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# *In Vitro* and *In Vivo* Evaluation of *Bauhinia variegata* Extracts to Prevent Coxsackievirus B3 Infection

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## Abstract

Coxsackievirus B3 (CVB3) is one of the most viral agents that cause myocarditis in human, particularly in infants and young children. However, up to date, there are no vaccine or antiviral agents to prevent and/or treat the disease caused by this virus. The aim of this study was to determine the antiviral activities of *Bauhinia variegata* extracts against CVB3 infection *in vitro* and *in vivo*. Five extracts from *B. variegate* leaves were tested for their antiviral activity against CVB3 *in vitro* by applying three different strategies using MTT and $TCID_{50}$ assays. The antiviral activity *in vivo* was performed by monitoring of morbidity, mortality, the heart index, virus titers, and pathologic scores. In addition, measuring the activities of Aspartate Transaminase (AST), Creatine Kinase (CK), and Lactic Dehydrogenase (LDH) enzymes in infected mice with CVB3. Our results suggested that the methanol extract had the highest impact on viral infection *in vitro* as compared to others and it may work via blocking of the viral receptors. Moreover, this extract reduced the morbidity and mortality, the virus titers, and pathological area in the heart tissues of infected mice. Also, it maintained AST, CK and LDH enzymes at normal levels in the sera of the infected mice, when compared with infected control. In conclusion, the methanol extract of *B. variegata* leaves may play potential role in the treatment of CVB3 infection.

**Keywords:** Coxsackievirus B3; Antiviral; *In vitro*; *In vivo*; MTT; $TCID_{50}$

**Abbreviations:** CVB3: Coxsackievirus B3; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; TCID50: 50% Tissue Culture Infectious Doses; AST: Aspartate Transaminase; CK: Creatine Kinase; LDH: Lactic Dehydrogenase; RNA: Ribonucleic Acid; DMSO: Dimethyl Sulphoxide; EMEM: Eagle's Minimum Essential Medium; GMK: Green Monkey Kidney Cell Line; HEPES: 4-2-Hydroxyethyl-1-Piperazineethanesulfonic Acid; PI: Post Infection; RBV: Ribavirin; BW: Body Weight; HW: Heart Weight; SD: Standard Deviation

## Introduction

Coxsackievirus B3 (CVB3) is a member of genus Enterovirus within the *Picornaviridae* family. RNA of CVB3 can be found in the cardiac tissue of 40-50% of patient with dilated cardiomyopathy [1,2]. Some cases of dilated cardiomyopathy may be requiring to heart transportation or progress to death [3]. The World Health Organization reported that there are 21 viruses which can cause viral myocarditis in human; CVB3 is one of the major viral aetiolgical agents inducing myocarditis, particularly in infants and young children [4,5]. However, up to date, there are no vaccines or specific antiviral agents against CVB3 infection in clinical use. It is important to develop new antiviral agents to prevent and control CVB3 infection in human. The aim of the current study was to search for new anti-CVB3 agents from *Bauhinia variegata* plant.

*Bauhinia variegata*, *Caesalpiniaceae* family, has been reported to have several activities: antidiabetic [6], anti-rotavirus *in vitro* and *in vivo* [7,8], anti-inflammatory [9], antimicrobial [10], nephroprotective [11], and anticancer [12]. The phytochemical screenings of crude extract of *B. variegate* leaves revealed the presence of carbohydrate, glycosides, protein, saponins, triterpenoids, and steroids [13]. In the current study, we have evaluated the antiviral activity of five extracts from *B. variegata* leaves against CVB3 infection *in vitro*, and selected the most potent extract to evaluate against CVB3 infection in mice.

## Materials and Methods

### Plant collection and extract preparation

During May and June 2011, *Bauhinia variegata* leaves at early reproductive stage were collected from Botanical Garden of the National Research Centre (NRC), Giza, Egypt and were kindly identified by Dr. Mona Marzok, Researcher at National Research Center (NRC) and Mrs. Tersea Labib, taxonomist at Orman botanical garden, Giza. The plant leaves were air-dried under shade at room temperature. Crude extract was obtained from the plant powder by soaking in methanol and evaporated to dryness in a rotary evaporator at 40°C. One portion of the crude extract was used to prepare chloroform, ethyl acetate or n-butanol extracts. The residue remained in water was used as aqueous extract. All solvents were removed from the extracts by dryness in a rotary evaporator at 40°C. 100 mg of each lyophilized extract was dissolved in 0.5 ml Dimethyl Sulphoxide (DMSO) to prepare stock solutions at a concentration of 10 mg/ml. The stock solutions were sterilized by membrane filtration (Millipore 0.45 μm and 0.22 μm) and diluted to different concentrations (7.8, 15.6, 31.25, 62.5, 125, 250, 500, and 1000 μg/ml) in EMEM with 100 units/ml penicillin, 100 μg/ml streptomycin and 2% of inactivated fetal bovine serum. Various solutions were stored at +4°C until use.

### Cell line and virus

Green Monkey Kidney cell line (GMK) and Coxsackievirus B3 (Nancy) were used which were kindly provided by National Reference Center of the Enterovirus Laboratory, Faculty of Medicine, Slovak Medical University, under the government project SAIA. GMK cells

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were seeded in 96-well plates in Eagle's Minimum Essential Medium (MEM) containing 10% heat inactivated Fetal Bovine Serum (FBS), 100 units/ml penicillin, 100 µg/ml streptomycin and 1% HEPES (4-2-hydroxyethyl-1-piperazineethanesulfonic acid). The cells were incubated in 5% $CO_2$ incubator. CVB3 stock was prepared in GMK cells as described Bopegamage et al. [14]. The viral titers were determined in GMK cell monolayers as $TCID_{50}$/0.1 ml (50% tissue culture infectious doses/0.1 ml) using standard Spearman Kärber formula [15]. In brief, monolayer of GMK cells (24 h culture in Roux bottles) was inoculated at 0.1 Multiplicity of Infection (MOI) with virus (10 ml of $10^4$ $TCID_{50}$/ml, i.e., 103 U/ml to each 500 ml Roux bottle). Adsorption was done by incubation at 37°C for 30 min, medium MEM supplemented with 2% bovine serum and ATB (PNC 100 U/ml, STM 100 µg/ml) was then added. Cultures were incubated at 37°C and observed daily. When 100% CPE was observed, which was on the second day post-infection (p.i.), the cultures were harvested by freeze-thawing three times and centrifuged at 3000 rpm, 4°C for 10 min (Heraeus Minifuge T, Sepatech). Supernatants were divided into aliquots and stored at -80°C as virus stock. The virus was propagated twice, in the same way, to achieve a stable titer of $10^{12}$ U/ml.

Virus stocks were titrated on GMK cells in 96-well microtitration plates by making tenfold dilution (eight wells per dilution). Plates were incubated at 37°C in a $CO_2$ incubator and the results were read daily until day 7 of incubation under the light microscope. Titers were expressed as Tissue Culture Infectious Dose ($TCID_{50}$), and stored at -80°C until further use.

### *In vitro* experiments

**Cytotoxicity assay:** The non-toxic concentrations of the *B. variegata* extracts on GMK cells used in the antiviral experiments was assayed by colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method according to Nabil et al. [16]. Briefly, GMK cell lines were plated in 96-well µl plates at a concentration of $5 \times 10^4$ cells per well and incubated at 37°C under 5% $CO_2$ atmosphere. After 3 days of incubation, the growth medium was removed and the extract dilutions made in EMEM with 2% FBS plus antibiotics were added to cells. Each dilution was added in triplicate and three wells with only medium was included as cell control. After 48 h incubation at 37°C under 5% $CO_2$ atmosphere, the MTT assay was performed. The supernatant with/without extract was discarded and 100 µl of MTT solution (5 mg/ml) were added to wells and kept for 4 h at 37°C. Afterward, the MTT solution was removed and replaced with 50 µl DMSO. After 30 min incubation at 37°C, absorbance at a wavelength of 540 nm was measured using ELISA reader (MRX microplate reader, Dynex technologies, USA). The percentage of cytotoxicity is estimated as follows: $(A-B/A) \times 100$, where A and B refer to the mean of three optical densities of cell control and treated cells, respectively.

**Antiviral assay:** Antiviral activity of *B. variegata* extracts on CVB3 by MTT method (in three different strategies) was measured as described in our previous study Shaheen et al. [17]. To perform the first strategy (virucidal), 100 µl of three different non-toxic dilutions of each extract were incubated separately with 100 µl of CVB3 suspension ($10^6$ $TCID_{50}$/0.1 ml) for 1 h at 37°C in $CO_2$ incubator. 100 µl of the previous mix was added to GMK cell lines and after 1 h, the mix was removed and 200 µl of fresh medium were added to each well. While in the second strategy (treatment before infection), 100 µl of three different non-toxic dilutions of each extract were incubated with GMK cell lines for 24 h at 37°C in $CO_2$ incubator, then the extracts were discarded and replaced with 100 µl of CVB3 suspension ($10^6$ $TCID_{50}$/0.1 ml). After 1 h, the unabsorbed viruses were removed and 200 µl of fresh medium were added to each well. In the third strategy (treatment after infection), 100 µl of CVB3 suspension ($10^6$ $TCID_{50}$/0.1 ml) was incubated with GMK cell lines for 1 h at 37°C in $CO_2$ incubator. After that, the virus suspension was removed and the cell lines were incubated with three different non-toxic dilutions of each extract.

The virus (untreated infected cells) and cells (untreated uninfected cells) controls were included in all assays. All plates were incubated at 37°C in incubator with $CO_2$ for 72 h; the cytopathic effect was monitored daily under inverted microscope and measured by the MTT assay described above. The percentage protection was calculated as described by Shaheen et al. [17].

Antiviral activity of *B. variegata* extracts on CVB3 by measurement of cytopathic effect (in three different strategies) was carried out by the method described by us in our previous work by Shaheen et al. [17]. In brief, all extract at 300 µg/ml except butanol extract at 10 µg/ml were used for $TCID_{50}$ determination. We prepared 10-fold dilutions of CVB3 in EMEM medium and 100 µl of the viral dilutions ($10^{-4}$ -$10^{-9}$) was treated with 100 µl of each extract separately and in three different protocols as described above in the antiviral MTT assays. Positive control (virus dilutions without plant extracts) and negative control (cell lines with only medium) were included. Virus dilutions with or without extracts were added onto cell lines in four parallel wells. All plates were incubated for 3 days at 37°C in $CO_2$ atmosphere, and then the cytopathic effect was checked daily under light microscope. The titration of the virus was calculated and expressed as $TCID_{50}$ by using Spearman Kärber method [15]. The differences between the values of treated and untreated virus were used to determine the reduction in virus titers.

### *In vivo* experiments

**Cytotoxic effect of the methanol leaf extract in mice**: Forty BALB/c male mice (4 weeks old), were purchased and maintained at the animal house of National Research Center, Dokki, Giza, Egypt. The animals were divided into five groups (n=8/group). Four groups were treated by four different concentrations (400, 300, 200, 100 mg/kg/body weight) of methanol extract for 7 days by oral gavage. Negative control group (n=8) was included (fifth group). Mice were observed daily for any deaths until day 21 after treatment.

**Protective efficacy in mice:** Forty BALB/c male mice (4 weeks old) were used to determine whether the crude extract inhibit CVB3 myocarditis in mice. The mice were divided randomly into 5 groups (8 mice/group). Four groups were injected intraperitoneally with CVB3 at concentration of $10^6$ $\log_{10}$ $TCID_{50}$. The remaining 8 mice were used as negative control and injected intraperitoneally with the same volume of 0.9% NaCl solution. Day 1 Post Infection (PI), the infected mice were divided and treated daily for 7 days as follows: Group A (n=8) were treated with methanolic leaf extract at 100 mg/kg body weight; group B (n=8) were treated with methanolic leaf extract at 50 mg/kg body weight; group C (n=8) was treated with 0.9% saline solution and used as infected control; group D (n=8) was injected intraperitoneally with ribavirin (RBV) at 10 mg/kg body weight and used as a positive control. Morbidity (diminished vitality, trembling, loss of appetite, and ruffled fur) the mortality was checked daily during the 7-days experiment.

Four mice from each group were weighted and sacrificed at 7 day post infection (p.i.). Blood samples were collected from the orbital region and serum was separated by centrifugation at 12.000 rpm for 10 min to determine the activities of Lactic Dehydrogenase (LDH), Creatine Kinase (CK), and Aspartate Transaminase (AST) using commercially available kits (Biosystem, Spain; Spinreact, Spain; and

Randox, UK). The hearts of each group were harvested and weighted to determine the heart index (the ratios of Body Weight (BW)/Heart Weight (HW)). Afterward, the hearts of each group were divided into two parts; one part was homogenized in 1.5 ml of EMEM. Virus was collected by freezing and thawing the homogenates and centrifugation at 1200 × g for 15 min. Virus titers was performed on GMK cell monolayers using plaque assay technique according to Bishop and Koch. The second part of the hearts were fixed in 10% formalin solution, sectioned (4 µm thick), and stained with hematoxylin-eosin. Sections of hearts were investigated under a light microscope for signs of myocarditis as described previously [18]. The remaining four mice in each group were observed to determine the mortality in each group. Animal experiments were conducted according to the guidelines of the Institutional Animal Ethics Committee.

**Statistical analysis:** Data were represented as mean ± Standard Deviation (S.D.). Comparison between difference groups were performed using One-way Analysis of Variance (ANOVA) test as comparison between more than two parametric groups with Dunnett and Duncan as multiple comparison. A probability value (p value) less than or equal to 0.05; was considered significant. All statistical calculations were done using computer program SPSS (Statistical Package for Social Science) statistical program version (16.0). Graphs were done using SPSS statistical program version (16.0) and Microsoft Excel program version 2010.

## Results

### Cytotoxicity of *Bauhinia variegata* extracts *in vitro*

The cytotoxicity of *Bauhinia variegata* extracts on GMK cells was investigated by calculation of $CC_{50}$ which is 711 and 901.3 µg/ml for methanol and aqueous extracts, respectively. The cytotoxicity of ethyl acetate and butanol was increased with $CC_{50}$ of 474.8 and 466 µg/ml, respectively. Whereas the chloroform extract showed the less cytotoxicity effect on GMK with $CC_{50}$ more than 1000 µg/ml (Table 1).

### Antiviral of *Bauhinia variegata* extracts *in vitro*

**Virucidal activity:** When virus treated with extract for 1 h prior to viral infection, the methanol, ethyl acetate, butanol, and aqueous extracts showed slight effect on virus replication with TI of 0.4, 2.3, 1.9, and 0.7 for respectively (Table 1). Whereas chloroform extract showed significant inhibitory effect against virus infection with TI of 8.6 resulting in reduction of virus titers (1.75 log $TCID_{50}$).

**Extract treatment before infection:** When the extracts pre-incubated with cells for 24 h prior to infection we observed slight protective effect at chloroform, ethyl acetate, butanol, and aqueous extracts with TI of 1.5, 0.3, 0.4, and 0.6, respectively. The strong antiviral activity was shown for the methanolic extract against CVB3 infection with TI of 22.2 and 4.75 log $TCID_{50}$ reduction of virus titer (Table 1).

**Extract treatment after infection:** Subconfluent cells were infected with virus for 1 h before cell treatment with extracts. The results showed that the methanol and butanol extracts have weak effect against virus infection with TI of 0.5 and 0.6 respectively. The therapeutic index of chloroform and aqueous extracts was increased to 3.8 and 3 respectively. The higher antiviral activity was shown at ethyl acetate against virus infection with TI of 13.5 and 3 log $TCID_{50}$ reductions of virus titers (Table 1).

### Antiviral of *Bauhinia variegata* extracts *in vitro*

**Toxicities *in vivo*:** Oral gavage treatment with the methanol extract at 200, 300, 400 mg/kg body weight/day for 7 days showed mice mortality ranged from 25-37.5% whereas no mortality was observed in the group treated with the extract at 100 mg//kg body weight/day (Table 2).

### Antiviral effects of methanolic leaf extract against Coxsackie virus B3 infection in mice

**Mortality, Morbidity, and HW/BW ratios *in vivo* experiments:** Clinical signs such as diminished vitality, weight loss, ruffled fur, loss of appetite were observed in treated infected mice, in the infected controls without treatment and in the sham infected controls. All mice of the infected control became morbid on day 3 p.i. whereas only 87.5%, 50%, and 37.5% were observed in the groups treated with ribavirin, methanol extract at 50 mg/kg, and methanol extract at 100 mg/kg, respectively.

| Extract | $CC_{50}$ (µg/ml)[a] | Virucidal | | | Treatment before infection | | | Treatment after infection | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $IC_{50}$ (µg/ml)[b] | TI[c] | R | $IC_{50}$ (µg/ml)[b] | TI[c] | R | $IC_{50}$ (µg/ml)[b] | TI[c] | R |
| Methanol | 711.19 | 1866 | 0.4 | 0 | 32.0 | 22.2 | $10^{4.75}$ | 1285 | 0.5 | 0 |
| Chloroform | >1000 | 433 | 8.6 | $10^{1.75}$ | 2500 | 1.5 | $10^{0.25}$ | 973 | 3.8 | $10^{0.75}$ |
| Ethyl acetate | 474.8 | 203 | 2.3 | $10^{0.25}$ | 1631 | 0.3 | 0 | 35 | 13.5 | $10^{3}$ |
| Butanol | 466 | 248 | 1.9 | $10^{0.25}$ | 1234 | 0.4 | 0 | 721 | 0.6 | 0 |
| Aqueous | 901.3 | 1269 | 0.7 | 0 | 1577 | 0.6 | 0 | 298 | 3 | $10^{0.25}$ |

**Abbreviations:** $CC_{50}$: The concentration of extract required killing 50% of viable cells; R: Reduction of virus titer was calculated as the difference between treated and untreated virus.
[a]Concentration of extract that is cytotoxic to 50% of cells.
[b]Concentration of extract that inhibits viral infectivity (Cytopathic Effect) by 50%.
[c]Therapeutic index=$CC_{50}/IC_{50}$=The mean values of triplicate experiments.

**Table 1:** The cytotoxicity and anti-coxsackievirus B3 of *Bauhinia variegata* extracts with the mode of action on GMK cells determined by MTT method.

| Group of mice | Concentrations/kg body weight/day | Number of dead animals | Survival rate | Mortality rate |
|---|---|---|---|---|
| Control | 0.00 | 0.00 | 100% | 0% |
| Methanolic extract of *Bauhinia variegata* | 100 mg | 0 | 100% | 0% |
| | 200 mg | 2 | 75% | 25% |
| | 300 mg | 2 | 75% | 25% |
| | 400 mg | 3 | 62.5% | 37.5% |

**Table 2**: Cytotoxicity results of methanolic extract of *Bauhinia variegata in vivo*.

| Group | Morbidity (%) | Mortality (%) | HW/BW Ratios (Mean ± SD) | Virus Titration ($log_{10}$PFU/ml, means ± SD) | Pathologic Scores (Mean ± SD) |
|---|---|---|---|---|---|
| Normal control group | 0 | 0 | 4.21 ± 0.02 | 0 | 0 |
| Infected group | 100 | 100% | 6.12 ± 0.03 | 6.42 ± 0.01 | 3.25 ± 0.10 |
| Ribavirin (1 mg/mL) | 87.5 | 25% | 5.81 ± 0.02** | 3.45 ± 0.03** | 2.75 ± 0.35** |
| *Bauhinia variegata* methanolic extract 50 mg/kg | 50 | 0 | 4.31 ± 0.02** | 2.36 ± 0.02** | 1.0 ± 0.01** |
| *Bauhinia variegata* methanolic extract 100 mg/kg | 37.5 | 0 | 4.30 ± 0.03** | 2.27 ± 0.01** | 0.75 ± 0.05** |
| *P* value[a] | | | 0.001 | 0.001 | 0.001 |

**Abbreviations:** HW/BW ratios: Heart Weight/Body Weight Ratios; PFU: Plaque Forming Unit; SD: Standard Deviation.
[a]*P* value of the comparison between the all different groups without the normal control group.
**$P \leq 0.01$ value of the comparison between each group with the infected group.

**Table 3:** Effect of methanolic extract of *Bauhinia vareigata* on morbidity, mortality, the heart index, virus titers, and pathologic scores after 7 days from inoculation of BALB/ mice with CVB3.

| Group of mice | AST | LDH | CK |
|---|---|---|---|
| Normal control group | 36.8 ± 1.14 | 160.6 ± 2.52 | 113.6 ± 1.15 |
| Infected group | 62.2 ± 1.24 | 210.3 ± 3.31 | 162.4 ± 3.24 |
| Ribavirin (1 mg/mL) | 52.5 ± 3.75** | 187.5 ± 3.22** | 145.6 ± 2.22** |
| *Bauhinia variegata* methanolic extract 100 mg/kg | 38.5 ± 1.12** | 163.4 ± 1.87** | 117.4 ± 2.13** |
| *Bauhinia variegata* methanolic extract 50 mg/kg | 39.1 ± 2.27** | 167.6 ± 1.01** | 120.5 ± 2.03** |
| [a]*P* value | 0.001 | 0.001 | 0.001 |

**Abbreviations:** AST: Aspartate Aminotransferase; LDH: Lactate Dehydrogenase; CK: Creatine Kinase; Values expressed in U/L are mean ± SD for n=4 mice per group.
[a]*P* value of the comparison between the all different groups without the normal control group.
**$P \leq 0.01$ value of the comparison between each group with the infected group.

**Table 4:** Effect of methanolic extract of *Bauhinia variegata* at two doses (100 and 50 mg/kg body weight) on AST, LDH, and CK in coxsackievirus B3-induced myocarditis in mice.

On day 9 p.i., the mice of infected group began to die and 100% of deaths were found on day 13 p.i. The ribavirin reduced the mortality to 25% whereas the animals treated with the methanolic extract at 50 and 100 mg/kg body weight/day as well as the normal control animals did not show any deaths. On the other hand, the methanolic extract at both dosages was observed to significantly reduce the HW/BW ratios compared with those in ribavirin and infected control groups (Table 3).

**Effects of methanol extract on LDH, AST, and CK in infected mice:** The activities of AST, CK and LDH enzymes as myocardial injury markers, were measured using commercial kits. Our data suggest that the level of these enzymes was significantly lower in the serum of mice treated with the crude extract at concentrations of 50 and 100 mg/kg body weight, compared with the infected mice without treatment (Table 4).

**Effect of methanol extract on myocarditis in infected mice:** We investigated the *in vivo* effect of methanol extract of *B. variegata* in CVB3 infected BALB/c male mice. H&E staining showed that methanol extract treatment had decreased the scores of the cardiac pathology (Table 3). Moreover, the myocardium damage, including infiltration and necrosis, was significantly lower than the untreated infected control group at day 7 in the heart sections of CVB3-infected mice (Figure 1).

## Discussion

In the present study, the antiviral effects of *Bauhinia variegata* against CVB3 have been demonstrated *in vitro* then based on the obtained results, the most effective extract was selected to be tested *in vivo*. *In vitro*, the antiviral activity was performed in three different ways in order to examine whether the extracts effect on virus entry and/or viral life cycle after entry into host cell. Our results findings suggested that the all extracts showed some inhibitory effect against virus infection where the methanolic extract showed the significant reduction of CPE compared with untreated infected cells, especially if added to cells 1 h prior infection. We suggest that this extract prevented the CPE of CVB3 infection by blocking/changing the viral receptor located on the surface of host cells and thereby prevented the virus entry into host cells. These results agree with our previous study that the methanolic extract of *B. varirgata* was the most effective than chloroform, ethyl acetate, butanol, and aqueous extracts of the same plant against rotavirus *in vitro* [7].

As we know, this data represent the first evidence for the antiviral activity of *B. variegate* against CVB3 *in vitro* and *in vivo* system. Saha et al. [19] reported that the methanol leaf extract contains carbohydrate, glycosides, protein, saponins, triterpenoids, and steroids. In our previous study, the methanolic leaf extract of *B. variegata* contained 28.67 mg of phenol and 4.19 mg of flavonoid/100 mg of plant leaves. Interestingly, several reports demonstrated that the phenol and flavonoid compounds have antiviral activity against CVB3 *in vitro* and *in vivo* [16,20,21]. Thus the anti-antiviral effect this extract may be due to presence of one or more of these active constituents and further experimentation is needed to test those constituents individually against CVB3.

Based on this results methanolic extract was selected as promising extract against CVB3 *in vivo*. *In vivo*, our results demonstrated that 100 mg/kg and 50 mg/kg body weight are two safe doses for antiviral evaluation. Our data showed that the virus titers were decreased significantly in the hearts of mice treated with the methanol extract at the both dosages, compared with infected control. Reduction in

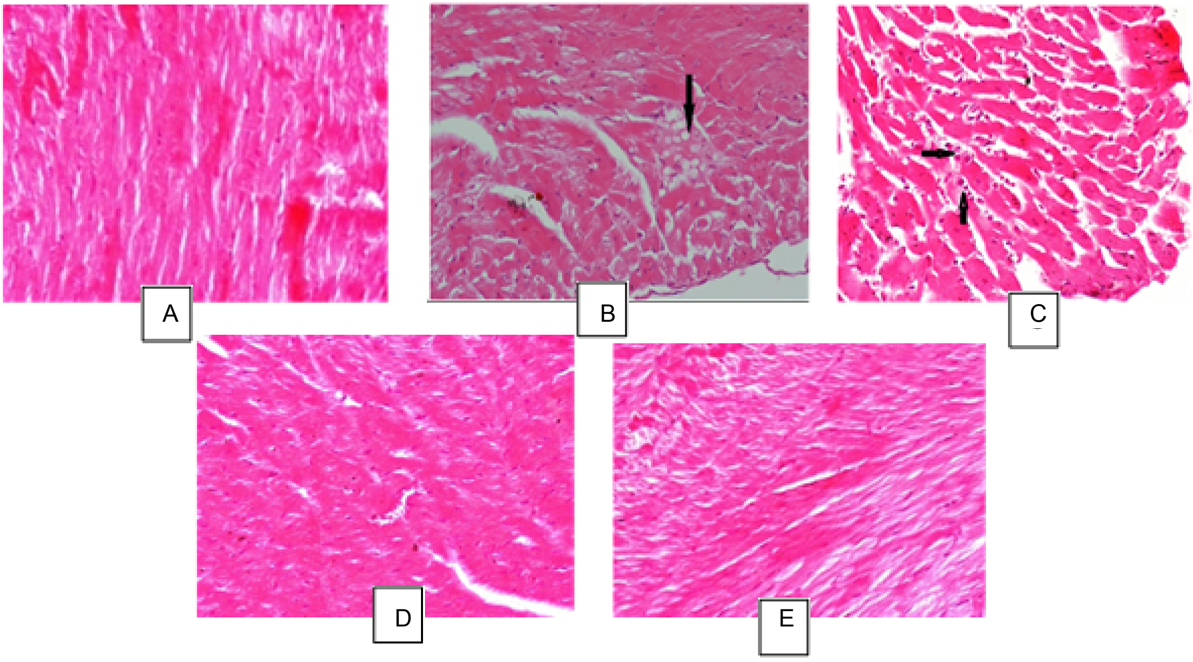


**Figure 1:** HE-stained sections of heart tissues from different groups of mice. (A) Negative control, (B) Infected control, (C) RBV group, (D) Methanol extract of Bauhinia variegata at 100 mg/kg group, (E) Methanol extract of B. variegata at 50 mg/kg group.

virus titers may lead to improvement in the morbidity and mortality. Furthermore, the oral administration of methanol extract after infection of mice with CVB3 protected the infected mice from severe acute heart infection and thereby it prevented the elevation of CK, ALT, and LDH. This finding agree with our previous study methanolic extract of *B. variegate* protected the mice from the harmful effect of rotavirus reducing the morbidity, mortality, severity diarrhea with duration of recovery as well as intestinal lesion scores when compared with those in infected untreated group [8].

We have not studied the mechanism of antiviral action *in vivo* and how reduction of virus replication is affected by the extract in heart tissues of the infected mice but there are several hypothesis. Among them, methanolic extract inhibited the virus replication by blocking him Coxsackievirus and Adenovirus Receptor (CAR) which represent the first primary step to virus entry into host cells [22,23]. Several drugs such as WIN compounds have been reported to inhibit the interaction between CVB and CAR [24]. We expect also that our extract inhibited the virus replication by interfering with cellular proteins which interfere with viral replication. Gao et al. [25] demonstrated that proteasome inhibitor MLN353 interfered with cellular proteins in CVB3 infected mice reducing mortality, myocardial injury, and viral replication. The extract might also inhibit the viral replication by interfering with the viral proteins after entry into host cells. Several compounds such as TBZE-029, guanidine hypochloride, and HBB have been reported to inhibit the synthesis of viral RNA by interacting with the viral protein 2°C, resulting in prevention of virus-induced cell lysis [26,27]. So, further studies are needed to explore the antiviral mechanisms of the crude extract *in vivo*.

## Conclusion

We demonstrate that all extracts of *Bauhinia variegata* have some inhibitory effect on CVB3 infection *in vitro*. Methanol extract showed the highest anti-CVB3 activity among the studied extracts. Moreover, oral administration of methanolic extract can reduce morbidity, mortality, virus titers, and the severity of CVB3-induced myocarditis *in vivo*. Thus, this extract may play an important role in the treatment of myocarditis induced by coxsackievirus B3.

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### Conflict of Interests

The author declares no conflict of interest.

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