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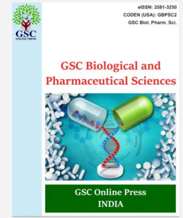

(Research Article)

Protective effects of ethanol-water leaf extract of *Jatropha gossypiifolia* on mouse virus-induced hepatitis (MHV) in male Swiss mice (*Mus musculus*)

Dezoumbe Donhai Honoré [1], James Bayoï [2, *], and Djoulde Darman Roger [1]

[1] Department of Agriculture, Livestock and Postharvest Technology, National Advanced School of Engineering Maroua, P.O Box 46, Maroua, Cameroon.

[2] Department of Biological Sciences, Faculty of Science, University of Maroua, P.O. Box 814 Maroua, Cameroon.

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Abstract

The aim of this study was to evaluate in vivo hepatoprotective activity of the ethanol-water extract of *Jatropha gossypiifolia* leaves (EJG) against mouse-induced hepatitis (MHV) in *Mus musculus* mice. This experiment was carried out with 30 male mice, divided into 6 batches, one control non-contaminated and five batches infected with MHV extracted from wild-type mice liver. Four batches of the infected mice were treated with either EJG (75, 150 and 300 mg/kg) or lamivudine (10 mg/kg) used as reference treatment. The fifth infected batch was untreated. The mice of all the batches were monitored every 12 hours for 5 days, including weight measurements and behavioral assessments. After 10 days of the MHV liver extract inoculation, all the subjects were sacrificed, the serum was collected and the biochemical parameters were measured. The results showed that MHV inoculation induced significant increase ($p < 0.05$) of relative weight of liver, heart, kidney and spleen in negative control, as well as serum values of ASAT, ALAT, C-reactive protein (CRP) and bilirubin compared with the control group. EJG at the concentrations of 75, 150, 300 mg/kg induced a significant reduction ($p < 0.05$) in relative organ weights, ASAT, ALAT, CRP and bilirubin contents compared with the negative control. In conclusion, EJG could protect the organs and has a potent hepatoprotective effect against MHV-induced liver damage in mice.

Keywords: *Jatropha gossypiifolia*; Mouse hepatitis virus (MHV); Liver damage; Organs protection; Hepatoprotective effect

1. Introduction

The liver plays an important role in bile synthesis, metabolic function, toxin degradation and synthesis of new substances in the body [1]. Liver damage is one of the most common diseases in the world with poor prognosis, mainly including hepatic steatosis, cholestasis, hepatitis, hepatic fibrosis, cirrhosis, even liver cancer and so on [2]. Viral hepatitis is a global public health problem, comparable to that posed by other major communicable diseases such as acquired human immunodeficiency syndrome (AIDS), tuberculosis or malaria [3].

The World Health Organization estimates around 240 million people worldwide with chronic hepatitis B of which approximately 600,000 die each year [4]. Infection due to Hepatitis B Virus (HBV) is much more common than that due to Human Immunodeficiency Virus (HIV) which affects approximately 35.5 million people [5]. Worldwide, 2 billion people have markers of past or present HBV infection [6]. Sub-Saharan Africa is recognized as an area of high HBV endemicity [7], with an estimated prevalence of 8% in West Africa and 5-7% in central, eastern, and southern Africa [4].

Prevention of liver exposure to damaging factors and good lifestyle habits are the most effective approaches [8], but patient compliance is very low. Therefore, there is an urgent need to develop new effective and low-toxicity drugs for

* Corresponding author: James BAYOÏ

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the treatment of liver disease. Modern cytokine therapy has been shown to be effective [9, 10, 11, 12]. But this modern treatment is not widely available and very expensive in Cameroon, so it is imperative to find a palliative against this scourge of society. Popular reports indicate that *Jatropha gossypiifolia* leaf known as Maagani-balmol powder is used in the traditional pharmacopoeia to treat "Sawaara", a condition reminiscent of hepatitis B infection. Various medicinal properties of *J. gossypiifolia* species are reported by traditional medicine. Some properties related to *J. gossypiifolia* are also common to other species of the *Jatropha* genus [13], where human and veterinary uses are described. Different parts of this plant, such as leaves, stems, roots, seeds and latex, are used in different forms of preparation (infusion, decoction and maceration, among others), by different routes and forms (oral, topical, baths, etc.). The most frequent reports refer to its anti-inflammatory, antidiarrheal, antiophidic, analgesic, antipyretic, antimicrobial, healing, antianemic, antidiabetic and antihemorrhagic activities, among many other examples [13].

The strong use of the leaves of *Jatropha gossypiifolia* against liver damage has been explored with the study of the complex mechanisms reported. Therefore, the current study objected to investigate the potential hepatoprotective effect of ethanol-water leaf extract of *Jatropha gossypiifolia* against MHV-induced liver damage in Swiss mice

2. Material and methods

2.1. Plant material

The mature fresh leaves of *Jatropha gossypiifolia* were collected from the edge of the Kaliao Mayo river, Maroua, Far North Cameroon. The leaves were identified by botanical experts of the Department of Biological Sciences, Faculty of Science, University of Maroua.

2.2. Animal material

Male white mice *Mus musculus* of Swiss strain, 8 - 12 weeks old and weighing between 20 - 30 g were used. They were bred at the animal house of the National Veterinary Laboratory (LANAVET) in Garoua-Cameroon.

2.3. Animal welfare

The experiments on animal study were conducted under the standard guidelines of National Research Centre for the use and care of laboratory animals. Animals were kept in standard metal cages (6 mice per cage) and at normal room temperature. They were acclimated for 2 weeks. The ventilation conditions were good and the lighting was natural. The animals were feed with standard diet and tap water *ad libitum*.

2.4. Preparation of viral solution

The viral solution was prepared according to the technique of Devisme [14] and Andral *et al.* [15], with some modifications. Murine hepatocytes were isolated from the liver of wild-type mice which is natural reservoir of Mouse Hepatitis Virus (MHV-3) (*Coronavirideae* family). This virus is commonly used to induce viral hepatitis in laboratory mice. The livers from sacrificed wild-type mice were recovered and placed in crucibles, after which mechanical dissociation was performed to rupture the liver capsule and highlight viral mouse hepatitis (MHV). After filtration using a 70 µm nylon and decantation, hepatocytes were separated from other liver cells by centrifugation at 4000 rpm for 15 minutes. The obtained supernatant was kept and used as viral solution. To demonstrate the presence of MHV-3 virus, 200 µl of the viral solution extracted from wild-type mouse liver was inoculated onto embryonated eggs for cell culture. Eggs were fresh, 5 to 6 days old and daily observed. The formation of aggregate in vitelline-inoculated eggs after five days of incubation confirmed the presence of MHV-3 virus in the wild-type mouse liver extract (viral solution).

2.5. Preparation of hydroethanolic extract

The leaves were washed and sun-dried to a constant weight. The dried leaves were pulverized with a mechanical blender, sieved and transferred to a sterile container. The extraction was carried out as previously reported by Dezoumbe *et al.* [16]. One hundred grams of leaf powder were mixed with 1 L of 70% (v/v) ethanol-distilled water solution. The mixture was left to macerate under intermittent shaking at room temperature for 24 hours. After that, the mixture was filtered and rotary evaporated at 40-50°C for the extract concentration.

2.6. Formulation of hepatoprotective treatments

The extracts were dissolved in distilled water, then the mixture was homogenized by shaking (5-10 min) with a magnetic stirrer. The obtained solutions were stored in a closed plastic jar and placed under refrigeration conditions

after each session of oral administration of EJG extract to the animals. The volumes of solution to be administered to the animals were calculated using the following formula:

$$V = \frac{D \times P}{C}$$

- V = volume of solution to be administered (mL)
- D = dose of the treatment (mg/kg)
- P = weight of the animal (kg)
- C = concentration of the extract (mg/mL)

2.7. Experimental protocol

The antiviral activity was evaluated according to the method described by Devisme [14]. The virus solution prepared from wild-type mice liver extract was diluted in sterile phosphate buffer to obtain the desired concentration and suboptimal virus dose. Each mouse was injected 200 µL per 20 g intraperitoneally on day 0 with either sterile phosphate buffer for uninfected control mice or phosphate buffer containing the viral solution for infected mice. Following MHV-3 inoculation (viral solution), every 12 hours for 5 days, the mice were monitored, including weight measurement and behavioral assessment, in order to identify any signs of animal distress and to euthanize them if necessary. According to the established protocol, all mice were sacrificed after 10 days post-inoculation of the virus. Treatment doses were administered 02 days after the onset of symptoms. The doses used to evaluate protective effects of *J. gossypiifolia* leaf extracts (EJG) corresponded approximately to the doses prescribed to patients by traditional physicians. Treatment in humans calls for the ingestion of approximately 10 mg of plant powder twice daily. Considering that the average biological weight of a human being is 70 kg, and that the quantities of hydroethanolic extracts that can theoretically be obtained by maceration from 10 g of *Jatropha gossypiifolia* powder is about 0.6 g (yield 6 %), the treatment in humans therefore consists in the daily ingestion of 25 mg/kg of *Jatropha gossypiifolia* body weight of ethanol-water extract. This value has been multiplied by 3 to compensate for a possible difference in bioavailability between humans and laboratory mice, and the value obtained of about 75 mg/kg of *Jatropha gossypiifolia* extract (EJG). These values were multiplied by 2, then by 4, to find the intermediate and maximum dose respectively. Therefore, three doses 75, 150, and 300 mg/kg of ethanol-water extract of *Jatropha gossypiifolia* leaves were selected for treatment of three groups of 6 mice each (EJG75, EJG150, EJG300). The fourth group of 6 mice received 200 µL of the solvent that corresponded to the negative control (NeG or TN), the fifth group received lamivudine tenofovir (10 mg/kg) as a positive control (PG or TP), and a final group that received just 200 µL of solvent as a normal control (NG or TNor). Each experiment was duplicated on groups of males. Each group was normally fed and watered before the sacrifice. At each sacrifice, blood was drawn through the jugular vein to measure liver transaminases (ALAT and ASAT), C-reactive protein (CRP), and blood bilirubin.

2.8. Preparation of serum and evaluation of some biochemical parameters

The animals were fasted overnight before blood sampling. They were then anesthetized with thiopental and blood samples were taken by cardiac puncture at the level of the right atrium after opening the rib cage. The blood was collected in dry tubes and centrifuged at 3000 rpm for 5 min to obtain the serum. The liver, heart, spleen, kidney, and lung were removed, rinsed in 0.9% NaCl, wrung out on filter paper, and weighed using an electronic balance. The relative weight of each organ was determined by the formula:

$$\textbf{Relative organ weight}\ (\%) = \frac{\text{Absolute organ weight (g)}}{\text{Animal body weight } (g)} \times 100$$

The serum obtained was used for biochemical assays, performed according to the protocols provided with the commercial Biolabo Diagnostics kits revised in October 2007. These are: bilirubin, C-reactive protein (CRP), alanine aminotransferase (ALAT), and aspartate aminotransferase (ASAT).

2.8.1. C-reactive protein assay

The CRP assay was performed using the agglutination method described by Amsellem [17]. CRP-Latex particles are coated with anti-human CRP antibodies. The CRP-Latex reagent is standardized to detect serum CRP levels around 6 mg/L, which is considered to be the lowest concentration of clinical significance. The assay was performed according to the instructions given in the Kit (Table 1). The mixing of the latex reagent with the CRP-containing serum leads to an antigen-antibody reaction that results in an easily visible agglutination within 2 minutes. The presence or absence of

visible agglutination indicates the presence or absence of CRP in the serum sample. The concentration C-reactive protein expressed in µg/mL was calculated using the following formula:

C-reactive protein (µg/mL) = (1/Dilution) x 6

Table 1 C-reactive protein assay

	Dilution			
	1/2	**1/4**	**1/8**	**1/16**
NaCl 9 g/L (µL)	100	100	100	100
Serum (µL)	100	-	-	-
Serum 2-fold diluted (µL)	-	100	-	-
Serum 4-fold diluted (µL)	-	-	100	-
Serum 8-fold diluted (µL)	-	-	-	100
Transfer to a circle on the test slide:				
Diluted serum (µL)	50	50	50	50
Reagent (flacon R1) (µL)	50	50	50	50

2.8.2. Determination of total bilirubin

The determination of serum bilirubin was performed according to the method described by Jagroop *et al.* [18]. Total bilirubin reacts with 2,4-dichloroaniline to form azobilirubin. Bilirubin bound to albumin is released by a detergent. The procedure used for the determination of total blood bilirubin is presented in Table 2. The bilirubin concentration expressed in mg/L were calculated by the formula below:

Bilirubin concentration (mg/L) =Absorbance × 12.5

Table 2 Bilirubin determination procedure

	Assay	**Blank**
Serum (µL)	100	-
Reagent (µL)	1000	1000
Distilled water (µL)	-	100

The mixtures were homogenized and left for 10 minutes at room temperature, protected from light. Then, the absorbance of the samples was read against that of the blank sample within 60 minutes.

2.8.3. Determination of transaminases

The determination of transaminases was conducted according to the method described by Guder *et al.* [19].

ALAT (alanine aminotransferase)

The enzyme ALAT catalyzes the transamination reaction between L-alanine and 2-oxyglutarate. The pyruvate formed is reduced to lactate in the presence of lactate dehydrogenase (LDH). NADH is oxidized to NAD+ to allow the last reaction to take place.

ASAT (aspartate aminotransferase)

The ASAT enzyme catalyzes the transamination reaction between L-aspartate and 2-oxyglutarate. The oxaloacetate formed is reduced to Malate in the presence of Malate dehydrogenase (MDH). NADH is oxidized to NAD+ to allow the last reaction to take place.

The disappearance of NADH per unit time is monitored by measuring the decrease in absorbance at 340 nm.

Experimental procedure

The tubes were divided and processed as indicated in Table 3. After incubation, absorbance was read at 340 nm in the spectrophotometer after calibration of the apparatus with the blank. Further readings were taken at 60 s time intervals after the 1st reading and the change in absorbance per minute as well as the enzyme activity was calculated according to the following formula:

Activity of serum transaminase = $((\Delta Abs/min)_{Assay})/(\Delta Abs/min)_{Calibrant}) \times$ Concentration of calibrant

Table 3 Serum transaminase procedure

Reagents	ASAT		ALAT	
	Blank	Assay	Blank	Assay
distilled water (µL)	100	-	100	-
Serum (µL)	-	100	-	100
Enzymatic reagent (mL)	1	1	1	1

2.9. Statistical analysis

All measurements were done in triplicate. Data were processed by R version 3.2.0. Since the variables were quantitative, ANOVA one-way followed by Turkey's post-test were used to assess the dose-response relationship of the treatment. Significance of differences between treatment and control groups was considered at $p < 0.05$.

3. Results and discussion

3.1. Effects of ethanol-water leaf extract of *J. gossypiifolia* on relative organ weight

Table 4 shows the effect of *Jatropha gossypiifolia* extracts on the relative weight (%) of some organs. The values in the tables represent the averages of the relative weights of each organ as a function of dose; there were no significant difference between the treated batches compared with the control one. The results in table 4 show that the liver and heart were the most affected organs by the presence of MHV-3 virus in the mice. At the end of the experiment, the relative weights of heart (0.40±0.05, 0.39±0.05, 0.30±0.03 %, respectively) and liver (4.08±0.56; 4.21±0.54, 3.71±0.25%, respectively) of mice treated with 75, 150 and 300 mg/kg were lower than those of the negative control (0.50±0.05 and 4.80±0.57%, respectively). This was the same with the others organs. This may be explained by the ability of EJG to protect tissues against destruction by the virus. This may be attributed to the presence of phenolic compounds in EJG. Dezoumbe *et al.* [15] reported significant contents of total polyphenols (21.23 ± 0.95 mgGAE/g), flavonoids (12.53 ±0.58 mgQE/g) and condensed tannins (7.13 ± 0.12 mgCE/g) in EJG compared with other tested extracts. Many previous studies reported antimicrobial and antiviral properties of polyphenolic molecules [20, 21]. According to Okuda *et al.* [22], tannins and similar compounds may provide protection to liver cells from the damaging effects of lipid peroxide by reducing lipid peroxide levels.

Table 4 Effect of ethanol-water leaf extract of *Jatropha gossypiifolia* on relative weight (%) of the organs of treated and no treated Swiss mice

Organs	Treatments					
	NG	PG	NeG	EJG75	EJG150	EJG300
Heart	0.43 ± 0.05a	0.44±0.05a	0.50±0.05a	0.40±0.05a	0.39±0.05a	0.30±0.03a
Liver	4.00 ± 0.57a	4.10±0.57a	4.80±0.57a	4.08±0.56a	4.21±0.54a	3.71±0.25a
Lung	0.81 ± 0.11a	0.86±0.11a	1.10±0.11a	0.79±0.12a	0.89±0.26a	1.00±0.22a
Kidney	0.90 ± 0.11a	0.92±0.11a	0.98±0.11a	0.90±0.07a	0.89±0.03a	0.94±0.10a
Splen	0.55 ± 0.15a	0.53±0.15a	0.70±0.15a	0.59±0.13a	0.53±0.09a	0.62±0.29a

NG: normal group; PG: positive group; NeG: negative group, EJG: *Jatropha gossypiifolia* extract; 75: Dose of 75 mg/kg; 150: Dose of 150 mg/kg; 300: Dose of 300 mg/kg. In the same line, two or more mean values with the same letter are not significantly different ($p > 0.05$).

3.2. Effects of *Jatropha* extract treatments on mortality, general appearance and animal behavior

Other behavioral parameters were also considered, such as social isolation and lack of food intake by sick mice. Mice that had not controlled the infection showed a fever peak with a significant drop in body temperature. This is explained by the characteristic symptoms associated with them: jaundice, nausea and dizziness, digestive disorders, anorexia, cloudy yellow complexion, yellowish whites of the eyes [23].

3.3. Effects of hydroethanolic leaf extract of *Jatropha* treatments on some biochemical parameters

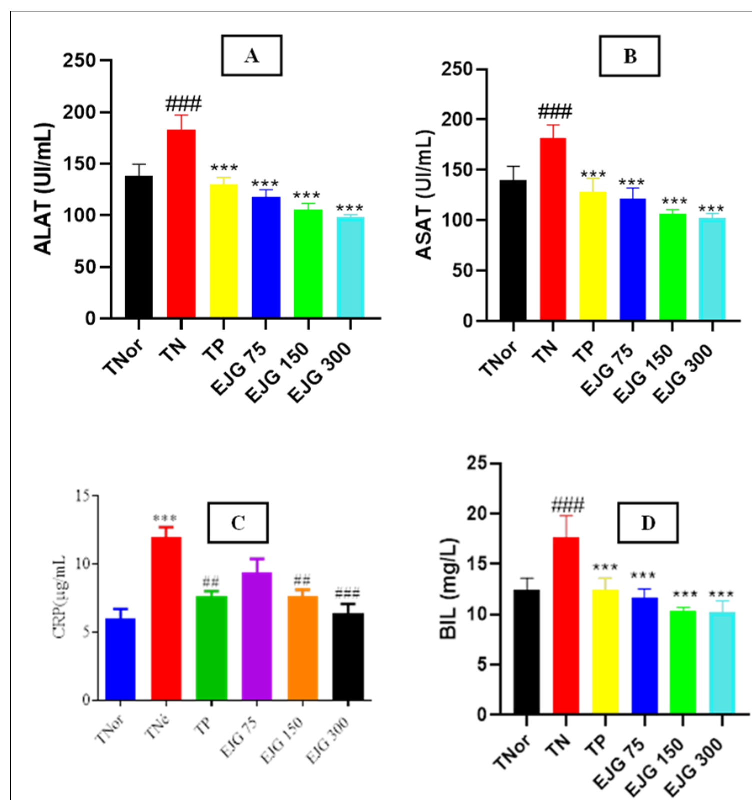

Figure 1 Effects of *Jatropha gossypiifolia* ethanol-water leaf extract on some biochemical parameters of male mice contaminated with MHV-3 extracted from liver of wild type mice. A: Alanine aminotransferase (ALAT), B: Aspartate aminotransferase (ASAT), C: C-reactive protein (CRP), D: Bilirubin (BIL). ###: p<0.01; ***: p < 0.05. TNor: Normal control; TN: Negative control; TP: Positive control; EJG: Ethanol-water extract of *Jatropha gossypiifolia* leaves; 75: Dose 75 mg/kg; 150: Dose 150 mg/kg; 300: Dose 300 mg/kg

Figure 1 presents the effect of *Jatropha gossypiifolia* leaf hydroethanolic extract on some biochemical parameters (ASAT, ALAT, Bilirubin and CRP). The administration of MHV-3 (viral solution) resulted in a significant increase ($p<0.001$) in ASAT, ALAT, CRP and Bilirubin in the negative control compared to the normal control. The increase in serum transaminases ASAT and ALAT activity testifies to the infectivity of the virus and the onset of liver damage [14]. Indeed, MHV-3 is a cytolytic virus causing primary lysis of infected hepatocytes [14]. Serum ALAT and ASAT activities, which are enzymes that were originally present in high concentrations in the cytoplasm, can be used to assess liver function. When the liver is damaged, these enzymes enter the bloodstream in proportion to the degree of damage [24]. There was also a significant ($p < 0.01$) increase in direct bilirubinemia. This phenomenon was particularly observed with rats from the intoxicated no treated control group (negative control). The sharp increase in biochemical parameters observed in negative control was similar with that of Sourabie *et al.* [25] who induced drug-induced hepatitis by carbon tetrachloride administration. These authors showed a disaggregation of hepatocyte membranes whose intra-cytoplasmic enzymes among others transaminases (AST/GOT and ALT/GPT) and Bilirubin will spill into the extracellular medium (blood plasma). All these disruptions lead to a significant elevation of serum enzyme markers and direct bilirubin observed with rats from the intoxicated control group.

On the other hand, treatment with ethanol-water extract of *Jatropha gossypiifolia* leaves (EJG) at different doses (75, 150, 300 mg/kg) significantly decreased ($p < 0.001$) the level of these parameters compared to the negative control. Indeed, administration of EJG to the infected mice in the test groups triggered a process of restoration of biochemical

markers. Administration of the extracts after the onset of infection symptoms restored hepatic parameters as the doses of the extracts increased. Our results are in line with those of Nurmuhammat et al. [26] showing that the aqueous extract of *Artemisia absinthium* L. (50, 100 and 200 mg/kg bw) has a significant, dose-dependent hepatoprotective effect against chemically (CCl4) and immunologically (BCG/LPS) induced liver damage in mice. Omeodu *et al.* [24] showed similar dose dependent reduction in blood serum levels of ASAT and ALAT when carbon tetrachloride-induced liver damage Wistar rats were treated with different doses of aqueous leaf extract doses (100, 150 and 200 mg/kg) of *Alchornea cordifolia*. The decrease in serum enzymes particularly ALAT (compared with negative control group) could be explained by the inhibition of intracellular enzyme leakage mediated by administration of EJG at different doses. Moreover, reduction in blood serum levels of ALAT and ASAT activities was more marked in EJG groups compared to PG (positive control group) which received reference hepatitis treatment (lamivudine at 10 mg/kg). This suggests that ethanol-water leaf extract of *J. gossypiifolia* may be used as alternative no chemical anti-hepatitis treatment.

Another biochemical marker for increased risk of inflammation may be blood serum level of CRP, which is a sensitive marker of systemic inflammation and is elevated in hepatitis; it is an acute-phase protein that has been identified as an important biomarker for various inflammation, degenerative and neoplastic diseases [27]. High levels of CRP have been found in blood during almost every disease associated with inflammation or tissue destruction, particularly in patients with various inflammatory diseases [28]. A sustained increase in serum CRP levels suggests sustained production and stimulation of acute-phase proteins during disease progression. In the current study, the association of CRP and hepatitis was investigated. It was observed that treatment with the hydroethanolic extract of *Jatropha gossypiifolia* leaves showed a significant reduction in CRP levels compared with the negative control group. Administration of the extracts restored serum CRP concentration in the blood. Our results showed that the 75 mg/kg dose had no effect on CRP concentration, whereas the 150 and 300 mg/kg doses respectively triggered the restoration of hepatocyte inflammation. This illustrates the ability of these extracts to protect the integrity of hepatocyte membranes against viral infection.

4. Conclusion

The main focus of the current study was to valorize use of *Jatropha gossypiifolia* leaves on liver protection against viral hepatitis. Extracts of *J. gossypiifolia* leaves (EJG) were prepared and their hepatoprotective activities were evaluated by testing in vivo the response of mice to the treatment. *J. gossypiifolia* extracts at doses of 75, 150, 300 mg/kg showed significant antiviral activity ($p<0.05$) with reduction of symptoms in more than 60% of *Mus musculus* mice infected with viral solution of MHV-3. Biochemical analysis showed a statistically significant difference in ASAT, ALAT, Bilirubin and C-reactive protein parameters at doses 75 and 150mg/kg; on the other hand, we noted a highly significant reduction in ASAT, ALAT, Bilirubin and C-reactive protein at the dose of 300 mg/kg compared to the negative controls. The pharmacological activities in liver protection provided in this work showed updated information on the pharmacology of *Jatropha gossypiifolia* leaves regarding its clinical application.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interests to be disclosed.

Statement of ethical approval

All experiments on animal study were conducted under the standard guidelines of National Research Centre for the use and care of laboratory animals.

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