacological content.

5. CONCLUSION

Current study suggests that homeopathic preparations of *Chelidonium majus* reduced the toxic effects of HAART in mice. Cholesterol and triglyceride levels decreased, and higher weight gain and better AST and ALT levels were recorded. Evaluated parameters indicate that *C. majus* may be decreasing HAART-induced hepatotoxicity.

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.

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