

INHIBITION OF COXSACKIE VIRUS B3 IN MICE USING METHANOLIC EXTRACT OF *CALLIANDRA HAEMATOCEPHALA*

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ABSTRACT

Coxsackie virus B3 (CVB3) represents current major threats to public health and considers as an important viral pathogen related to viral myocarditis. We determined the safety of methanolic extract of *Calliandra haematocephala* in non-infected mice then two safe doses were selected to be evaluated in infected mice with CVB3 by determining the morbidity, mortality, heart to body weight ratio (HW/BW), virus titers in heart tissue. The effect of the extract on the heart tissues and the activities LDH, AST, and CK enzymes in the mice infected with CVB3 were also determined. Our results recorded that the methanolic extract at 100 mg/kg body was safe dose in mice and didn't shown significant changes in functions or histological structures of liver and kidney in non-infected mice and therefore we used 100 mg/kg and 50 mg/kg to be evaluated in infected mice with CVB3. We observed that the methaolic extract of *Calliandra haematocephala* leaves at the two doses decreased the morbidity, mortality, HW/BW, virus titers, necrosis and mononuclear cell infiltration. The levels of LDH, AST, and CK enzymes were also reduced in the treated infected mice compared with those untreated infected mice. This result suggested that the methaolic extract of *Calliandra haematocephala* may represent a potential antiviral drug to treatment viral myocarditis

Keywords: *Callindra*, antiviral, *in vitro*, *in vivo*, CVB3

INTRODUCTION

Enteroviruses, including coxsackie-, polio-, echo-, and enteroviruses 68-71, are characterized by small RNA viruses in the picornaviridae family (Rajtar *et al.*, 2008). Coxsackieviruses can be divided into group A and B based on the difference in the pathogenicity in mice. The group A coxsackieviruses include 23 serotypes whereas the group B coxsackieviruses include 6 serotypes (Mahy, 2008; Melnick, 1996). Coxsackieviruses can cause wide variety of illness in human including myocarditis, diabetes, common cold, neurological disorders, cardiomyopathy, and inflammation (Galbraith *et al.*, 1997; Fohlman *et al.*, 1993; Muir *et al.*, 1996; Ramsingh *et al.*, 1997). Heart diseases caused by viral myocarditis have been reported in infant, children, and adult. Although there is many viruses such as adenovirus, Epstein-Barr virus, parvovirus, Herpes virus type 6, cytomegalovirus, hepatitis C virus cause myocarditis (Bowles *et al.*, 2003; Kindermann *et al.*, 2008; Kuhl *et al.*, 2005a,b; Kyto *et al.*, 2005; Mahrholdt *et al.*, 2006; Matsumori, 2005; Matsumori *et al.*, 2006), however coxsackieviruses and specially type B3 consider the main ethiological pathogen for myocarditis (Blauwet, 2010; Kuhl *et al.*, 2005^a; Mahrholdt *et al.*, 2006). Coxacki B3 virus is responsible for more than fifty percent of all viral myocarditis cases (Shen *et al.*, 2009). However up to now, there are no specific drugs or vaccines available to clinical treatment of CVB3 infection. Development of antiviral agents from medicinal plants are increasing day by day in the worldwide because it consider as cheap and safe sources for both animal and human. So, the aim of the present work was to evaluate the antiviral activity of methanolic extract obtained from *Calliandra haematocephala* leaves against coxsackie B3 virus *in vivo*.

Calliandra haematocephala belongs to the family Fabaceae (Bailey, 1976; Benson, 1957; Williamson, 1981) is native to tropical America and widely distributed in various regions such as Australia, South Florida, Malaysia, and Egypt (Williamson, 1981). Phytochemical screening of the methanolic extract of *C. haematocephala* leaves is reported to contain carbohydrate, protein, alkaloids, flavonoid, steroid, and glycoside (Gupta *et al.*, 2013). *C. haematocephala* is reported also to contain betulinic acid (Nia *et al.*, 1999). The betulinic acid act as

an anti-inflammatory, anti-tumor and anti-HIV agents (Potier, 1991; Kashiwada *et al.*, 1996). *C. haematocephala* showed various pharmacological properties such as antibacterial (Nia *et al.*, 1999), Anti-oxidant (Moharran *et al.*, 2006), and gastroprotective activities (Barbosa *et al.*, 2012).

MATERIAL AND METHODS

Plant collection and identification

C. haematocephala leaves were collected from the National Research Centre Botanical Garden during the period May and June 2012, and was kindly identified by, Mrs. Tersea Labib, taxonomist at Orman Botanical garden, Giza and Dr. Mona Marzok, researcher in National Research Center (NRC).

Extraction with different organic solvents

The fresh leaves of *C. haematocephala* were dried at room temperature in shade and grinded to fine powder. Air dried fine powdered of *C. haematocephala* leaves was extracted by methanol in percolator at room temperature till exhausted, and evaporated till dryness in rotary evaporator at 40 °C then kept in refrigerator until use.

Cell culture and virus

Green monkey kidney (GMK) and coxsackievirus B3 (Nancy) strain were obtained from the Slovak Medical University. GMK cells were cultivated in Eagle's minimum essential medium (EMEM) (Lonza, Belgium) supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 units/ml penicillin, 100 µg/ml streptomycin, and 1% HEPES (4-2-hydroxyethyl-1-piperazineethanesulfonic acid). All reagents were purchased from Lonza, Belgium. The medium used for the cytotoxicity and antiviral assays contained 2% of the appropriate fetal bovine serum. Viral CVB3 stocks were prepared in GMK cells and kept at -80°C until used.

Virus titration

Virus was titrated on GMK cells in 96 well microtiter plates as described previously (Bopegamage et al., 2003). Tissue culture infectious doses/ml (TCID₅₀/ml) were calculated using Kärber method (Karber, 1931).

Cytotoxicity determinations in vivo

BALB/c male mice, 4 weeks old (17-20 g) were obtained from animal house of National Research Center, Cairo, Egypt. Mice were divided into 6 groups of 10 mice. Five groups were treated by different concentrations of methanolic extract of *C. haematocephala* (100, 250, 500, 750 and 1000 mg/kg body weight/day), one control group. Mortality of mice was controlled daily during the 8 weeks.

Effect of methanolic extract of *C. haematocephala* leaves on liver and kidney function and structure sub-chronictoxicity

Biochemical measurements and the serum glucose level were analysed by an enzymatic colorimetric method. The liver function tests such as the activities of serum albumin, total bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and gamma glutamyltransferase (γ-GT) levels as well as serum glucose and kidney function tests such as creatinine and urea nitrogen, and total protein levels were also measured spectrophotometrically by standardized commercial kits as described by (Webster, 1974; Friedman and Young, 1997; Bergmyer et al.1986a; Bergmyer et al. 1986b; Belfield and Goldberg, 1971; Szasz, 1969; Trinder, 1959; Tabacco et al., 1979; Bartels and Bohmer, 1971; Cannon et al. 1974). All kits were purchased from BIODIAGNOSTIC (29 El-Tahrer St. - Dokki- Giza - Egypt).

Antiviral activity

Forty BALB/c male mice, 4 weeks old (17-20 g) obtained from animal house of National Research Center in Cairo were used in this study. These mice were divided into 5 groups of 8 each. Of which, four groups of mice were infected intraperitoneally with CVB3 (10⁴ TCID₅₀/0.1 ml) and the remaining group was used as negative control and was similarly injected intraperitoneally with 0.1 ml of PBS (phosphate saline buffer) without viruses. After additional 24 hours, twenty four of inoculated mice were orally given methanolic extract (100 mg/kg body weight/day, n=8 & 50 mg/kg body weight/day, n=8) or injected intraperitoneally with ribavirin, which were used as positive control at a dose of 10 mg/kg (n=8) daily for 7 days. Eight inoculated mice were treated with 0.9% NaCl solution daily and used as infected control. The negative control (n=8) were given orally 0.9% NaCl solution daily. The morbidity (such as trembling, loss of appetite, diminished vitality, ruffled fur, and weight loss) and mortality were recorded daily during the 7-d experiment.

Mice were sacrificed on day 7 (4 in each group) after viral exposure. Blood was collected from the eye sockets and separated into serum to determine the lactic dehydrogenase(LDH) (Biosystem, Spain), creatine kinase(CK) (Spinreact, Spain), and aspartate transaminase(AST) (Randox, UK) activities by using commercially available kits. The ratio of BW/HW as the heart index was determined by measuring the body weight (BW) and heart weight (HW) of each mice. The heart was divided into two parts, one part of the hearts of mice in each group was homogenized in 1.5 ml of test medium followed by frozen and thawed twice to release CVB3 from heart tissue then centrifuged at 1200xg for 15 min. Virus titers was determined by the plaque assay with GMK cells (Bishop and Koch, 1969). The other part of the hearts of mice in each group were fixed in 10% buffered then sectioned (4µm thick) and stained with hematoxylin-eosin. Sections of hearts were scored for myocardial necrosis as described previously (Zhang et al., 2006). Photographs for myocarditis signs were taken under an inverted microscope using camera (Cannon). The remaining four mice of each

group were checked daily to determine the changes in body weight and any deaths.

Statistical analysis

All data obtained were expressed as mean, ± S.D., and examined by one way analysis of variance (ANOVA), CoStat Computer Program. A probability values of less than 0.05 was regarded as statistically significant.

RESULTS AND DISCUSSION

Cytotoxicity determinations in vivo

For the acute toxicity study, 100, 250, 500, 750 and 1000 mg/kg body weight of the *C. haematocephala* methanolic extract were administered through orally to mice and obvious symptoms of toxicity and mortality were monitored for 8 weeks. Dose 100 mg/kg body weight of methanolic extract induces 10% mortality of mice. Administration of *C. haematocephala* methanolic extract at concentrations of 250, 500, 750 and 1000 mg/kg body weight recorded mortality mice by 30 % (Tab. 1). So, 100 mg of methanolic extract/kg body weight was selected as safe dose to determine the biochemical indices in mice. Unfortunately, no literatures were found concerning the cytotoxicity of methanolic leaf extract of *C. haematocephala*. However, ethanolic leaf extract of another species of genus *Calliandra*(*Calliandra portoricensis*) was found to be safe with LD₅₀ = 150mg/kg (Onyema et al., 2012).

Table 1 Mortality rates of mice after administration of extract at different concentrations

Plants	Concentrations/kg body weight	Number of mice in each	Number of dead animals	Mortality rate
Control	0.00	10	0	0%
Methanolic extract of <i>C. haematocephala</i> leaves at different concentrations	100 mg	10	1	10%
	250 mg	10	3	30%
	500 mg	10	3	30%
	750 mg	10	3	30%
	1000 mg	10	3	30%

The effect of methanolic extract of *C. haematocephala* leaves on liver and kidney function and structure Sub-chronictoxicity

This study represent the first report concerning the safety of methanolic leaf extract of *C. haematocephala*. The effects of daily oral administering dose at 100mg/kg body weight of the extract for 8 weeks on albumin, bilirubin and glucose levels are shown in Tab. 2. The results showed that the extract did not cause any significant changes on the level of the albumin, bilirubin and glucose as compared to control group. Aminotransferases (ALT and AST) are produced in the liver and are good markers of liver cells damage (Rej, 1989). Administration of healthy mice with *C. haematocephala* methanolic extract at 100 mg/kg body weight caused significant decrease in the levels of liver function enzymes AST by 7.13 unit/ml (18.91%) and ALT by 7.21 unit/ml (13.70%), The reduction in the level of AST and ALT at the 1st week was temporary and the level increased to the normal level at the 2nd week and persisted to the end of the experiment(Tab. 3). The level of ALP was decreased in 1st week of treatment by 14.19 units/ml and then slightly increased from the 2nd week until the 8th week as compared to control group. However the extract didn't caused significant changes in the level of GGT along the experiment (Tab. 3).

Table 2 Effect of methanolic extract of *C. haematocephala* on liver function indices and glucose level in normal mice at different durations

Parameters	Control	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week	7 th week	8 th week
Albumin g/dL	3.65±0.32 ^a	3.48±0.23 ^a	3.81±0.13 ^a	3.86±0.57 ^a	3.81±0.40 ^a	3.91±0.41 ^a	3.84±0.32 ^a	3.76±0.43 ^a	3.16±0.14 ^a
Bilirubin mg/dL	0.50±0.08 ^{ab}	0.47±0.06 ^{ab}	0.53±0.04 ^{ab}	0.53±0.06 ^{ab}	0.42±0.06 ^b	0.53±0.07 ^{ab}	0.51±0.05 ^{ab}	0.62±0.09 ^a	0.55±0.05 ^{ab}
Glucose mg/dL	82.40±2.61 ^{ab}	77.73±5.67 ^b	76.93±2.70 ^b	82.88±2.93 ^{ab}	81.74±2.76 ^{ab}	77.47±2.77 ^b	77.17±4.70 ^b	79.23±3.62 ^{ab}	86.53±3.52 ^a

- Data are means ± SD of five mice in each group.
- ^{ab} significance values at p≤ 0.02 for bilirubin and ≤ 0.007 for glucose.

Table 3 Effect of methanolic extract of *C. haematocephala* on liver function enzymes in normal mice at different durations

Parameters	Control	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week	7 th week	8 th week
AST Unit/ml	37.70±2.89 ^a	30.57±1.56 ^b	35.98±2.86 ^a	36.06±1.65 ^a	36.06±1.41 ^a	34.17±1.69 ^{ab}	33.74±1.28 ^{ab}	33.96±1.6 ^{ab}	36.73±2.59 ^a
ALT Unit/ml	52.61±2.66 ^a	45.40±2.17 ^b	50.01±1.42 ^a	48.62±2.20 ^a	50.15±1.78 ^a	51.29±2.42 ^a	51.18±1.86 ^a	51.01±1.77 ^a	53.73±3.15 ^a
ALP Unit/L	145.75±2.6 ^a	131.56±9.93 ^a	133.35±9.1 ^a	139.14±5.6 ^a	138.09±6.34 ^a	136.47±8.2 ^a	135.25±9.1 ^a	137.19±4.7 ^a	137.38±5.10 ^a
γ-GT Unit/L	15.21±2.55 ^a	15.14±2.57 ^a	14.60±3.04 ^a	15.87±2.91 ^a	15.29±2.50 ^a	15.23±2.21 ^a	15.91±1.87 ^a	15.92±2.39 ^a	15.53±2.35 ^a

- Data are means ± SD of five mice in each group.
- ^{a,b}the significance values at p≤ 0.002 for AST and ≤ 0.001 for ALT

Kidney function indicators, changes in creatinine, urea and total protein were observed after administration of mice with the extract at dose 100 mg/kg body weight daily for 8 weeks. Tab.4 shows that the changes were insignificant. They are normally present at low levels in the blood so if the liver cells are damaged, it would be expected that some of the enzymes leak into the blood and increase in

levels. If there is tissue damage, some of these biomolecules find their way into the serum probably by leakage through altered membrane permeability (Akanji and Yakubu, 2000).

Table 4 Effect of methanolic extract of *C. haematocephala* on kidney function parameters in control mice at different durations

Parameters	Control	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week	7 th week	8 th week
Urea mmol/L	5.17±0.33 ^{ab}	3.89±0.45 ^b	4.13±0.78 ^{ab}	3.92±0.38 ^b	5.53±0.91 ^a	5.55±1.08 ^a	4.56±0.77 ^{ab}	4.64±0.34 ^{ab}	4.53±0.19 ^{ab}
Creatinine μmol/L	35.25±2.50 ^a	32.85±1.88 ^a	34.75±2.66 ^a	35.00±2.58 ^a	35.25±3.86 ^a	34.00±2.58 ^a	32.75±2.50 ^a	34.54±2.64 ^a	35.75±1.71 ^a
Total protein g/dL	6.79±0.27 ^a	6.83±0.23 ^a	7.32±0.80 ^a	6.75±0.68 ^a	7.38±0.29 ^a	7.44±0.32 ^a	6.89±0.41 ^a	7.04±0.38 ^a	6.71±0.32 ^a

- Data are means ± SD of five rats in each group.
- ^{a,b}the significance values at p≤ 0.004.

Microscopic examination of liver of control mice showed normal structure of the central vein, hepatocytes, and blood sinusoids (figure 1, A). Histopathological examination of sections of liver of the treated mice with the methanolic extract of *C. haematocephala* for 1-8 weeks showed the normal histological structure (figures 1, B-I) and normal architecture suggesting no morphological disturbances.

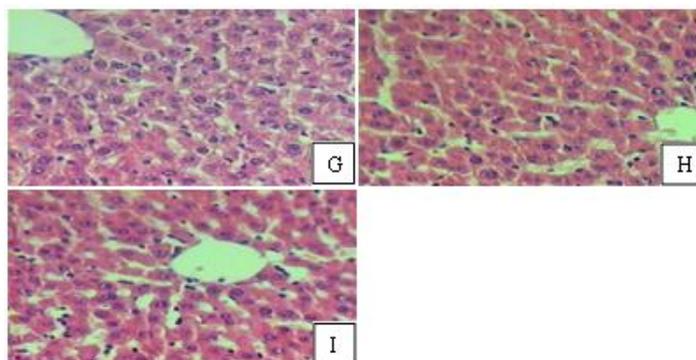
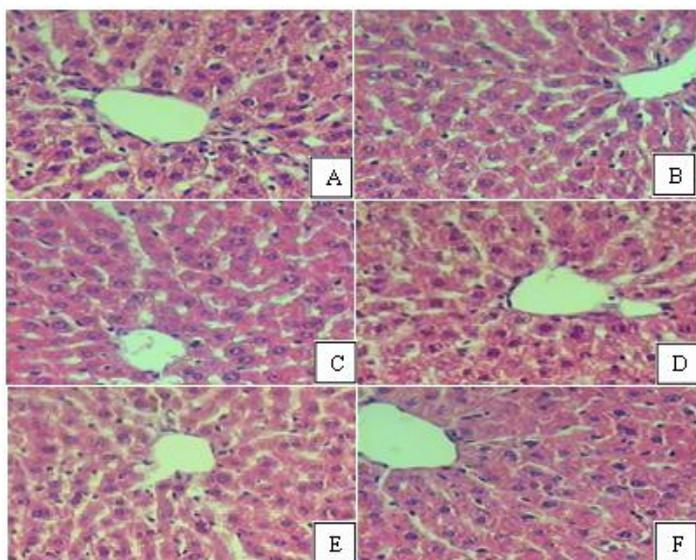


Figure 1 Sections of liver of mice treated with *C. haematocephala* methanolic extract A) normal control, B) mice treated for 1 week, C) mice treated for 2 weeks, D) mice treated for 3 weeks, E) mice treated for 4 weeks, F) mice treated for 5 weeks, G) mice treated for 6 weeks, H) mice treated for 7 weeks and I) mice treated for 8 weeks showed normal structure (H & E x 150).

Sections of cortical tissue of the kidney of control mice showed normal mice corpuscles, proximal convoluted tubules, and distal convoluted tubules (figure 2, A). Histopathological investigation of sections of kidney of the treated mice with the methanolic extract of *C. haematocephala* for 1-8 weeks showed the normal histological structure (figure 2, B-I).

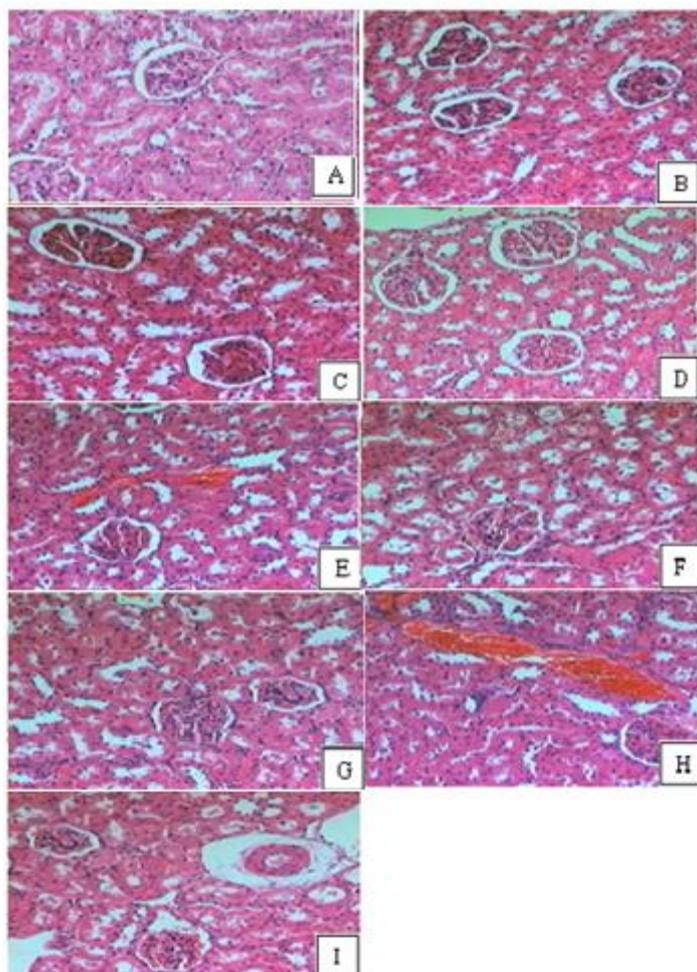


Figure 2 Sections of kidney of mice treated with *C. haematocephala* methanolic extract A) normal control, B) mice treated for 1 week, C) mice s treated for 2 weeks, D) mice treated for 3 weeks, E) mice treated for 4 weeks, F) mice treated for 5 weeks, G) mice treated for 6 weeks, H) mice treated for 7 weeks and I) mice treated for 8 weeks showed normal structure (H & E x 150).

Morbidity, mortality, and HW/BW ratios in vivo.

By day 3 after viral exposure, some mice especially in the viral control group showed morbid. The morbidity was 87.5% (7/8) in infected mice group while it reduced to 12.5% (1/8) and 25% (2/8) in mice treated with total alcohol of *C. haematocephala* at the doses of 100mg/kg and 50 mg/kg respectively. The mortality was found on day 9, some of mice especially in the viral infected control group began to die, and by 14 day, all of them had died. But there was no any deaths in the mice treated with the methanolic extract of *C. haematocephala* at the two doses 100 mg/kg and 50 kg/kg (Tab. 6).

The HW/BW ratios were significantly increased in the infected control mice without treatment than that of those treated mice with the methanolic extract of *C. haematocephala* at the two dosages (100 mg/kg and 50 kg/kg) compared with those in the normal control group (Tab.6).

Virus titers of hearts in vivo experiment

The virus titers were significantly decreased in the hearts of mice group treated with the methanolic extract of *C. haematocephala* leaves than that of those in the viral infected control group where the virus titers 4.32 ± 0.23 in the myocardial cells of the infected mice but significantly reduced to 2.41 ± 0.85 and 2.87 ± 0.44 in the myocardial cells treated with 100 and 50 mg methanolic extract of *C. haematocephala* leaves (Tab. 6), indicating that the methanolic extract of *C. haematocephala* possess potent antiviral activity against coxackie B3 virus. The exact mechanism of viral inhibition by our extracts in treated mice is not clear. However, we suggest that the methanolic leaf extract may be inhibited the virus replication by blocking the coxsackievirus and adenovirus receptor (CAR) on host cells. CAR receptor is the primary receptor of CVB3 (Bergelson et al., 1997; Tomko et al., 1997) or by binding the extract to viral protein 1 (VP1) on the outer membran of the virus and prevented its attachment to receptor on host cells. the WIN compounds, WIN 54954, are antiviral agents by interacting with the hydrophobic pocket on the surface of the Coxackie B3 virus virion and inhibit interaction of the virion with the viral receptor (CAR) and thus prevent the virus attachment to the target cell receptors (Pevear et al., 1989). Finally, we suggest that the extract may be inhibited the viral replication by inhibition of RNA transcription or translation one or more of unstructured proteins (2A, 2B, 2C, 3A, 3B, 3C, and 3D) of the viral genome in the infected cells. Nitric oxide (NO) have been reported to inhibit the coxsackievirus replication via inactivation of proteases 2A and 3C by S-nitrosylation (Badorff et al., 2000; Saura et al.,1999). In another study, compounds, such guanidine hypochloride, HBB, MRL-1237 and TBZE-029 inhibited RNA synthesis by interacting with the viral protein 2C which led to protection of infected cell from lysis (De Palma et al., 2007 and 2008).

Table 6 Effect of methanolic extract of *C. haematocephala* leaves on mortality, the heart index, virus titers, and pathologic scores in BALB mice infected with CVB3 7 days after inoculation

Group	Morbidity (%)	Mortality (%)	HW/BW ratios (means± S.D.)	Virus titration (means± S.D.)	Pathologic scores (means±S.D.)
Negative control group	0	0	4.21±0.02	0.00±0.00	0.00±0.00
Infected control group	87.5	100	6.12±0.03	4.32±0.01	3.21±0.10
Ribavirin (1 mg/mL)	25*	25*	5.81±0.02*	3.45±0.03*	2.62±0.35*
Methanolic extractof <i>C. haematocephala</i> 100 mg/kg	12.5*	0	4.31±0.01*	2.41±0.03*	0.75±0.03*
Methanolic extractof <i>C. haematocephala</i> 50 mg/kg	25*	0	4.75±0.04*	2.87±0.03*	0.97±0.02*

Means± S.D. values obtained from four replicate samples in each group. *p value is less than 0.01, as compared with the viral infected control

Effect of methanolic extract of *C. haematocephala* leaves on the activities of LDH, AST, CK.

The changes in the activities of lactic dehydrogenase (LDH), aspartate transaminase (AST), and creatine kinase (CK) is an indicator to injury to heart (Baba et al., 1976). We observed that the activities of LDH, AST, and CK enzymes were significantly increased in the serum of viral infected group than those observed in mice treated with methanolic extract of *C. haematocephala* at the two dosage (at 100mg/kg and 50 mg/kg), when they were compared to that in the normal control (figure 3). These results indicated that the methanolic leaf extract protected the cardiac tissue from the harmful effect of CVB3 and decreased the leakage of these enzymes to blood stream and therefor their levels in the serum of treated mice were found at normal values when compared with infected control mice.

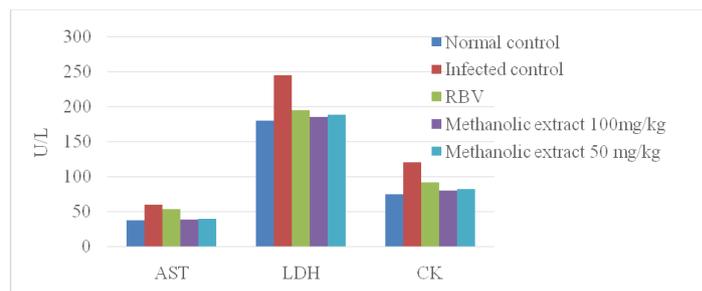


Figure 3 Effect of methanolic extract *C. haematocephala* leaves on the AST, LDH, and CK activities in the serum of infected mouse on the 7th day post-infection. *p=0.01, compared with the viral infected control.

Pathological findings

The hearts of normal control group showed that the cardiac muscle cells have arrangement structure with clear nucleolus in addition to there were no necrotic cardiomyocyte and mononuclear cell infiltration in the normal myocardium. Tissue damage of cardiac muscle cells and scores of necrosis and infiltration were observed in viral infected control and ribavirin control while these signs were absent in the groups treated with methanolic extract from *C. haematocephala* leaves at the two doses 100mg/kg and 50mg/kg body weight/day(Figure 4).

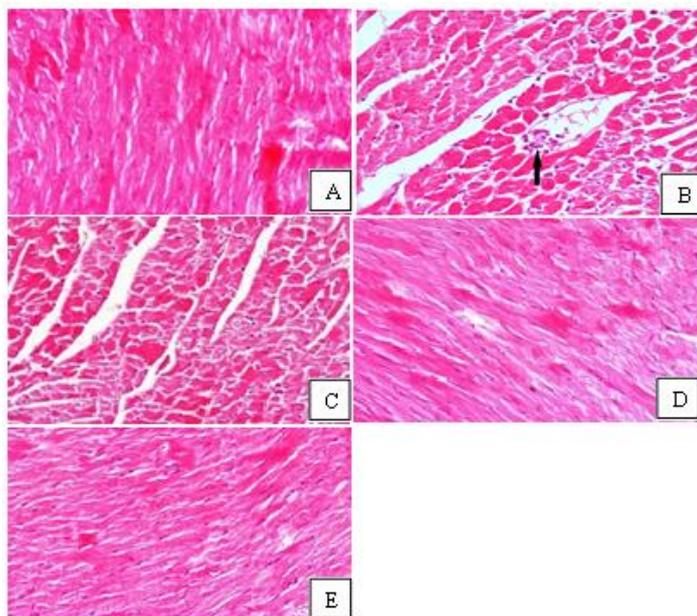


Figure 4 HE-stained sections of heart muscles from different groups. (A) normal control, (B) infected control showed mononuclear cell inflammation and myocardial necrosis, (C) RBV group, (D) *C. haematocephala* 100 mg/kg group, (E) *C. haematocephala* 50 mg/kg group.

As far as we know, this study represent the first report concerning evaluation of the antiviral activities of methanolic leaf extract of *C. haematocephala*. However, leaves of another species of genus *Calliandra*, *Calliandra polytirsia*, has been reported to have anti-hepatitis C virus activities (Wahyuni et al., 2013). In addition, several plants or their constituents have been reported to possess antiviral activities against CVB3 *in vivo*. Among them, astragaloside IV from the roots of *Radix Astragali* plant was found to possess potent antiviral activities against CVB3 in mice (Zhang et al., 2006). Calycosin-7-O-b-D-glucopyranoside from the dried root of *Astragalus membranaceus* plant exerted significant antiviral activities against CVB3 both *in vitro* and *in vivo* (Zhu et al., 2009). Wang et al. (2009b) demonstrated that the Phyllaemblicin B, isolated from the root of *Phyllanthus emblica* plant, exerts significant antiviral activities against CVB3. 20(S)-Protopanaxatriol, isolated from the root of *Panax notoginseng*, has been reported to have antiviral effects on CVB3 virus-induced myocarditis in mice (Wang et al., 2012). Wang et al. (2009a) found that Salidroside, extracted from *Rhodiola rosea* plant, possesses antiviral activities against CVB3 in mice and it may represent a potential therapeutic agent for viral myocarditis. Total flavonoid extracts, isolated from *Selaginella moellendorffii* plant, have been found to exhibit an effective antiviral activity against CVB3 infection *in vitro* and *in vivo* (Yin et al., 2014).

CONCLUSION

In the present study, we evaluated the safety as well as the antiviral activity of the methanolic extract of *Calliandra haematocephala* leaves in mice. The antiviral activity was evaluated by determination of the morbidity, mortality, HB/BW ratios, virus titers, pathological scores, and AST, CK, and LDH activities. Our results showed that the extract is safe for medication use at 100 mg/kg body weight where neither mortality, nor significant alterations in enzymes and morphological structures of liver and kidney organs. For antiviral activity, the infected mice treated with methanolic extract of *C. haematocephala* leaves at 100 mg/kg and 50 mg/kg showed significant decreasing of mortality, HB/BW ratios, virus titers, pathological scores, and AST, CK, and LDH activities than those in the viral infected control. These results indicate that the methanolic extract of *C. haematocephala* may represent as a potential antiviral agent for treatment of myocarditis causing coxsackievirus.

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