Volume 1, Issue 2, pp. 21-30; December 2012
Online International Journal of Medicinal Plants Research

Full Length Research

Available Online at http://www.onlineresearchjournals.org/OIJMPR

Thrombocytosis and Anti-inflammatory Properties, and Toxicological Evaluation of *Carica papaya* Mature Leaf Concentrate in a Murine Model

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Downloaded 11 October, 2012 Accepted 14 November, 2012

Management of thrombocytopenia is by drugs and blood products, both of which are costly. Conversely, Sri Lankan traditional medicine use mature leaf concentrate of *Carica papaya* to treat this condition. This claim was scientifically validated. Adult Wistar rats (N=6/group) with Hydroxyurea-induced thryombocytopenia (model established for the first time), were orally administered, once daily on 3 consecutive days with three doses of fresh mature leaf concentrate of *C papaya* (0.18, 0.36 and 0.72 ml/100g), while controls received water. Standard protocols were used to establish their platelet, WBC and RBC counts. Effects of mature leaf concentrate of *C papaya* on carrageenan induced oedema in rats, on rat erythrocyte membrane stabilization, and on acetic acid-induced vascular permeability in mice, as well as acute toxicity studies were conducted using standard methodology. High dose of mature leaf concentrate of *C papaya* in thrombocytopenic rats significantly (P<0.05) increased platelets by 76.5%, WBC by 30.51% and RBCs by 9.08%, when compared with controls. High dose of mature leaf concentrate of *C papaya* also significantly (P<0.5) inhibited careegenan induced rat paw oedema and impaired *in vivo* vascular permeability in mice (by 82%), while inducing maximum (10.11%) membrane stabilizing activity of rat RBCs at 8mg/ml of mature leaf concentrate of *C papaya*, suggestive of effective anti-inflammatory activity. Administration of high dose of mature leaf concentrate of *C papaya* on 3 consecutive days neither provoked overt signs of toxicity nor stress, where hepatotoxicity, renotoxicity, hematotoxicity and neurotoxicity were also ruled out. Thus freshly prepared mature leaf concentrate of *C papaya* is orally active, effectively increases rat platelet, WBC and RBC counts with no acute toxicity, and possesses potent anti-inflammatory activity, that overly justify claims of traditional medicine.

Key words: Acute toxicity, anti-inflammatory activity, *Carica papaya* mature leaf concentrate, platelet counts, thrombocytopenia, thrombocytopenic rat model.

INTRODUCTION

Thrombocytopenia is a condition associated with the lower production of platelets than the normal numbers in the bone marrow and is often multifactorial. Disorders, such as haematological malignancies, aplastic anaemia, an immune system malfunction, loss by splenic destruction are well known mechanisms of thrombocytopenia [1]. The physiological range for thrombocytes in the normal healthy human is 150-400

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x 10^9 per liter of blood [2]. Thrombocytopenia causes few signs or symptoms with platelet counts in the range of 100 - 150 x 10^9/l that represent mild thrombocytopenia. Platelet counts of 50 - 100 x 10^9/l and levels less than $50x10^9$/l are categorized as moderate and severe thrombocytopenia, respectively [2]. In rare cases, the number of platelets may be so low that dangerous internal bleeding can occur. The most common reasons for thrombocytopenia are; defective production of platelets by the bone marrow, diminished platelet survival and sequestration of the platelets by the spleen. A combination of above facts also can lead to thrombocytopenia [2].

Diseases such as dengue, idiopathic thrombocytopenic purpura (ITP), malignancy, hypersplenism, aplastic anemia, leptospirosis, chickungunya, drug induced thrombocytopenic purpura (for example heparine induced thrombocytopenia), hemolytic uremic syndrome, Gaucher's disease etc result in a low thrombocyte count in blood [3]. Of the available methods for treating thrombocytopenia, the treatment method mainly depends on the disease severity. Blood transfusions, steroid treatment, splenectomy, immunosuppressive therapy, intravenous gamma-globulin therapy can be used to correct thrombocytopenia [4-6].

Nevertheless, due to certain side effects and the costs involved, the availability of treatment for thrombocytopenia is limited. Non-scientific data on the platelet increasing effect of *Carica papaya* leaf extract in dengue patients with thrombocytopenia are sited in copious internet sites, such as http://shine.yahoo.com/channel/health/papaya-leaf-jiuce-remedy-for-dengue- fever-252188/) [7].

However, published scientific reports are very limited on the effect of *Carica papaya* leaf extract on platelet number [3,8]. *Carica papaya*, belongs to the family of Caricaceae, and several species of Caricaceae have been used as remedy against a variety of diseases [9,10]. It is a dicotyledonaeplant, with a single stem growing from 5 to 10 meters (16 to 33 ft) height, with spirally arranged leaves confined to the top of the trunk which is common in most of the tropical regions of the world. The lower trunk is conspicuously scarred where leaves and fruit were borne [11]. Some of the phytochemical constituents found in *C. papaya* based on previous studies are alkaloids, methyl carpaine, dehydrocarpaines, pseudocarpaine, flavonoids, benzylglucosinolate, tannins and saponins [12,13].

Moderate and severe thrombocytopenia are associated with morbidity and mortality, the management of which is by drugs and blood products, both of which are costly as mentioned above. Conversely, some Sri Lankan traditional medical practitioners (though not documented) use mature leaf concentrate of *Carica papaya* to treat this condition. The platelet increasing effect of mature leaf concentrate of *Carica papaya* (MLCC), including the evaluation of its therapeutic use and toxic effects were yet to be investigated, and thus this study was undertaken to scientifically validate these claims.

MATERIALS AND METHODS

Collection of Plant Material

Mature leaves of *Carica papaya* (Red Lady variety) were collected from a single geographical location, in Ganemulla in the Gampaha district in Sri Lanka (longitude - 79° 57′ 0″ E, latitude - 7° 4′ 0″ N), from August to October 2010. The specimen was identified and authenticated by Dr H Kathriarachchi of the Department of Plant Sciences of the University of Colombo, Sri Lanka.

Preparation of Leaf Concentrate

Mature leaves of *C. papaya* were thoroughly washed, blotted dry and after removal of petiole and veins, leaf blades were crushed using a mortar and pestle for 10-15 minutes, without addition of water. The suspension was manually separated from the crushed leaf debris to avoid removal of the mucous. The final concentration of this preparation was 4 g leaf blade/ ml of concentrate.

An adult human individual under thrombocytopenic conditions is usually given 20 ml of MLCC once daily for three consecutive days (Available at www.scribd.com/doc/9882730/Papay-Leaf-Cure) [14]. Taking in to consideration the metabolic rate of rats, the human equivalent dose (HE) was approximately equal to the usually used daily dose (20 ml) by adult patients with thrombocytopenia [15]. The low dose (LD) was calculated by halving the human equivalent dose (HED) while the high dose (HD) was two folds of that of the human equivalent dose.

Experimental Animals

Healthy, adult male and female Wistar rats purchased from the Medical Research Institute, Colombo, Sri Lanka were used in this study. All animals received humane care. They were housed in plastic cages in the animal house of the Department of Zoology, University of Colombo under standard animal house conditions (temperature; 28 – 31°C, photoperiod; approximately 12 hours natural light per day, relative humidity : 50 – 55%). The animals were fed with pelleted food (VET HOUSE Ltd. Colombo, Sri Lanka) and clear drinking water *ad libitum.* All experiments were conducted with the ethical approval of the

Research, Ethics and Higher Degrees Committee, of the Institute of Biochemistry, Molecular Biology and Biotechnology, of the University of Colombo.

Establishment of Hydroxyurea Induced Thrombocytopenia in the Rat Model

Induction of thrombocytopenia was performed by the oral administration of freshly prepared Hydroxyurea (CYTODRX, CIPLA Ltd., India) dissolved in distilled water. Each rat received $1/10^{th}$ of the standard dose of 15mg/kg Hydroxyurea and thrombocytopenia was observed after 24hours [16].

Clinical Assessment and Evaluation of Thrombocytopenia

Clinical Investigation

Induction of thrombocytopenia was established by determining platelet counts with the use of Neubauer's improved Haemocytometer (HAWKSLEY Ltd; London, England) according to standard protocols [17]. Briefly, blood obtained from the tail bleed of rats was added to EDTA containing tubes. Twenty microliters of blood was mixed thoroughly with 0.38 ml of diluting fluid (1% ammonium oxalate freshly prepared) for at least 10 minutes. The haemocytometer filled with this mixture was incubated in a moist chamber for 10-20 minutes to allow the platelets to settle down. Round to oval shaped platelets were counted in the triple laminated middle 25 squares of the haemocytometer, using oil immersion light microscopy (NIKON, Tokyo, Japan) [17].

Platelet Increasing Effect of MLCC

The platelet increasing effect of MLCC was measured using 3 oral doses of extract (LD =0.18, HED=0.36 and HD=0.72 ml/100g) on 3 consecutive days under artificially induced thrombocytopenia in rats (N=6/ group). The effect of MLCC on normal (non thrombocytopenic) rats was tested by treating the test group (N=6) with LD of MLCC for 3 consecutive days (once daily) while the two control groups (platelet depleted and non-depleted) received distilled water (DW). Platelet counts of test and control groups were made daily, using tail bleed [17].

Effect of MLCC on White Blood Cell (WBC) and Red Blood Cell (RBC) counts of Thrombocytopenia Induced and of Normal Rats

Twenty four hours following MLCC treatment, 0.5 – 1ml of tail bleed was dispensed into EDTA containing tubes from rats of all 6 groups (normal control, normal test, thrombocytopenic control, thrombocytopenic low dose group, thrombocytopenic human equivalent dose group and thrombocytopenic high dose group). WBC and RBC counts of the blood samples were made according to standard protocols, using Neubauer's improved Haemocytometer [18]. Where appropriate EC50 values were calculated for increase of platelet, WBC and RBC counts [19].

Effect of MLCC on Carrageenan Induced Oedema

Carrageenan – induced rat paw oedema was utilized as the model for acute inflammation as previously described [20]. Rats (N=6/group) were treated with HED (0.36ml/100g) and HD (0.72ml/100g) of MLCC as treatment, distilled water as the negative control and 5mg/kg Indomethacin as the positive control. One hour later, each rat was injected with 0.05ml of 1% carrageenan into the sub-plantar surface of the right hind paw. The paw volumes were measured before and after 3 consecutive hours of treatment, using a digital plethysmometer (PAN LAB Ltd, Barcelona, Spain).

Effect of MLCC on Erythrocyte Membrane Stabilization

The membrane stabilizing effect of mature MLCC on rat RBCs exposed to heat was investigated by standard methodology [21]. A dilution series (10, 15, 20 and 25 µl/10ml) was prepared using the initial concentration of 4g/ml of MLCC from mature leaves and Aspirin of same concentrations as positive control were used in the assay of rat RBC membrane stabilizing activity monitored at 540nm.

Effect of MLCC on Acetic Acid- Induced Vascular Permeability in Mice

The *in vivo* vascular permeability test was carried out using standard methodology [22]. Briefly, two groups of mice (N=6/group) were orally treated with high dose of MLCC (0.72ml/100g) and distilled water, respectively. Each animal was injected intravenously (to the tail vain) with 1% solution of Evans blue dye (0.1ml/10g) 1h after the oral administration of MLCC / distilled water. Thirty minutes later, the mice were intraperitoneally injected with 0.1 ml/10g of body weight of freshly prepared 0.7% acetic acid solution. After 30 minutes, mice were sacrificed and 10 ml of saline was injected to the peritoneal cavity. The collected peritoneal fluids were then centrifuged at 4000rpm for 10min. The absorbance of the supernatant was measured at 540nm using a spectrophotometer.

Effect of Leaf Concentrate of C. papaya made up of Mature leaves vs. Immature leaves on Percentage Increase of Rat Platelets

Mature and immature leaves of *C. papaya* were collected

from a single plant in the same geographical location as mentioned above, and leaf concentrates was prepared accordingly. Since the high dose (HD – 0.72ml/100g) of mature leaves appeared to be the most effective dose to increase platelets, the same dose of the immature leaf concentrate was administered on 3 consecutive days to thrombocytopenia induced rats (N=6).

Evaluation of Acute Toxicity

Two groups of artificially induced thrombocytopenic rats were treated either with 0.72ml/100g of MLCC or DW, once daily for 3 consecutive days to evaluate the toxicity of Hydroxyurea. Whereas, two groups of normal rats (platelet non-depleted), one treated with LD of MLCC (test group) and DW (control group), once daily for three consecutive days were used to evaluate toxicity of MLCC.

Following treatment they were continuously observed for overt signs of toxicity (salivation, diarrhoea, tremors, ataxia, yellowing of hair, loss of hair, lethargy, sleepiness, postural abnormalities and behavioral changes), stress (fur erection and exophthalmia), aversive behavior (biting paws, intense grooming behavior, scratching behavior and licking of tail) and mortality. The consistency of faeces and the color of urine were recorded daily for each group.

On day 3 post treatment, 1.5 ml of a tail bleed from each rat was collected under mild ether anesthesia using aseptic precautions. The platelet [17], white blood cell (WBC) and WBC differential (DC) counts [18] and Packed Cell Volume (PCV) [23] of fresh blood was determined using standard techniques. Serum parameters (SGOT, SGPT, urea, creatinine levels) were determined using Randox kits (RANDOX LABORATORIES Ltd., Co. Antrium, U.K) and the spectrophotometer (JASCO V560, Jasco Corporation, Tokyo, Japan) as per manufacturer's instructions.

The bar and bridge tests [24] were carried out to find out the neurotoxic effects of MLCC on test rats. The reaction times were recorded using a stop watch. Rats were weighed on day 0 and on day 3 post treatment using an animal balance (MP 6000, CHYO BALANCE CORPORATION, Tokyo, Japan). They were sacrified, their liver, spleen, kidneys, heart and adrenal glands immediately excised, blotted free of blood and weights determined using an electronic balance (SHIMADZU LIBRORER SERIES, SHIMADZU CORPORATION, Tokyo, Japan). Subsequently, the dry organ weights were calculated.

Statistical Analyses

Data is presented as mean $\pm$ SD, and was analysed using One way ANOVA followed by Tukey's pair wise comparison, and Mann-Whitney U-test. The significance level was set at $P \leq 0.05$.

Phytochemical Screening

Phytochemical screening of MLCC was carried out for alkaloids, flavonoids, saponins, tannins and steroids [25].

RESULTS

Effect of Oral Administration of MLCC on Percentage Increase of Rat Platelets

The low and human equivalent doses of orally administered MLCC significantly ($P \leq 0.05$) increased the platelets by 14.2% and 14.3% while an increase of 76.5% was detected with the high dose, compared with the control (Figure 1). The effect on platelet counts of the oral administration of different doses of MLCC on normal, non-thrombocytopenic and thrombocytopenia induced rats are shown in Table 1. EC_{50} value of MLCC in percentage platelet increase in thrombocytopenic rats was calculated to be 0.47ml/100g. The treatment of normal, non-thrombocytopenic rats with low dose (0.18 ml/100g) of MLCC showed a significant ($P \leq 0.05$) percentage increase of platelets by 19.17% when compared with the control that received distilled water (1.3%).

Effect of Oral Administration of MLCC on Percentage Increase of WBC

The low and human equivalent doses of MLCC showed a percentage increase of WBC by 15.09% and 15.14%, respectively, in thrombocytopenia induced rats which did not significantly differ from the control group of rats that received distilled water (Figure 1). Conversely, when compared with the control, a significant increase of WBC (One way ANOVA followed by Tukey's pair wise comparison; [$P \leq 0.05$]) by 30.51% was observed with the MLCC high dose only (Figure 1). The EC_{50} value of MLCC was 0.361 ml/100g in WBC percentage increase. The treatment of normal, non-thrombocytopenic rats with low dose (0.18 ml/100g) of MLCC showed a significant ($P \leq 0.05$) percentage increase of WBC by 19.35% when compared with the control that received distilled water (1.3%).

Effect of Oral Administration of MLCC on Percentage Increase of RBC

When compared with the control, a significant increase of RBC (One way ANOVA followed by Tukey's pair wise comparison; $P \leq 0.05$) by 17.0% and 9.08% was observed with the MLCC low and high doses, respectively (Figure 1). In contrast, the human equivalent dose with a percentage increase of 7.55% did not significantly differ from the control

Table 1. Effect of oral administration of Mature Leaf Concentrate of *Carica papaya* (MLCC) on percentage increase of rat platelets.

Study group	% Increase of platelets
Normal, non-thrombocytopenic groups	
Normal control group (distilled water)	1.3087 ± 0.2188
Normal test group (low dose of MLCC)	*19.173 ± 3.975
Thrombocytopenia induced groups	
Thrombocytopenia induced control group (distilled water)	0.000 ± 0.000
Thrombocytopenia induced MLCC low dose (LD) group	*14.2733 ± 1.2922
Thrombocytopenia induced MLCC human equivalent (HE) group	*14.2767 ± 3.5918
Thrombocytopenia induced MLCC high dose (HD) group	*[a,b]76.495 ± 4.0968

Values are expressed as means ± SEM (N = 6); *P ≤ 0.05 as compared with the control (One way ANOVA followed by Tukey's pair wise comparison); [a]P ≤ 0.05 as compared with LD (One way ANOVA followed by Tukey's pair wise comparison); [b]P ≤ 0.05 as compared with HE (One way ANOVA followed by Tukey's pair wise comparison).
MLCC – *Carica papaya* Leaf Concentrate, LD – Low Dose, HE – Human Equivalent dose, HD – High Dose

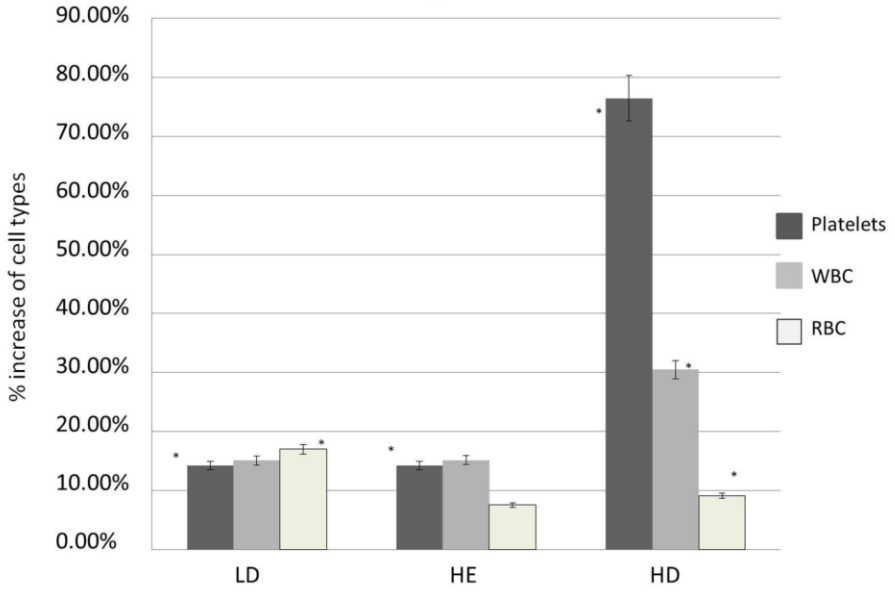

Figure 1. Effect of mature leaf concentrate of *Carica papaya* (MLCC) on thrombocytopenia induced rat platelet, WBC and RBC percentage increase (mean ± SEM, N=6/group). As compared with the control (distilled water), the low dose (LD), human equivalent dose (HE), and the high dose (HD) of MLCC significantly increased rat platelet counts, while the HD also increased both WBC and RBC counts, significantly (One way ANOVA followed by Tukey's pair wise comparison; *P ≤ 0.05).

(P>0.05) and the EC_{50} value of the MLCC was 0.386 ml/100g. A significant (P ≤ 0.05) percentage increase of RBC by 13.83% was noted with the treatment of LD (0.18ml/100g) MLCC when compared to the control that received distilled water (1.3%).

Effect of Oral Administration of MLCC on Carrageenan Induced Oedema

It was evident that the MLCC extract exhibited a significant anti-inflammatory activity against carrageenan – induced oedema with the use of both the human equivalent dose (0.36 ml/100g) and the high dose (0.72ml/100g) (Figure 2). Compared to the vehicle (distilled water), four hours post challenge with MLCC, the HE and the HD significantly reduced the rat paw oedema by 65.38% (P< 0.05) and 19.35% (P<0.01), respectively, while the positive control, Indomethacine, showed 82.35% (P< 0.05) reduction.

Effect of MLCC on Erythrocyte Membrane Stability

Use of MLCC at a concentration of 8 mg/ml exhibited significant (P<0.05) maximum membrane stability of

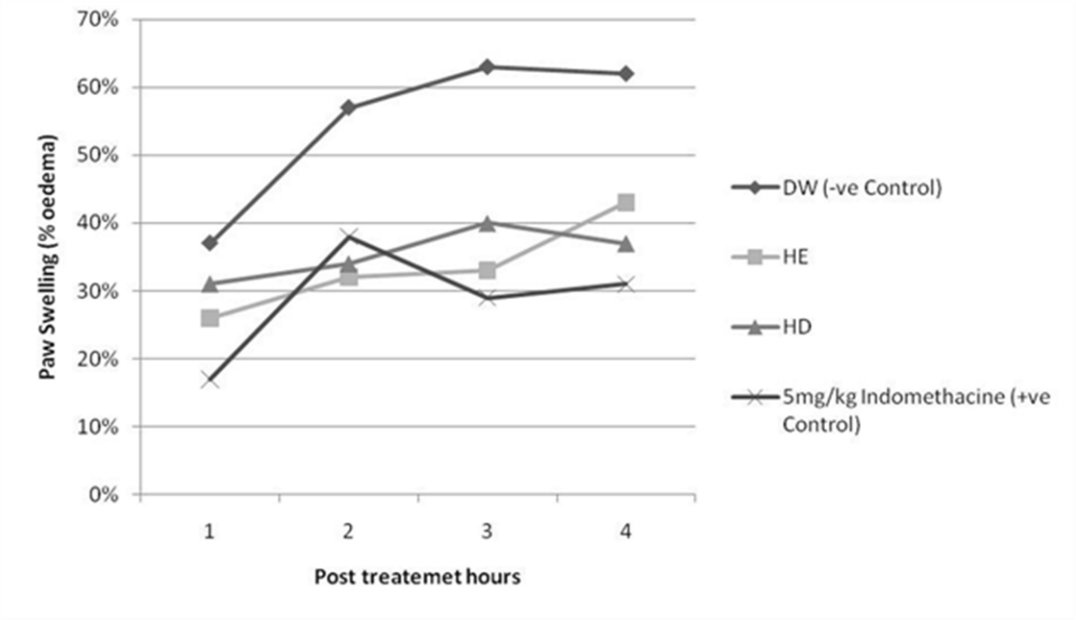

Figure 2. Effect of oral administration of mature leaf concentrate of *Carica papaya* (MLCC) on rat paw oedema (mean ± SEM, N=6/group). MLCC human equivalent dose (*P ≤ 0.05) and the high dose (**P≤ 0.01) significantly inhibited paw oedema as compared to the negative control (distilled water) (One way ANOVA).

heat treated rat RBC by 10.11% as compared to distilled water, where the positive control (Aspirin) showed 25.1% stability at the same concentration (Fig 3).

Effect of MLCC on Acetic Acid-Induced Vascular Permeability

A profound (by 82%) and significant (P < 0.05) impairment of *in vivo* vascular permeability induced by acetic acid in mice with the HD of MLCC was apparent when compared with distilled water (negative control) (Fig 4).

Effect of MLCC made up of Mature Leaves vs. Immature Leaves on Percentage Increase of Rat Platelets

Both the concentrated leaf extracts made up of mature and immature leaves of the same plant showed a high potential of increasing platelets by 73.8% and 71.3% respectively, with the oral administration of HD for 3 consecutive days but with no significant difference in the % increase of rat platelets between the two preparations (P ≥ 0.05).

Toxicological Studies

Evaluation of Toxicity of Hydroxyurea

The oral administration of Hydroxyurea did not provoke any overt signs of toxicity, stress and aversive behavior, also, no significant (P ≥ 0.05) toxicity was observed associated with the oral administration of Hydroxyurea on serum parameters (SGOT, SGPT, Urea and Creatinine) between the treated and the control rats (data not shown). The dry weights of vital organs in the treated rats did not significantly (P ≥ 0.05) differ with the control group.

Evaluation of Toxicity of Acute Administration of MLCC

Acute treatment with high dose of MLCC (0.72 ml/100 g) did not elicit any overt signs of toxicity, stress and aversive behavior in rats. Both serum and heamatological parameters showed no significant (P ≥ 0.05) difference between the treated and control groups (data not shown). Treatment did not significantly (P ≥ 0.05) alter the body weights (data not shown). The dry weights of vital organs did not significantly (P ≥ 0.05) differ between treated and control rats.

Results of Phytochemical Screening

Phytochemical screening of the MLCC revealed the presence of alkaloids, flavonoids, saponins, tannins and steroids.

DISCUSSION

The present study, for the first time, examined the

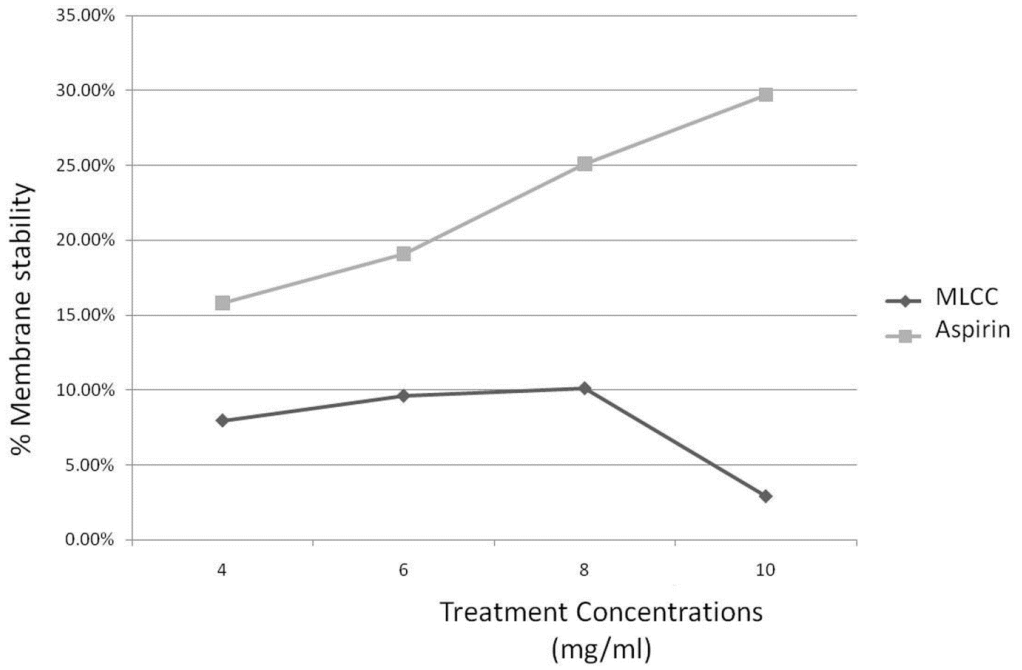

Figure 3. Effect of mature leaf concentrate of *Carica papaya* (MLCC) on percentage membrane stabilization (mean ± SEM, N=3/dilution). Membrane stabilizing profiles of MLCC high dose and Aspirin (positive control) showed significant membrane stability (*P ≤ 0.05) of heat treated rat RBC as compared to the control (distilled water) (One way ANOVA).

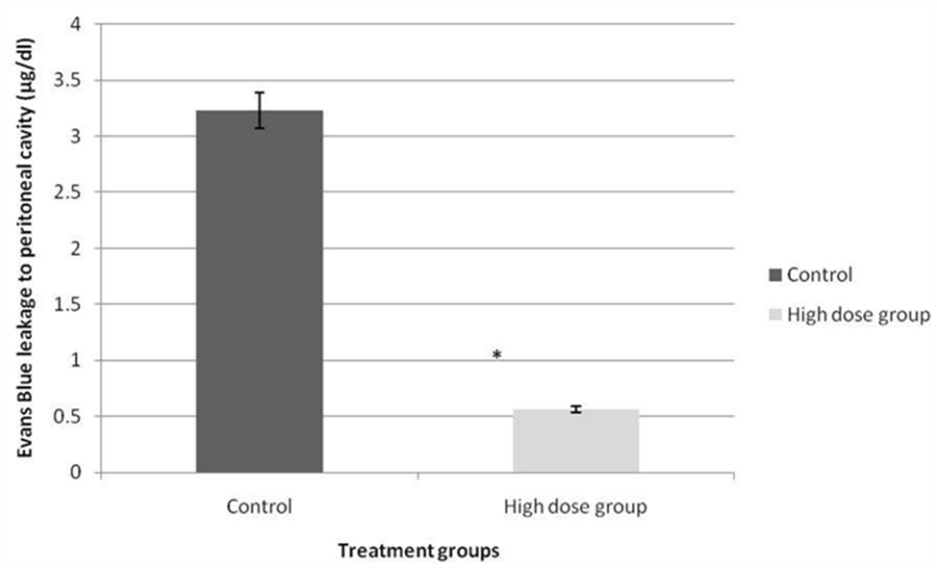

Figure 4. Effect of mature leaf concentrate of *Carica papaya* (MLCC) HD on Acetic acid-induced vascular permeability (mean ± SEM, N=6/group). The MLCC significantly (*P ≤ 0.05) reduced Evans Blue dye leakage by 82% as compared to the control (distilled water) (One way ANOVA).

platelet increasing effect of the oral administration of freshly prepared, mature leaf concentrate of *Carica papaya* (MLCC) in both normal, non-thrombocytopenic and thrombocytopenia induced rats. Importantly, this study, for the first time established a rat model for thrombocytopenia by using Hydroxyurea. As this drug is claimed to be highly toxic [26], a low dose (one tenth of the human equivalent dose) of Hydroxyurea was used to induce thrombocytopenia in rats. This was an important therapeutic investigation as there is

no effective treatment method for thrombocytopenia at present.

The most common thrombocytopenic conditions found in Sri Lanka are dengue fever, chikungunya, where thrombocytopenia is a result of platelet destruction, sequestration and importantly by bone marrow suppression, leptospirosis associated with platelet destruction and bone marrow suppression and sepsis mainly caused by platelet destruction [27-29]. The results showed that the oral administration of freshly prepared, mature leaf concentrate of *C. papaya*, significantly ($P \leq 0.05$) increased the platelet counts in both normal, non-thrombocytopenic and thrombocytopenia induced rats. The most potent platelet increasing effect of MLCC was elicited by the high dose (0.72ml/100g) of oral administration consecutively for 3 days. Interestingly, the high dose of MLCC also increased WBC and RBC significantly ($P \leq 0.05$). The carrageenan-induced paw edema test is widely accepted as a sensitive phlogistic tool for investigating potential anti-inflammatory agents, particularly the nonsteroidal type.[30] Inflammatory reactions produced by carrageenan have been shown to be due to a step-wise release of vasoactive substances such as histamine, bradykinin and serotonine in the early phase and prostaglandins in the acute phase MLCC showed a potent and significant anti-inflammatory activity in this model [31].

During inflammation, lysosomal hydrolytic enzymes are released into the sites which cause damage of the surrounding organelles and tissues leading to a variety of disorders [32]. Lysosomes play a major role in the inflammatory reaction and there is a close similarity between the erythrocyte and lysosomal membrane system [33]. The MLCC extract also exhibited membrane stabilizing property, as it offered significant protection of rat RBC membrane against lysis induced by heat. This indicates that the MLCC could stabilize the lysosomal membranes to inhibit the release of proteolytic enzymes. It is noted as a biphasic response with both positive and negative effects towards the erythrocyte membrane stability by whole MLCC. In other words, a particular concentration showed maximal protection against heat induced lysis of rat RBC and higher concentrations of drug caused lysis. The plant therefore may be regarded as a natural source of membrane stabilizer that warrants future in depth analysis.

Vascular permeability change participates in pathophysiology of inflammation with leakage of vascular contents to interstitial tissue [34]. This was assessed by the amount of Evans blue dye which extravasated to peritoneal fluid in acetic acid induced peritonitis in the mice. The concentrated extract of *C. papaya* significantly reduced vascular permeability by 82%. The extract contains chemical components which could interfere with metabolism or the targets of released vascular active mediators as aforementioned. Therefore, these results collectively suggest that the anti-inflammatory effects of the MLCC were caused by membrane stabilization as well as inhibitory effects on oedema, and on the vascular permeability responsible for the inflammation.

The most common thrombocytopenic diseases also show inflammatory conditions along with thrombocytopenia. Dengue is one of the most common thrombocytopenic diseases, caused by a mosquito vector. It has two types *i.e* classics dengue fever (DF) and dengue hemorrhagic fever (DHF). Classic DF is characterized by sudden onset of fever plus two or more of the following: retro-orbital pain, myalgias, arthralgias, rash, hemorrhagic manifestation, and inflammation [35]. Whereas, dengue hemorrhagic fever, a less common, more severe manifestation of the infection, is defined by fever, thrombocytopenia, hemorrhagic manifestations, and increased vascular permeability [36]. Chickungunya, a much similar thrombocytopenic condition to dengue is also associated with the manifestation of systemic inflammation [27,37]. Leptospirosis associated with thrombocytopenia also show systemic inflammation ,[38] whereas sepsis is identified with alteration in vascular permeability, coagulation and inflammation [39].

This study reiterated that the MLCC, irrespective of whether obtained from mature or immature leaves of *C. papaya*, has the potential to be developed as a plant based therapeutic agent for thrombocytopenia. Therefore, depending upon the availability and the ease of preparing MLCC, either type of the leaf can be used without disparity. An acute toxicity study of MLCC was carried out to confirm its safety for oral administration over a period of 3 days. The MLCC was well tolerated by rats showing no overt signs of toxicity, stress, aversive behavior or behavioral changes.

Further, hepatotoxicity, renal toxicity, haematotoxicity, neurotoxicity were also ruled out. Also, the MLCC failed to alter the body weights and the weights of vital organs of the test rats. Preliminary phytochemical analysis performed showed the presences of alkaloids, flavonoids, tannins and steroids in the MLCC, of which either a single type, or several phytochemicals in synergy, may play a role in increasing platelets under induced thrombocytopenia. It is planned to carry out bioactivity directed isolation of the active phytoconstituent(s) responsible for the platelet increasing and anti-inflammatory effects of the mature leaf concentrate of *C. papaya,* under the next phase of this study.

The platelet increasing effect of the oral administration of freshly prepared MLCC from the mature leaf concentrate of *C. papaya*, evident from the present study, does not directly pinpoint to an exact

mechanism/s of action. However, under normal healthy body conditions, platelets are produced from megakaryocytes within 4 to 6 days [40]. Nevertheless, in this study a dramatic increase of platelets was observed within only 72 hours (3 days). Under normal healthy body conditions spleen tends to hold one third of the platelets produced by megakaryocytes [8]. The smooth muscle contraction of the spleen, release stored platelets in to the circulation. That the crude papaya latex induce rat uterine contractions has been previously recorded [41]. As such it is plausible to hypothesise that the platelet increasing effect of the MLCC is due either to megakarypoietic / thrombopoietic stimulatory activity, and or to induced splenic contractions. A synergistic effect of these two mechanisms may also operate.

Therefore, it is imperative to study the effects of MLCC on thrombopoises/Megakaryopoises, and to investigate the potential of MLCC on inducing splenic contractions. Of the 3 doses of MLCC compared for the effectiveness in increasing platelets in the current study, the high dose (double the human equivalent dose) induced the most marked and significant increase of platelets, WBC and RBC without any acute toxicity. Accordingly, the high dose of MLCC appears to be the effective or potent dose. Nevertheless, a wider range of doses of MLCC should be tested to arrive at a valid inference.

CONCLUSION

In conclusion, this study scientifically claimed for the first time that the MLCC prepared by the mature leaves of *C. papaya* can be orally administered, is safe (non-toxic) for a period of 3 days and is orally active, effectively increasing platelet, WBC and RBC counts in normal (non-thrombocytopenic) and thrombocytopenic rats, and furthermore possesses potent anti-inflammatory activity, that overly justify claims of traditional medicine.

Hence, the MLCC may be a potential candidate for further research leading to the development of a herbal therapeutic agent for thrombocytopenia and associated inflammatory disease conditions, manifested in diseases such as dengue, chickungunya, leptospirosis, sepsis etc.

ACKNOWLEDGEMENT

Financial support was provided by the Institute of Biochemistry Molecular Biology and Biotechnology, and the Department of Zoology, University of Colombo, Sri Lanka. Relevant staff of the Institute of Biochemistry Molecular Biology and Biotechnology, and the Department of Zoology, both of the University of Colombo, Sri Lanka is acknowledged.

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