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Full Length Research Paper

Anti-oxidant vitamins, phytochemicals and proximate composition of the ethanol extract of the leaves of *Musa paradisiaca*

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The leaves of *Musa paradisiaca* are used in the treatment of diverse ailments including diabetes mellitus and diarrhoea and hence, the anti-oxidant vitamins, phytochemicals and proximate composition of the ethanol extract of its leaves were investigated using standard methods. The results show that the concentrations of vitamins A, C and E in the extract were found to be 1.972 ± 0.013 µg/g, 0.686 ± 0.030 and 1.884 ± 0.042 mg/100 g, respectively. The qualitative phytochemical screening of the extract showed the presence of alkaloids, flavonoids, proteins, carbohydrates, saponins, tannins, fats and oil, steroids and terpenoids. Glycosides, reducing sugars, resins and acidic compounds were not detected in the extract. Similarly, the quantitative phytochemical analyses of the extract showed that the concentrations of alkaloids, flavonoids, saponins, tannins and steroids were found to be 0.374 ± 0.026, 0.581 ± 0.010, 0.198 ± 0.004, 1.464 ± 0.071 and 0.271 ± 0.007 mg/g respectively while the percentage contents of moisture, fibre, ash, fats, proteins and carbohydrates were found to be 44.51 ± 0.21, 3.86 ± 0.07, 2.58 ± 0.06, 2.23 ± 0.05, 16.81 ± 0.12 and 27.86 ± 0.17 respectively. In conclusion, the findings of this study indicate that the leaves of *M. paradisiaca* possess nutritional and health benefits and thus, substantiate their medicinally multidimensional applications in different parts of the world.

Key words: *Musa paradisiaca*, anti-oxidant vitamins, phytochemicals, proximate composition.

INTRODUCTION

For a long period of time, plants have been valuable sources of natural products for maintaining human health especially in the last decade with more intensive studies for natural therapies. About 80% of the individuals from developed countries use traditional medicine which has compounds derived from medicinal plants (Karadi et al., 2011). Plants are important sources of many biologically active compounds. Plants used in traditional medicine provide an interesting and still largely unexplored source for the development of new drugs (Cos et al., 2006).

Musa paradisiaca Linn (Figure 1) (Musaceae) commonly known as plantain is a perennial tree-like herb widely distributed in the tropics. Due to its enriched food value and versatile medicinal potential, it is one of the most important fruits and vegetable crops of several countries. The fruits, leaves, peels, root and stalks of the plants have been used orally or topically as a medicine for treating diarrhoea and dysentery, healing of intestinal lesions in colitis, lithiasis, inflammation, pains, snakebite and ulcer. They equally possess hypoglycaemic, hypolipidaemic and anti-oxidant effects. A constituent, hydroxyanigorufone obtained from the plant was shown

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Figure 1. The leaves, fruits and flowers of *M. paradisiaca* Linn.
Source: www.tropicalspicegarden.com

to be a potential cancer chemo-preventive agent (Weremfo et al., 2011; Onyenekwe et al., 2013). Despite the avalanche of medicinal effects exhibited by *M. paradisiaca*, there is still paucity of information on its nutritional and phytochemical constituents. For this reason, the aim of this study was to evaluate the anti-oxidant vitamins, phytochemicals and proximate composition of the ethanol extract of the leaves of the plant.

MATERIALS AND METHODS

Plant

Fresh and apparently uninfected leaves of *M. paradisiaca* were harvested from the Botanical Garden of the University of Nigeria, Nsukka. The leaves were identified by Prof. (Mrs.) May Nwosu of the Department of Botany, University of Nigeria, Nsukka where the voucher specimens were deposited in the herbarium.

Preparation of the extract

Fresh leaves of *M. paradisiaca* were washed with distilled water and spread on a clean mat in a well-ventilated room with regular turning to enhance even drying and avoid decaying. The leaves were shade-dried for three weeks and homogenised into fine particles using an electric blender. A known weight (50 g) of the ground leaves was macerated in absolute ethanol for 36 h at room temperature. The mixture was thereafter filtered, concentrated in a rotary evaporator, dried in a boiling water bath and weighed.

Chemicals and reagents

The chemicals and reagents used for this study were of analytical grade and included the following: 99% (v/v) methanol (BDH Chemicals Ltd., Poole, England), absolute ethanol (BDH Chemicals Ltd., Poole, England), chloroform (BDH Chemicals Ltd., Poole, England), petroleum ether (BDH Chemicals Ltd., Poole, England), acetone (BDH Chemicals Ltd., Poole, England), dilute tetraoxo-sulphate (VI) acid, 0.5% α, α-dipyridine, 2% (v/v) hydrochloric acid, 1% (w/v) picric acid, boric acid, oxalic acid, trichloroacetic acid,

Table 1. Anti-oxidant vitamin content of the ethanol extract of the leaves of *M. paradisiaca*.

Anti-oxidant vitamin	Concentration
A (µg/g)	1.972 ± 0.013
C (mg/100 g)	0.686 ± 0.030
E (mg/100 g)	1.884 ± 0.042

Table 2. Qualitative phytochemical constituents of the ethanol extract of the leaves of *M. paradisiaca*.

Phytochemical constituent	Inference
Alkaloids	+
Flavonoids	+
Glycosides	ND
Proteins	+
Carbohydrates	+
Reducing sugars	ND
Saponins	+
Tannins	+
Fats and oil	+
Resins	ND
Steroids	+
Terpenoids	+
Acidic compounds	ND

+ = present; ND = not detected.

acetic anhydride, methyl orange, Dragendorff's reagent, Mayer's reagent, Wagner's reagent, Millon's reagent, Fehling's solution, 5% (w/v) ferric chloride solution, potassium sulphate, copper sulphate, potassium hydroxide solution, sodium hydroxide solution, aluminium chloride solution, lead subacetate solution, ammonium solution, Molisch's reagent and distilled water.

Anti-oxidant vitamin content

The concentration of vitamins A, C and E of the extract were determined using the methods described by Pearson (1976).

Phytochemical analyses

Qualitative phytochemical analyses were carried out on the extract according to the procedures outlined by Harborne (1998) and Trease and Evans (1989). Quantitative phytochemical analyses were carried out to determine the concentration of the following: alkaloids and flavonoids by the methods of Harborne (1998); tannins by the method of Swain (1979); steroids by the method of Okeke and Elekwa (2003); and saponins by the method of Obadoni and Ochuko (2001).

Proximate composition

The percentage content of moisture, fibre, ash, fats, proteins and carbohydrates of the extract was determined according to the methods described by AOAC (1990).

Statistical analysis

The results of the analysed data were expressed as means of three replicates ± standard deviations (SD). The bar charts were constructed using Excel graphic plots. This analysis was done using the computer software known as Statistical Package for Social Sciences (SPSS), version 18.

RESULTS

Anti-oxidant vitamin content of the ethanol extract of the leaves of *M. paradisiaca*

As shown in Table 1, the concentrations of vitamins A, C and E in the extract were found to be 1.972 ± 0.013 µg/g, 0.686 ± 0.030 and 1.884 ± 0.042 mg/100 g respectively.

Qualitative phytochemical constituents of the ethanol extract of the leaves of *M. paradisiaca*

The qualitative phytochemical screening of the extract showed the presence of alkaloids, flavonoids, proteins, carbohydrates, saponins, tannins, fats and oil, steroids and terpenoids (Table 2). Glycosides, reducing sugars, resins and acidic compounds were not detected in the extract.

Quantitative phytochemical constituents of the ethanol extract of the leaves of *M. paradisiaca*

The quantitative phytochemical analyses of the extract showed that the concentrations of alkaloids, flavonoids, saponins, tannins and steroids were found to be 0.374 ± 0.026, 0.581 ± 0.010, 0.198 ± 0.004, 1.464 ± 0.071 and 0.271 ± 0.007 mg/g respectively as shown in Figure 2.

Proximate composition of the ethanol extract of the leaves of *M. paradisiaca*

Figure 3 shows that the percentage contents of moisture, fibre, ash, fats, proteins and carbohydrates in the extract were found to be 44.51 ± 0.21, 3.86 ± 0.07, 2.58 ± 0.06, 2.23 ± 0.05, 16.81 ± 0.12 and 27.86 ± 0.17 respectively.

DISCUSSION

The concentrations of the anti-oxidant vitamins in the ethanol extract of the leaves of *M. paradisiaca* were found to be in the order of vitamin A < vitamin C < vitamin E (Table 1). The presence of these anti-oxidant vitamins

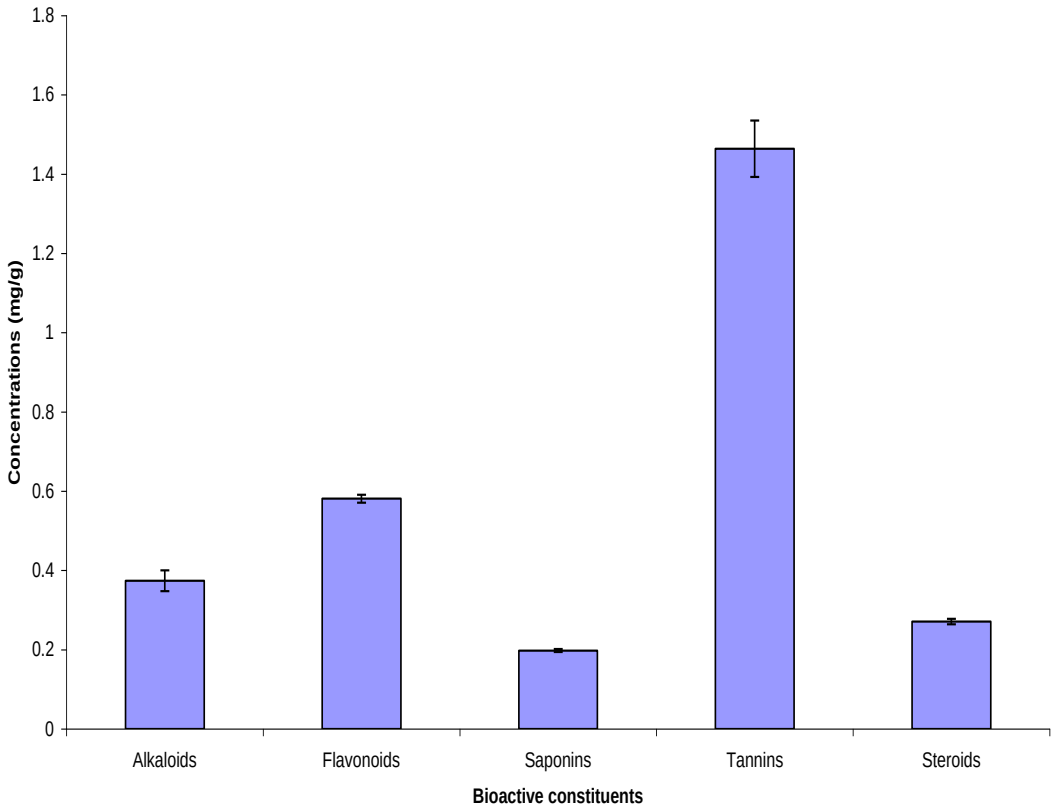

Figure 2. Quantitative phytochemical constituents of the ethanol extract of the leaves of *M. paradisiaca*.

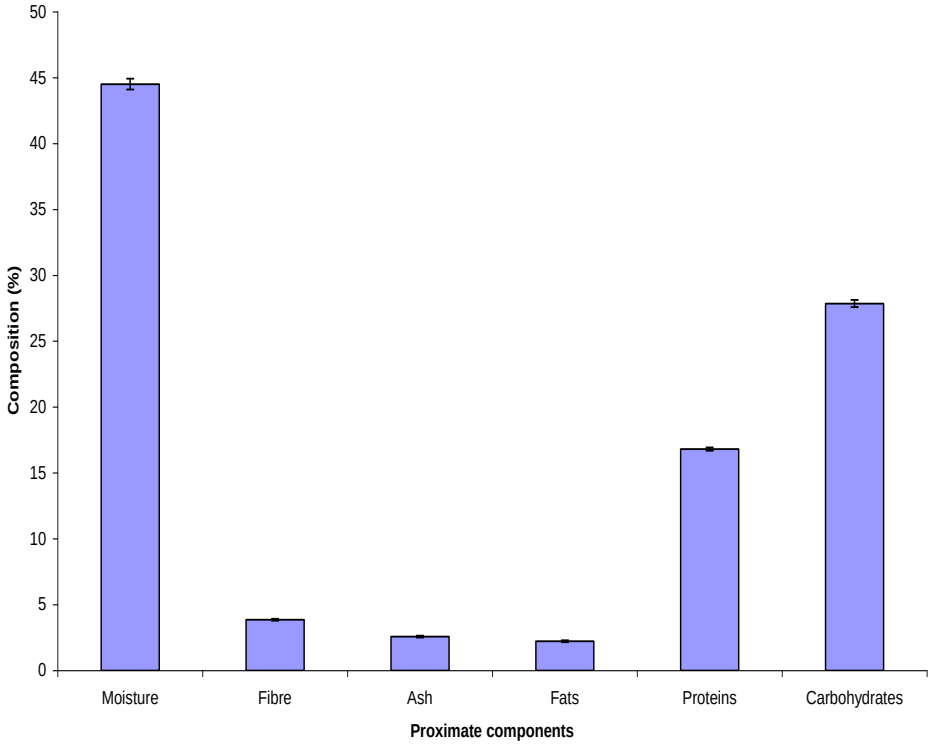

Figure 3. Proximate composition of the ethanol extract of the leaves of *M. paradisiaca*.

in the plant part studied indicate that *M. paradisiaca* leaves might be useful in curbing the harmful effects of free radicals in human health. Anti-oxidants are first line of defense against free radical-mediated damage and are therefore, critical for maintaining optimum health and wellbeing. The need for anti-oxidants becomes even more critical with increased exposure to free radicals (Yusuf and Muritala, 2013).

Qualitative phytochemical tests carried on the extract revealed the presence of tannins, saponins, flavonoids and alkaloids among others (Table 2). This agrees with the work of Onyenekwe et al. (2013) who reported the presence of the aforementioned phytochemicals in the stem extrude of *M. paradisiaca*. Quantitative phytochemical analyses of the extract showed that the concentration of tannins was the highest and that of saponins was the lowest (Figure 2). Alkaloids, tannins and saponins have been reported to have medicinal properties (Agoreyo et al., 2012). The presence of these phytochemical constituents in the extract shows that the leaves of *M. paradisiaca* have medicinal property. Sofowora (1993) reported the roles of these phytochemicals as analgesic, anti-inflammatory, anti-hypertensive and anti-microbial. Saponins and tannins also exhibit cytotoxic effects and growth inhibition making them suitable as tumour-inhibiting agents (Asl and Hossein, 2008).

Proximate analysis of the extract showed the presence of relatively high and low percentage contents of proteins and fats respectively (Figure 3). Although, the high percentage moisture content of the leaves of *M. paradisiaca* as ascertained in the present study may undermine their shelf life, their relatively low fat content and high protein content make them recommendable for the individuals who are watching their weights and the general public as their protein content could be used to supplement the proteins from stable food.

In conclusion, the results of the present study generally imply that the leaves of *M. paradisiaca* possess nutritional and health benefits and thus, substantiate their medicinally multidimensional applications in different parts of the world. However, further studies on the comparisons of the data of the present study and those of other nutritionally and medicinally promising plants are recommended.

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Review

Bacterial meningitis: An update review

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Bacterial meningitis is still considered serious life-threatening disease in spite of the decline in the morbidity and mortality in the last decade. The expeditious diagnosis of the disease, a prompt empiric antibiotic treatment and the proper adjunctive therapy are corner stones for the successful management of the disease. The objective of this review is to provide the health care professionals with an update of reference for bacterial meningitis diagnosis and treatment. In addition, the various types of vaccines and the empiric chemoprophylaxis treatment are reviewed.

Key words: Neisseria meningitides, cerebrospinal fluid (CSF) examination, polymerase chain reaction, chemoprophylaxis.

INTRODUCTION

Meningitis is an inflammatory disease of the meninges membranes that cover the brain and spinal cord. The inflammation and swelling may extend through the membranes of the pia mater, arachnoid or subarachnoid (Mace, 2008). Meningitis can be classified into infectious and noninfectious disease. Noninfectious meningitis can emerge from administration of certain drugs such as non-steroidal anti-inflammatory drugs, immunoglobulins or some antibiotics. It can also develop from diseases like sarcoidosis and neoplastic meningitis. Infectious meningitis can be further sub-divided to non-bacterial and bacterial (pyogenic) meningitis. Non-bacterial meningitis is typically caused by viral or fungal infections (Mace, 2008). Bacterial meningitis is characterized by the significant polymorph nuclear changes in the cerebrospinal fluid (CSF). This review focuses only on acute bacterial meningitis; the common causes and effective methods of management.

EPIDEMIOLOGY

The introduction of conjugate vaccines and the prophylactic antibiotic treatment during pregnancy caused a change in the epidemiology of bacterial meningitis (Brouwer et al., 2010; World Health Organization (WHO), 2010). In spite of the advances in medical care with the introduction and widespread use of antibiotics, meningitis still has high morbidity and mortality rates. According to WHO, the incidence of bacterial meningitis is exceeding 1.2 million cases each year worldwide (WHO, 1988). In Saudi Arabia, bacterial meningitis epidemics usually occur

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usually occur after Al Hajj and Omrah seasons. Al Mazrou et al. (2004) evaluated the patterns of the disease in a retrospective study (1999 to 2002) and found that 58% of the cases were among local population and 48% of it was reported at the holy areas of Makkah and Al Madinah.

In the United States, the incidence of meningitis was decreased to 1.38 cases in 2006 to 2007 in exchange for two out of every hundred thousand people in 1998 to 1999 (Thigpen et al., 2011). However, the attack rates are very age-specific with a higher extent among newborn infants and elderly. The attack rates for newborn infants are in the range of 400 cases per 100,000 in exchange for 20 per 100,000 in older infants (≤ 2 years) while this reduces to only 1 to 2 per 100,000 in adults (Loring, 2004). Furthermore, males are affected slightly more than females. The incidence and the proportion of deaths among bacterial meningitis diagnosed cases are dependent on area and country, the causative micro-organism and age (Centre for Disease Control (CDC), 2013). The reported mortality rate of meningitis ranges from 3 to 33%. Major of mortality predictors include over-60-age, immunocompromised status, low Glasgow coma scale score and infection with Gram negative bacteria. The common morbidity associated with meningitis typically encompasses neurological sequelae, such as hearing loss, mental disability or weakness of a limb (Tang et al., 1999; Rosenstein et al., 2001).

ETIOLOGY

The causes of acute bacterial meningitis are dependent on age and the clinical setting under which the infection occurs. Infection with *Haemophilus influenzae, Streptococcus pneumoniae*, *Neisseria meningitidis*, group B streptococcus (*Streptococcus agalactiae*) or *Listeria monocytogenes* is considered responsible for more than 80% of cases of bacterial meningitis (Porto, 2012*)*. The existing underlying disease states together with a patient's age highly influence the etiology of the disease. Table 1 shows the common bacterial pathogens according to age and Table 2 shows a list of causative microorganisms and their common host characteristics.

PREDISPOSING FACTORS

Predisposing factors for meningitis include head trauma, immunosuppression, central nervous shunts, cerebrospinal fluid fistula/leak, neurological patients, alcoholism, sinusitis, otitis media, pharyngitis, bacterial pneumonia, splenectomized patients, sickle cell disease and congenital defects. Risk factors for meningitis can be summarized as follows:

1. Age (Geiseler et al., 1980): (extremes of age: elderly (age > 60 years); young children (age < 5 years), especially infants (age < 2years/newborns).
2. Demographic/socioeconomic (Choi, 1992; Chaves-Bueno and McCrcken, 2005): (male gender, African American ethnicity, poor populations, crowding (military recruits and crowded dormitories).
3. Exposure to pathogens (Mace, 2008): recent colonization, (household/close contact with meningitis patient), contiguous infection: sinusitis, mastoiditis, otitis media or bacterial endocarditis, intravenous drug abuse or dural defect: status post neurosurgery, central nervous system (CNS) trauma, congenital defect, ventriculoperitoneal shunt, other CNS devices or cochlear implants).
4. Immuno-compromizing factors (Geiseler et al., 1980; Schutze et al., 2002): Post splenectomy, hematologic disorders such as sickle cell disease or thalassemia major, malignancy, diabetes, alcoholism/cirrhosis or HIV.
5. Drugs (Porto, 2012*)*: Nonsteroidal anti-inflammatory drugs (NSAIDs), trimethoprim-sulfamethoxazole or immunosuppressive drugs.
6. Disease (Porto, 2012*)*: Systemic lupus erythematosus.

CLINICAL PRESENTATION

Clinical manifestations are nonspecific and vary depending upon the patient's age. The classic triad of symptoms in meningitis in adults includes fever, stiff neck and altered mental status. Approximately 44% of cases present with this classic triad. The majority of patients show at least two of the following four symptoms: fever, headache, neck stiffness and altered mental status (Tunkel et al., 2009; van de Beek et al., 2004). Positive Kernig's and Brudzinski's signs of meningeal irritation may be also seen in a number of patients. Other symptoms such as nausea, vomiting, cardiorespiratory arrest, focal CNS signs, photalgia and seizures may frequently occur (Tunkel et al., 2009; Bamberger, 2010). In newborns and infants, the common symptoms include fever, poor feeding, vomiting, lethargy, diarrhea and sometimes apnea. They can be limited to temperature instability and the presence of bulging fontanel in newborns (Tunkel et al., 2009). In older children, in addition, photalgia and mental disorder may occur. Sometimes the symptoms may be limited to only seizures especially in pediatric patients with pneumococcal meningitis. In Neisseria-caused-meningitis, rash and petechiae are common in more than half of the cases (Tunkel et al., 2009). For elderly and immunocompromised patients, signs may be masked with the common age- or immunodeficiency-related symptoms. Such groups of patients can present with lethargy and mental disorder as the common early signs (Tunkel et al., 2009). Other symptoms such as headaches, photalgia, seizures, rash, nausea and vomiting

Table 1. Common bacterial meningitis pathogens in different age groups.

Age/predisposing factor	Pathogens	Less common organisms
Newborns (<1 month)	*Group B streptococcus (S. agalactie), E. coli, L. monocytogenes*	*L. monocytogenes*, *Herpes simplex*, type 2
1-3 months	*Group B streptococcus (S. agalactie), E. coli, L. monocytogenes, S. pneumonia, N. meningitides, H. influenza*	Viruses
3 months-50 years	*Pneumonia, N. meningitides*	*E. coli* , Viruses
More than 50 years	*Pneumonia, N. meningitides, L. monocytogenes, gram-negative bacilli*	*L. monocytogenes*; aerobic, Gram-negative bacilli, viruses

Source: Mace (2008).

are also seen. Physical examination may reveal signs of meningeal irritation, stiff neck and positive Kernig and Brudzinski signs.

DIAGNOSIS

Combining the laboratory tests together with the investigational local meningeal irritation signs, confirmed by physical examination and the patient's medical history can confirm the diagnosis of acute bacterial meningitis; however, the key factor remains the CSF examination. When meningitis is suspected, patients' CSF should be examined after collection by a lumbar puncture (LP) unless uncorrected coagulopathy or cases known to cause elevated intracranial pressure are present. The high intracranial cases include seizure, focal neurologic deficits and/or head trauma. The presence of cardiopulmonary instability is considered another contraindication for LP, and in such cases brain imaging is often recommended (Tunkel et al., 2004; Chavez-Bueno and McCracken Jr., 2005). CSF analysis should highlight differential white blood cell counts and the level of protein and glucose. Gram stain and culture should be performed to confirm the presence and to deter-mine the type of bacterial infection. In bacterial meningitis, CSF analysis findings include: high white blood cell (WBC) with values exceeding 1,000 cells/mm^3 with predominance of neutrophils, high CSF protein (> 100 mg/dl) and low CSF glucose (usually < 40 mg/dl) due to the inflammatory process. Table 3 provides a summary of the findings. Normal or near normal findings may be seen in young children, immunocompromized and partially treated patients (Fraser et al., 1947). Determination of the causative microorganism allows for the selection of the most effective treatment. Gram staining of CSF is recommended as a fast, inexpensive and accurate tool to determine the bacterial etiology since CSF cultures can take up to 48 h. Positive gram stain is reported in 60 to 80% of untreated cases of bacterial meningitis and in 40 to 60% of partially treated cases. The sensitivity according to the causative organism varies from 90% for pneumococcal- or staphylococcal caused meningitis to less than 50% in Listeria-caused meningitis (Seehusen et al., 2003).

Polymerase chain reaction (PCR) and latex agglutination test may be applied to confirm a diagnosis of bacterial meningitis when negative culture results are obtained in patients with prior antibiotic treatment as it does not depend on the presence of viable bacteria.

Agglutination test should not be used alone as it is not sensitive to *N. meningitidis* (Hall et al., 1995). Serum procalcitonin and C-reactive protein can be useful to give a primary indication of bacterial infection though they lack bacterial species specificity. They are beneficial for differential diagnosis of bacterial and viral meningitis (Brouwer et al., 2010).

MANAGEMENT

Patients suspected of having meningitis must be diligently cared for. Meningitis management involves fast and appropriate diagnosis, antimicrobial therapy, adjunctive and supportive therapy, chemoprophylaxis for contacts and vaccination for prevention. Table 4 represents the recommendations for empirical therapy by age group and specific risk factor. In addition, a list of common bacterial meningitis organisms is presented in Table 5.

ANTIMICROBIAL THERAPY

Often, the selection of antimicrobial agents is based on a variety of factors which include: (a) patients age; (b) clinical setting; (c) presumed immune status; (d) CSF count; (e) ability to achieve concentration in the CSF above the minimum inhibitory concentration; (f) gram stain; (g) culture and sensitivity of CSF and blood isolate. The choice of the antimicrobial agent should be made

Table 2. Microorganisms and common host characteristics.

Organism	Common host characteristic
Streptococcus pneumoniae	Normal; could be impaired
Neisseria meningitides	Normal
Hemophillusinfluenzae	Normal
Listeria monocytogenes	Normal (infants), often immunocompromised in older adults
Streptococcus agalactiae	Colonization during delivery (infants). Gastrointestinal source of bacteremia (older adults)

Source: Bleck, (2013).

Table 3. Common CSF findings in acute meningitis.

Finding	Viral	Bacterial
WBC (Cells/mm^3)	100-1,000	>1,000
Neutrophils (%)	20-40	≥80
Glucose (mg/dl)	Normal	≤40
Blood/CSF ratio	Normal	≤0.4
Protein (mg/dl)	Normal	>100
Positive Gram stain	NA	60%-95%
Positive culture	NA	>95%
Lymphocyte predominance	Yes	NA
Polymerase chain reaction	Enterovirus, Herpesvirus	Under investigation for *pneumonia, N. meningitides and H. influenza*

Source: Porto (2012).

with as much information as possible but treatment should not be delayed while waiting for test results (Brouwer et al., 2012; van de Beek et al., 2012). As soon as the microorganism has been identified, and its *in vitro* susceptibilities known, a modification of therapy may be applied if necessary. Table 6 summarizes the recommended antimicrobial therapy for each pathogen-specific acute meningitis. The blood brain barrier (BBB) characteristics and the physicochemical properties of the antimicrobial agent such as logP and pKa are considered the most critical factors affecting adequate CSF concentrations (Sinner and Tunkel, 2004; Nau et al., 2010).

CSF penetration

The first determinant in the ability of the drug to treat bacterial meningitis effectively is its ability to cross BBB. The CSF has a unique environment and as a result has different pharmacokinetic parameters than in other areas of the body. Generally, antimicrobial agents are not significantly metabolized in the CSF and because of that, concentrations of most drugs primarily depend on penetration and elimination through the BBB that is affected by the following factors (Andes and Craig, 1999):

Lipid solubility: Lipophilic agents, such as the fluoroquinolones, chloramphenicol, rifampin and sulfonamides are able to enter the CSF via passive diffusion which allows them to reach peak CSF concentrations more rapidly, maintain adequate CSF concentrations and reach CSF half-lives similar to those in serum, regardless of the presence or absence of meningeal inflammation. In contrast to hydrophilic agents, such as β-lactams and vancomycin, these depend on the opening of tight junctions for entry and as a result have poor penetration and delayed onset of peak CSF concentrations (Lutsar et al., 1998; Chowdhury and Tunkel, 2000; Sinner and Tunkel, 2004).

Molecular weight: Fluoroquinolones and rifampin have low molecular weights and simple structures, which result in a better CSF penetration compared with larger compounds with more complex structures, such as vancomycin (Lutsar et al., 1998; Chowdhury and Tunkel, 2000; Sinner and Tunkel, 2004).

Ionization: Drugs with high ionization have poor CSF penetration. In bacterial meningitis, the pH of CSF is lower than that of plasma, so drugs such as β-lactam antibiotics which are weak acids and highly ionized in the physiologic pH of plasma have poor penetration into the CSF and tend to pass from the CSF into the plasma instead of in the reverse direction (Lutsar et al., 1998; Chowdhury and Tunkel, 2000; Sinner and Tunkel, 2004).

Protein binding: Only unbound fractions of antimicrobials enter the CSF; a high degree of protein binding in the

Table 4. Recommendations for empiric therapy by age group and specific risk factors.

Age-Group	Therapy
Neonate<1 month	Ampicillin plus aminoglycoside or ampicillin plus cefotaxime
Infant (1-23 months)	Third-generation cephalosporin (cefotaxime or ceftriaxone) plus vancomycin
Pediatric and adult (2 to 50 years)	Third-generation cephalosporin (cefotaxime or ceftriaxone) plus vancomycin
Elderly (>50 years)	Third –generation cephalosporin (cefotaxime or ceftriaxone) plus ampicillin plus vancomycin
Penetrating head trauma, post neurosurgery or cerebrospinal fluid shunt	Vancomycin plus cefepime or ceftazidime or meropenem
Skull Fracture	Vancomycin+ ceftriaxone or cefotaxime

Table 5. Common bacterial meningitis organisms.

Organism	Age	Risk factors	Proportion of cases	Case fatality
S. pneumoniae	All ages	Immunoglobulin alternative complement deficiency, asplenia, alcoholism	57%	17.9%; higher if immunocompromised
N. meningitidis	Aged 11 to 17 years and younger adults	Multiperson dwellings, travel to Sub Saharan Africa	17%	10%
L. monocytogenes	Neonates and adults	Cell-mediated immunodeficiencies (e.g. steroids, HIV, alcoholism), newborns	4%	18%
H. influenzae	Children and adults	Newborns	6%	7%
Group B streptococcus	Neonates	86% of cases are in patients aged G2 months	17%	11%
Gram-negative rods (*E. coli,* K. *pneumoniae*)	Adults	Nosocomial infection; only 3% from community	33% of all nosocomial meningitis	35% nosocomial; 25% community acquired

Source: Bartt (2012).

binding in the serum (for example, with ceftriaxone) limits the degree of CSF penetration (Lutsar et al., 1998; Chowdhury and Tunkel, 2000; Sinner and Tunkel, 2004).

Mode of administration

Mode of administration of the drug can be by intermittent or continuous intravenous administration. The standard clinical practice of intermittent administration usually leads to higher peak CSF concentrations but may not maintain concentrations above the minimal bactericidal concentration (MBC) for the entire dosing interval. In contrast, continuous infusion administration maintains concentrations above the MBC during nearly 100% of the dosing interval, although a lower peak CSF concentration is attained (Sinner and Tunkel, 2004). The mode of administration has been a concept of considerable debate but fewer clinical failures were seen in infections treated with continuous intravenous infusion of antibiotics that act by time-dependent killing (for example, β-lactams) and even with aminoglycosides that exhibit concentration-dependent killing (Kasiakou et al., 2005).

Table 6. Recommendations for antimicrobial therapy for pathogen-specific acute meningitis.

Microorganism	First choice	Alternative agents
S. pneumonia	Vancomycin plus ceftriaxone or cefotaxime	Meropenem, fluoroquinolone
Neisseria meningitides	Ceftriaxone or cefotaxime	Penicillin G, ampicillin, fluoroquinolones, aztreonam
GBS (*S. agalactiae*)	Ampicillin or penicillin G+/-aminoglycoside	Cefotaxime or ceftriaxone
Listeria monocytogenes	Ampicillin or penicillin G+/-aminoglycoside	meropenem
Haemophilusinfluenzae	Ceftiaxone or cefotaxime	Chloramphenicol, cefipime, meropenem, fluoroquinolone
S. aurues		
Methicillin-sensitive	Ampicillin+gentamicin	NA
Ampicillin-resistant	Vancomycin+/-rifampin	NA
Vancomycin resistant	linezolid	NA
P. aeruginosa	Ceftazidime or cefepime +/- aminoglycoside	Azteronam, fluoroquinolone, meropenem+/-aminoglycoside

Source: Bartt (2012).

Table 7. Recommended doses for certain antibiotics.

Antibiotic	IV Dosage (children)	IV Dosage (adult)
Amikacin	20-30 mg/kg/day ÷ every 8 h	15 mg/kg/day ÷ every 8 h
Ampicillin	200-400 mg/kg/day ÷ every 6 h	12 g ÷ every 4 h
Cefepime	150 mg/kg/day ÷ every 8 h.	6 g ÷ every 8 h
Cefotaxime	225-300 mg/kg/day ÷ every 6-8 h	8-12 ÷ g every 4-6 h
Ceftazidime	150 mg/kg/day ÷ every 8 h	6 g ÷ every 8 h
Ceftriaxone	100 mg/kg/day ÷ every 12 h	4 g ÷ every 12-24 h
Ciprofloxacin	NA	800 - 1200 mg ÷ every 8-12 h
Gentamicin	7.5 mg/kg/day ÷ every 8 h	5 mg/kg/day ÷ every 8 h
Meropenem	120 mg/kg/day ÷ every 8 h	6 g ÷ every 8 h
Nafcillin	200mg/kg/day ÷ every 6 h	9-120 g-every 4 h NOT sure of dosing here
Penicillin G	300,000 million unit/kg/day ÷ q 4-6 h	24 million units every 4 h
Rifampin	10-20 mg/kg/day ÷ q 12-24 h	600 mg q 24 h
Tobramycin	7.5 mg/kg/day ÷ every 8 h	5 mg/kg/day ÷ every 8 h
Trimethoprim-Sulfamethoxazole	10-20 mg/kg/day ÷ q 6-12 h	10-20 mg/kg/day q 6-12 h
Vancomycin	60 mg/kg/day ÷ q 6 h	30-60 mg/kg/day q 8-12 h

Antimicrobial pharmacodynamics in CSF

Knowledge of the pharmacodynamic properties of antimicrobials allows for appropriate optimization of bactericidal drug concentrations (Lutsar et al., 1998; Aronin, 2000). Bacterial killing is particularly important in the CSF in which there is a decreased immune response from relatively lower concentrations of antibody and complement and inefficient phagocytosis. The recommended doses for commonly used antibiotics are included in Table 7. Antibiotics may exhibit either time-dependent or concentration-dependent killing activities. Time-dependent antimicrobial activity (demonstrated by the β-lactam antibiotics and vancomycin) depends on the time that the drug concentration in CSF is above the MBC (T > MBC). An experimental study of cephalosporin-resistant pneumococcal meningitis showed that the T > MBC was the most important single determinant of ceftriaxone efficacy and correlated best with the bacterial kill rate supported by the direct linear relationship that was found between T > MBC and the bacterial killing rate (Lutsar et al., 1997). Aminoglycosides and fluoroquinolones exhibit concentration-dependent killing (Ahmed et al., 1997; Rodriguez-Cerrato et al., 2001), although fluoroquinolones, particularly trovafloxacin and gatifloxacin have been shown to have features of time-dependent killing in which the T > MBC was also considered a factor in bacterial killing (Kim et al., 1997; McCracken, 2000). The efficacy of concentration-dependent killing depends on attaining high peak CSF meningitis

Table 8. Therapy of common pathogen and duration of therapy.

Organism	Recommended therapy	Alternative therapy	Duration of therapy (days)
Streptococcus pneumoniae	**MIC< 0.1 mcg/ml:** (a) Penicillin G 4 Million Units IV q4h; (b) Ampicillin 2 g IVq4h **MIC 0.1-1 mcg/ml: (a)** Third-generation cephalosporin **MIC>2 mcg/ml: (a)** Vancomycin plus third-generation cephalosporin	**MIC< 0.1 mcg/ml: (a)** Third –generation cephalosporin or chloramphenicol MIC 0.1-1 mcg/ml; (b) Cefepime or meropenem **MIC > 2 MCG/ml:** Fluoroquinolone	10-14
Neisseria meningitidis	**MIC 0.1-1 mcg/ml: (a)** Penicillin G 4 Million Units IV q4h; (b) Ampicillin 2 g IVq4h **MIC 0.1-1 mcg/ml:** Third-generation cephalosporin	**MIC 0.1-1 mcg/ml:** Third –generation cephalosporin or chloramphenicol **MIC 0.1-1 mcg/ml:** Chloramphenicol, fluroquinolone, or meropenem	7
H. Influenzae	**Beta lactamase negative:** Ampicillin 2 g IV q4h **Beta lactamase positive:** Third-generation cephalosporin	**Beta lactamase negative:** Third –generation cephalosporin , cefepime, chloramphenicol or Fluoroquinolone **Beta lactamase positive:** Cefepime, chloramphenicol or fluoroquinolone	10-14
Streptococcus agalactiae	Penicillin G 4 Million units IV q4h; Ampicillin 2 gm IVq4h	Third –generation cephalosporin	14-21
Listeria monocytogenes	Penicillin G 4 million Units IV q4h; Ampicillin 2 g IVq4h	Trimethoprim- sulfamethoxazole or meropenem	>21

syndrome.

ADJUNCTIVE TREATMENT

As with any severe infection, physiological support is paramount while one is awaiting the effect of the definitive therapy. Fluid and oxygenation are keystones of supportive therapy and must be augmented by pressors and ventilation as required. Convulsions must be treated appropriately. Fluid management must be adequately addressed. Careful monitoring of the hydration status of the patient is critical as over hydration can result in the possibility of associated cerebral edema and increased intracranial pressure. As improvement is noted (after 24 to 48 h), liberalization of fluid intake may be allowed but intake and output as well as serum electrolytes should be continuously monitored (van de Beek et al., 2012).

Dexamethasone has been found to prevent sensorineural hearing loss after *H. influenzae* and pneumococcal meningitis without interfering with antimicrobial therapy. It is recommended on individual basis for children greater than 2 months of age, with consideration of the benefit and possible risk when the diagnosis of bacterial meningitis

Table 9. Empiric therapy for acute meningitis syndrome.

Source or syndrome	Common pathogens	Suggested empiric therapy
Community acquired		
Adult or child	*S. pneumonia, N. meningitides, L. monocytogenes, H. influenzae*	Vancomycin + ceftriaxone
Neonate	Group B streptococcus: *L. monocytogenes and S. pneumoniae*	Ampicillin +cefotaxime or aminoglycoside
Immunocompromised e.g. patients with HIV, asplenia, alcoholism, cancer, or patients older than 60 years)	*S. pneumonia, L. monocytogenes* Aerobic gram-negative bacilli (eg, Enterobacteriaceae family)	Vancomycin + ampicillin +extended-spectrum cephalosporin
Adjunctive therapy (community-acquired disease unless contraindicated)	-	Dexamethasone (before or with first dose of antibiotics)
Identified focus of infection		
Maxillary sinusitis or otitis	Streptococcus species, Gram-negative bacilli, Staphylococcus aureus, Haemophilus species	Vancomycin +metronidazole + extended-spectrum cephalosporin
Endocarditis	*Viridians streptococcus; S. aureus Streptococcus;* bovis HACEK group, Enterococci	Vancomycin + extended-spectrum cephalosporin
Nosocomial	Gram-negative bacilli, Staphylococci species	Vancomycin +extended-spectrum cephalosporin
Penetrating trauma or recent neurosurgical procedure (eg, shunt)	S. aureus and other species (especially MRSA),Enterobacteriaceae family Pseudomonas species	Vancomycin + metronidazole + extended-spectrum; Cephalosporinor Vancomycin + meropenem
Encephalitis (e.g., seizures, obtundation)	Herpes family (especially herpesvirus type 1)	Acyclovir

Source: Bartt (2012).

is proven or highly suspected on the basis of CSF analysis report, Gram stain or positive agglutination test results. It is preferable to start steroid therapy as soon as diagnosis is made or at least at the start of antimicrobial administration.

The practice guidelines of the Infectious Diseases Society of America include the recommendation of adjunctive use of dexamethasone with the start of antibiotic treatment in patients with suspected pneumococcal meningitis (Tunkel et al., 2004). The mode of administration is 10 to 20 min before or concomitantly with the first dose of an antibiotic. For children, a dose of dexamethasone 0.6 mg/kg/day intravenously divided into four doses is recommended for four days while 10 mg IV every 6 h is recommended for adults (van de Beek et al., 2012).

PROPHYLAXIS

Chemoprophylaxis

In the case of *H. influenzae* or *N. meningitidis*, people in close contact including family, health care professionals or school settings are considered at high risk of contracting the disease. Other types of bacterial meningitis including cases

caused by *S. pneumoniae* are considered to be less transmissible to close contacts. A two-day rifampin course of therapy is proved as the first-line regimen for chemoprophylaxis (Lieberman et al., 1990). The recommended dosage is 10 mg/kg every 12 h for children older than 1 month or 600 mg every 12 h for adults. Other option includes the use of single dose 500 to 750 mg ciprofloxacin or a single dose of ceftriaxone at a dose of 125 mg in children or 250 mg in adults given intramuscular (Darouiche et al., 1990; Schaad et al., 1995).

Vaccines

There are currently three vaccines available that target the most common bacterial causes of meningitis: *S. pneumoniae*, *H. influenzae*. These microorganisms are largely human pathogens, contain a polysaccharide capsule as the main virulence determinant of and that capsular types associated with meningitis are only a small subset of those that colonize the nasopharynx, these similarities are important for vaccine development (Mcintyre et al., 2012). *H. influenzae* vaccine is available as a single antigen conjugate vaccine and in combination with other vaccines. Despite the type of vaccine, the recommended dose is to be given at 2, 4 and 6 months or at 2 and 4 months. Pneumococcal vaccine can be classified as a pneumococcal conjugate vaccine (PCV) or a pneumococcal polysaccharide vaccine (PPSV). Approved PCV include: the 7-valent (PCV-7), the 10-valent (PCV-10) and the 13-valent (PCV-13). PCV is recommended for all children with ages younger than 5 years. PCV-7 was approved by Food and Drug Administration (FDA) in 2000 for use in infants and young children. It consists of seven serotypes conjugated to a carrier protein. These serotypes (which include 4, 6B, 9V, 18C, 19F, and 23F) have been found to be responsible for about 82% of meningitis cases caused by pneumococci and effective in reducing invasive infection by 79% (Seppa, 2011). However, an increase in the incidence of infection caused by other serotypes was observed (for example, 19A), that led to the development of the 10-valent and 13-valent pneumococcal conjugate vaccine. In 2009, the European Commission gave authorization for marketing of 10-valent PCV containing serotypes 1, 5 and 7F in addition to all serotypes of PCV-7. Shortly after that, the 13-valent was also introduced in 2010 (European Medicines Agency, 2013). This vaccine covers an additional three serotypes (which are 3, 6A and 19A). These 13 serotypes are responsible for 63% of invasive pneumococcal cases in children less than 5 years and 5 to 6 years with underlying medical conditions such as chronic lung disease, diabetes or heart disease. The main disadvantages of the 23-valent pneumococcal polysaccharide vaccine (PPSV23) are the inability to induce immunologic memory and effect on nasopharyngeal carriage.

The CDC Advisory Committee on Immunization Practices (ACIP) recommends the PPSV23 for children aged ≥ 2 years that have underlying medical conditions after completing all recommended doses of PCV13. A poster dose of PPSV23 given 5 years after the first dose is recommended for children with anatomic or functional asplenia. PPSV23 is also recommended for elderly subjects only if it is proved that they did not receive pneumococcal vaccination at least in the last five years. High risk populations such as subjects with chronic pulmonary or cardiovascular diseases, diabetes and/or immunodeficiency, in addition to nursing home residents and smokers, should be considered for PPSV23 vaccine.

Meningococcal vaccines are active against many strains of *N. meningitidis*. Immunization against meningococcal is not warranted as post exposure prophylaxis unless the strain is documented to have a capsular serotype represented in the vaccines (type A, B, C, Y or W-135). A marked reduction in *H. influenzae* meningitis has been associated with the use of *H. influenzae* vaccine directed against the type b capsular polysaccharide of this organism in children in developed countries since 1987.

Conclusion

Bacterial meningitis continues to carry high morbidity and mortality rates. Awareness of appropriate empiric and directed antimicrobial therapy regimens may help to lower the morbidity and mortality rates. Vaccinations, dexamethasone and chemoprophylaxis should be used judiciously in the appropriate patient population to provide the best patient care.

Conflict of interest

Authors reported none.

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Full Length Research Paper

Preclinical evaluation of the crude extract from the fruits of *Punica grantaum* L. (*Punicaceae*) for antimicrobial activity in *in vitro* and *ex vivo* experimental models: A comparative study

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Currently, natural products have been evaluated as sources of antimicrobial agents. Considering the increasing use of pomegranate, it has become important to establish a correlation between the phytocompounds and the antimicrobial properties of the crude extract used in Brazilian folklore medicine. The compositional analysis revealed the presence of classes of secondary metabolites of pharmaceutical interest, among them, tannins, flavonoids and phenolic compounds. The *in vitro* study was performed by broth micro-dilution susceptibility assay according to the protocols of the National Committee for Clinical Laboratory Standards and described the antibacterial and antifungal activities of the extract. The preeminent antimicrobial activities were recorded against *Staphylococcus aureus* and *Staphylococcus epidermidis*. In order to estimate the *ex vivo* antimicrobial activity of the extract, serum and granulomatous tissues were separately submitted to microbiological assay. The crude extract did not present any inhibition zone. Thus, it is possible to suggest that after the oral ingestion of the extract, bacteria present in the gastrointestinal tract of the animal, hydrolyze phytocompounds, which present antimicrobial potential in *in vitro* models but do not present sufficient serum and tissue concentrations to exert this activity in *ex vivo* and presumably in *in vivo* models.

Key words: Punicaceae, *Punica granatum* L., preclinical, antimicrobial, *in vitro*, *ex vivo*.

INTRODUCTION

Punica granatum L. (Punicaceae) is a large deciduous shrub or small tree which fruit (pomegranate), are common in the Mediterranean and has been therapeutically used as food in Brazil. The fruit is delimited by a leathery pericarp contained within are numerous arils, each as a single seed surrounded by a translucent juice-containing sac. Thin acrid-tasting membranes extend into the interior of the fruit from the pericarp, providing a lattice work for suspending the arils. The fruit itself gives rise to three parts: the seeds, about 3% of the weight of the fruit,

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about 3% of the weight of the fruit, the juice, about 30% of the fruit weight and the peels, which also include the interior membranes (Lansky and Newman, 2007).

In Ayurvedic medicine, pomegranate has been used for the treatment of a variety of ailments including parasitic infections, diarrhea and ulcers (Jurenka, 2008). The root and stem barks are reported to have astringent effect (Alper and Acar, 2004) and antihelmintic activity (Gracious et al., 2001). The fruit rind is traditionally used to treat dysentery (Chopra et al., 1995). The flowers serve as a remedy for diabetes mellitus (Katz et al., 2007). The pharmacological activities of pomegranate include antioxidation (Dikmen et al., 2011), anti-hepatotoxicity (Kaur et al., 2006), antiplasmodial activity (Dell'Agli et al., 2009), anticancer (Albrecht et al., 2004) and antimicrobial properties (Reddy et al., 2007).

In the last few years, many important functions of fresh fruits and vegetables have been reported and they are now recognized as being good sources of natural antimicrobial agents (Berk and Tepe, 2013). Polyphenols have been acknowledged to have health beneficial effects, owing to derived products such as tannins, flavonoids and phenolic compounds. According to recent reports, pomegranate is rich in polyphenols, mainly ellagitannins and gallotannins, such as punicalin, punicalagin, pedunculagin, punigluconin, granatin B and tellimagrandin I (Noda et al., 2002). However, the correlation between the phytocompounds and the antimicrobial properties of the crude extract from the fruits of *P. granatum* L., which is therapeutically used in Brazilian folk medicine has not been investigated. Therefore, this preclinical evaluation aimed to clarify the antimicrobial activity of pomegranate through *in vitro* and *ex vivo* models to confirm its potential.

MATERIALS AND METHODS

Plant

Fresh ripe fruits of *P. granatum* L. were harvested from an orchard located in the municipality of Ribeirão Preto (São Paulo State, Brazil) in June 27th, 2012 and authentified in the Department of Exact and Earth Sciences of Federal University of São Paulo.

Preparation of the crude extract

Fruits of *P. granatum* L. were previously washed in running water. Then, they were air dried at 50°C, crushed, milled in a knife mill to obtain 500 g and subsequently subjected to an extraction with water-ethanol solution (5.0 L, 70%) by maceration during seven days. The crude extract was filtered and concentrated on a rotatory evaporator unit at 60°C under reduced pressure, furnishing 127 g (yield 25.4%) of the crude extract.

Compositional analysis of the crude extract

The crude extract was preliminarily subjected to a qualitative analysis to detection of the secondary metabolites classes by chemical reactions characteristic for each substances (Delgado et al., 2013).

In vitro antimicrobial activity of the crude extract

Microbial strain

The crude extract was tested towards 5 reference bacteria, 2 *Gram*-positive strain: *Staphylococcus aureus* (ATCC 6538) and *Staphylococcus epidermidis* (ATCC 12228) and 3 *Gram*-negative strains: *Pseudomonas aeruginosa* (ATCC 15442), *Klebsiella pneumoniae* (ATCC 13883) and *Escherichia coli* (ATCC 10536) and 1 reference fungus, *Candida albicans* (ATCC 10231).

Experimental procedure

Broth micro-dilution susceptibility assay was used to determine the minimum inhibitory concentrations (MIC) according to the protocols of the National Committee for Clinical Laboratory Standards (National Committee for Clinical Laboratory Standards 1997a, b). All tests were performed in Mueller-Hinton broth (Merck, Darnstadt, Germany) and overnight broth cultures of each microbial strain were prepared. Microorganism suspensions at a final concentration of 2×10^6 CFU/ml were added to microwell plates and the plates were incubated at 37°C for 24 h. The crude extract was dissolved in 1% dimethyl sulphoxide (DMSO) and serial doubling dilutions were prepared over the concentration range 1 to 100 µg/ml. As positive controls, two-fold serial dilutions ranging from 100 to 1.6 µg/ml of amikacin, amphotericin B and chloramphenicol were used. All determinations were performed in triplicate and repeated twice. The MIC value was defined as the lowest concentration of the crude extract at which the microorganism does not present visible growth. MICs ≤ 250 µg/ml are considered of interest for plant extracts (Lopes et al., 2013).

Ex vivo antimicrobial activity of the crude extract

Bacterial strain

A penicillin-sensible *S. aureus* strain (ATCC 25923) was used for the *ex vivo* test to determine MIC and minimum bactericidal concentration (MBC_{100}) by using Mueller-Hinton broth (Merck, Darnstadt, Germany) and Salt Mannitol Agar (Merck, Darnstadt, Germany), respectively. The MBC_{100} value was defined as the lowest concentration of the crude extract at which at least 99% of the microorganisms did not present visible growth. The same strain was used to carry out the regression line assay and the microbiological assay.

Drugs

Amoxicillin trihydrate was obtained from Sigma Chemical Co. (St. Louis, MO, USA.). Physiological saline solution (0.9% NaCl) was administered to the control animals.

Animals

Twenty-four adult male Wistar rats (*Rattus norvegicus* albinus), weighing 175 ± 25 g, were acquired from the biotery of ABC Medical School. The animals were housed in polyethylene cages (n = 6) in a climate-controlled environment (25 ± 4°C, 55 ± 5% humidity) with a 12 h (07:00 to 19:00) day length and had *ad libitum* access to food (Labina, Purina) and water. This project was performed in accordance with the guidelines of the Ethics in Research

and the standards of use of laboratory animals in research of the Federal University of São Paulo (protocol #1356/12).

Granulomatous tissue model

All animals were anesthetized with a combination of ketamine 90 mg/kg/i.m. and xylazine 10 mg/kg/i.m and granulomatous tissue was induced as previously described (Mattos-Filho et al., 2006). Briefly, four sterilized polyurethane sponge discs (density 35 kg/m^3) were subcutaneously implanted in the back of all rats. These sponge discs (Proespuma Com. & Ind. Ltd., São Paulo, Brazil) were 12 mm in diameter and 5 mm in thickness, weighing 12.21 ± 0.73 mg.

Experimental groups

Seven days after the sponge introduction, all animals were assigned into three groups of eight animals each: amoxicillin (G1) 25 mg/kg *per os*, crude extract of *P. granatum* L. (G2) 300 mg/kg *per os* (Jain et al., 2013) and physiological saline (G3) 1.0 ml *per os* (0.9% NaCl). All drugs were administered in a single dose.

Surgical and sampling procedures

After 90 min of drug administration, blood samples were collected by cutting the carotid plexus of each animal under general anesthesia. Blood samples were centrifuged and 10 µl of serum was placed on three sterilized paper discs (6.25 mm) and dried at room temperature. Granulomatous tissues were delimited and surgically removed. All discs and two granulomatous tissue samples of each animal were placed on Muller-Hinton agar plates inoculated with 10^8 CFU/ml of *S. aureus* strain. After eighteen hours of incubation at 37°C, the inhibition zones were measured. Two granulomatous tissue samples of each animal were weighed and analyzed by a histological routine technique (HE). Granulomatous tissue weights, tissue and serum concentrations were submitted to the Kruskal-Wallis test and Dunn test (software Bioestat 1.0® for Windows©) in order to compare all groups.

Regression line

Amoxicillin and crude extract suspensions of 0.03, 0.05, 0.07, 0.10, 0.30, 0.50, 0.70, 1.0, 3.0, 5.0, 7.0 and 10 µg/ml were made by using drug-free serum of rats and 10 µl were placed onto dry paper-filter discs (6.25 mm). Three discs of each concentration were placed on the Mueller-Hinton agar, previously inoculated with 10^8 CFU/ml of *S. aureus* strain. The resulting inhibition zones were measured (mm) after eighteen hours of incubation at 37°C. These zones and the drugs concentrations were used to obtain the regression line (software Excel XP® for Windows©).

RESULTS

Compositional analysis of the crude extract

Through the compositional analysis, it was possible to identify major classes of secondary metabolites of pharmacological interest present in the crude extract from the fruits of *P. granatum* L. exhibited in Table 1.

In vitro antimicrobial activity of the crude extract

The *in vitro* antimicrobial activity of the crude extract from the fruits of *P. granatum* L. (*Punicaceae*) against different microorganisms is displayed in Table 2.

Ex vivo antimicrobial activity of the crude extract

MIC and MBC_{100} of amoxicillin against *S. aureus* (ATCC 25923) were, respectively 0.2 and 1.5 µg/ml. The limits of detection of the regression curve were 0.03 µg/ml (12 mm of inhibition zone diameter) and 10 µg/ml (31 mm of inhibition zone diameter). The relation between the diameter of inhibition zone (DIZ - in mm) and the concentration of amoxicillin (CA - in µg/ml) was DIZ = (3.23 × Ln (CA)) + 24.16, which showed a coefficient of regression (R) of 0.9851. This relation was used to estimate tissue and serum concentrations, considering the mean of tissue weights of each animal. The wet weight (mg) values (mean ± standard error of the mean) of the granulomatous tissue samples were 31.18 (± 1.98), 32.16 (± 2.16) and 31.88 (± 2.31), respectively for groups 1, 2 and 3. No statistically significant differences were observed among groups ($p > 0.05$) regarding the wet weight values. After seven days and ninety minutes, a delimited fibrous capsule involving the sponge was observed in all samples. Fibroblasts, mesenchymal cells and new capillary formation were verified in large scale. Infectious exudates were not observed in any of the granulomatous tissues. Table 3 exhibits amoxicillin serum and tissue concentrations of group 1. Groups 2 and 3 did not present any inhibition zone considering both serum and tissue samples during the microbiological assay.

DISCUSSION

A compositional analysis has been accomplished to verify the presence of major classes of secondary metabolites of pharmaceutical interest in the crude extract from the fruits of *P. granatum* L. and then these phytochemical results were attached to its preclinical antimicrobial evaluation. *In vitro* tests performed with extracts rich in tannins or pure tannins, and pomegranate is a rich source of tannins (Afaq et al., 2005), have identified several biological activities, among them, bactericide and fungicide (Chung et al., 1998). From the pericarp of the fruit, rich in tannins, it has been described the isolation of granatins A and B, punicalagin and punicalin, which must be primarily responsible for the antimicrobial effect (Catao *et al*., 2006).

Flavonoids and phenolic compounds, also present in the crude extract are essential for growth and reproduction of vegetables and are produced as a plant's response to damage caused by microorganisms. Their benefits are usually connected to two properties: inhibition

Table 1. Compositional analysis of the crude extract.

Metabolite	Result
Alkaloids	-
Anthraquinones	-
Carbohydrates	+
Cardiotonic heterosydes	-
Fixed oils and fats	-
Flavonoids	+
Glycosides	+
Phenolic compounds	+
Proteins and amino acids	+
Saponins	-
Tannins	+

Table 2. *In vitro* antimicrobial activity of *Punica granatum* L. (*Punicaceae*) species.

Micro-organism	Concentration of extracts (µg/ml)							
	4000	2000	1000	500	250	125	Control	MIC (µg/ml)
Staphylococcus aureus	-	-	-	-	-	+	+	250
Staphylococcus epidermidis	-	-	-	-	-	+	+	250
Escherichia coli	-	-	-	-	+	+	+	500
Candida albicans	-	-	-	-	+	+	+	500
Klebsiella pneumoniae	-	-	-	+	+	+	+	1000
Pseudomonas aeruginosa	-	-	-	+	+	+	+	1000

Table 3. *Ex vivo* antimicrobial activity of *Punica granatum* L.

Drugs	Serum (µg/ml)	Tissue (µg/ml)
Amoxicillin	2.23	3.89
Crude extract	0.00	0.00
Physiological saline	0.00	0.00

of certain enzymes and antioxidant activity (Cotelle, 2001). Therefore, they can protect other components of the vegetables, such as carotenoids and vitamin C, digestive enzymes and the intestinal epithelial cells of oxidation of free radicals produced in the stomach (McDougall et al., 2005). Thus, tannins, flavonoids and phenolic compounds must act synergistically to constitute the antimicrobial potential of pomegranate.

The crude extract from the fruits of *P. granatum* L. (*Punicaceae*) was evaluated for *in vitro* antimicrobial activity against six different microorganisms. The minimum inhibitory concentration (MIC) is determined by inoculating the organism into a series of test wells that contain a standard amount of broth and serial dilutions of the antimicrobial agent being tested. Following a period of incubation, the wells are examined for growth. The MIC number is the lowest concentration of drug that inhibits growth of the pathogen. Usually, successful treatment of infection is achieved by merely inhibiting multiplication of the microorganism and relying on a healthy immune system. Thus, the assay evinced broad spectrum antimicrobial property; contents of the ethanolic extract were sufficient to inhibit the growth of all the tested microorganisms. Among the selected microorganism cultures, the preeminent antimicrobial activity was recorded against *S. aureus* and *S. epidermidis*, which presented significant MIC values (Lopes et al., 2013).

All the tested strains evidenced sensibility towards the crude extract, causing damage to the morphology, as irregularities on its superficies and cell wall. Moreover, microorganisms which are responsible for diarrhea and typhoid diseases (*S. aureus* and *E. coli*), wound infection (*S. aureus*, *E. coli* and *P. aeruginosa*), respiratory disorders (*S. aureus*, *K. pneumoniae* and *P. aeruginosa*) and skin infections (*S. aureus*, *S. epidermidis* and *C. albicans*) were inhibited considerably, which in turn, prove the local effect of the extract. These results are in accordance with previous studies that investigated the *in*

vitro antimicrobial activity of *P. granatum* L. (Moorthy et al., 2013).

On the other hand, the *ex vivo* assay permitted to confirm *S. aureus* susceptibility through MIC and MBC_{100} values of amoxicillin (Koneman et al., 1997). As observed in previous studies, the microbiological method was accurate enough to measure drugs concentrations (Mattos-Filho et al., 2006). This method has the same precision as high performance liquid chromatography (HPLC) assay and it has been widely used for determining this type of test samples concentrations (Charles and Chulavnatol, 1993). The period of seven days used for the development of granulomatous tissue in the present study is adequate. Different periods (7, 14, 21 and 28 days) were observed for development of granulomatous tissues in rats and it has been concluded that the period did not interfere with the pharmacokinetics of the drugs (Groppo et al., 2004).

While amoxicillin has an intestinal absorption through passive diffusion (Sugawara et al., 1989) and through oligopeptide transporter system present mainly in kidney and intestine (Moore et al., 2000), there are no data regarding the bioavailability of any pomegranate extracts. The oral ingestion of the crude extract of *P. granatum* L. did not present any inhibition zone during the *ex vivo* microbiological assay, since pomegranate phytocompounds must be metabolized during digestion, suggesting that the bioactive compounds that should have provided an *ex vivo* activity may not be the same as those identified in the extract (Johanningsmeier and Harris, 2011). Thus, it shall be explained by the action of enzymes, such as tannase, an extracellular enzyme produced by bacteria in the presence of tannic acid, which hydrolyzes esters and side connections of tannins (Battestin et al., 2004). Based on these facts, it is suggested that the crude extract from the fruits of *P. granatum* L. (G2) did not evidence *ex vivo* antimicrobial activity, since after the oral ingestion of the extract, bacteria present in the gastrointestinal tract of the animal, hydrolyze phytocompounds, which present antimicrobial potential in *in vitro* models, but do not present sufficient serum and tissue concentrations to exert this activity in *ex vivo* and presumably in *in vivo* models. Thus, the compositional analysis evidenced secondary metabolites with known antimicrobial action. The *in vitro* results obtained, in turn, were promising, because the tested strains demonstrated sensibility towards the crude extract, causing damage to the morphology, as irregularities on its superficies and cell wall. However, pomegranate phytocompounds did not present sufficient serum and tissue concentrations to exert this activity in the *ex vivo* model proposed. Presently, the crude extract from the fruits of *P. granatum* L., considered a promising source for alternative treatment requires *in vivo* and clinical studies to confirm its antimicrobial capacity, promoting scientists to continue studies on safety, efficiency and standard quality issues for *P. granatum* L. (*Punicaceae*).

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Full Length Research Paper

Hepatoprotective effect of *Caesalpinia crista* Linn. against CCl_4 and paracetamol induced hepatotoxicity in albino rats

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This study was done to investigate the hepatoprotective effect of extracts of *Caesalpinia crista* against carbon tetrachloride and paracetamol induced liver toxicity in albino rats. Seeds of *C. crista* were subjected to ethanolic and aqueous extraction. Albino rats were exposed to carbon tetrachloride (3 ml/kg rat b.w) and paracetamol (3 g/kg rat b.w) in two different protocols. Seven groups (n = 6) of animals were used in each protocol. Olive oil was used as vehicle. Rats treated with extracts of *Caesalpinia crista* exhibited a significant reduction in CCl_4 and paracetamol induced increase in serum levels of alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), total bilirubin and also caused a significant increase in serum level of total proteins which was decreased by hepato-toxic compounds used. The protective effect of these extracts was comparable with Silymarin. Ethanolic extract of *C. crista* was able to normalize the biochemical levels and histopathological changes which were altered due to CCl_4 and paracetamol intoxication.

Key words: Hepatotoxicity, *Caesalpinia crista*, paracetamol, CCl_4.

INTRODUCTION

Liver is one of the largest organs in human body and chief site for intense metabolism and excretion. It has a surprising role in the maintaining, performing and regulating homeostasis of the body (Ward et al., 1999). The liver disorders are a world problem (Bruck et al., 1996). Due to excessive exposure to hazardous chemicals the free radicals generated will be so high such that they overpower the natural defensive system leading to hepatic hepatic damage and causes jaundice, cirrhosis and fatty liver, which remain one of the serious health problems (Zimmerman and Hayman, 1976). Hepatotoxicity rate has been reported, much higher in developing countries for example like India (8 to 30%) as compared to advanced countries (2 to 3%) with a similar dose schedule (Sharma, 2004).

The Indian system of medicine (Ayurveda) recommends

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a number of medicinal preparations for the treatment of liver disorders as there is an absence of a reliable liver protective drug in the modern system of medicine (Chatterjee, 2000). Natural remedies from medicinal plants are considered to be effective and safe alternative treatments for hepatotoxicity has been reported to be much higher in developing countries like India (8 to 30%) compared to that in advanced countries (2 to 3%) with a similar dose schedule (Sharma, 2004).

Caesalpinia crista belonging to the plant Caesalpiniaceae is a medicinal plant growing widely throughout India and tropical countries of the world (Kirtikar et al., 1999). It is a large straggling and very thorny shrub. Traditionally, in Ayurveda, this plant was used for the treatment of gynecological disorders, constipation, piles, skin diseases and ulcers (Williamson, 2002). Most widely used part is seed kernel which is reported as a rich source of norcassane and norcassane type diterpenoids (Kalauni et al., 2005). The stem part and root part constitutes two novel peltogynoids, 6-methoxypulcherrimin and pulcherrimin and one novel homoisoflavonoid 8-methoxybonducellin (Cheenpracha et al., 2005). Its seeds are reported as antipyretic, anti-inflammatory, anthelmintic and antimalarial antidiuretic, antianaphylactic, antibacterial, antidiarrhoeal, antiameobic and antiviral properties (Dhar et al., 1968). It has been reported that the methanol extract of *C. crista* seed and seed kernel possess anti-feedant and anthelmintic property (Jabbar et al., 2007). So after extensive literature survey, the present study was designed to assess the hepatoprotective activity of the different extracts of seeds parts of *C. crista* against liver damage and to authenticate the data by using different models for analyzing the hepatoprotective activity.

MATERIALS AND METHODS

Chemicals

All the chemicals used in this research were of analytical grade and obtained from local market of Jaipur and assay kits for the estimation of biochemical factor were purchased from span diagnostics.

Plant

The seeds of *C. crista* were procured from the local market of Ropar, Punjab in the month of December, 2011. The seeds were authenticated by Mr. Madan Pal, Ex. Eng., Horticulture Division No.-2, Chandigarh.

Preparation of extract

Shade dried part (seeds) of plant were powdered (200 g) coarsely and firstly extracted with petroleum ether for defatting and then ethanol by Soxhlet apparatus for 72 h and aqueous extracted was prepared by maceration process. The obtained extracts were concentrated until dryness under reduced pressure and controlled temperature (40 to 50°C) using flash evaporator and then preliminary phytochemical screening were performed (Kokate et al., 2009). Percentage yield of all extracts were calculated. The % yield of ethanolic and aqueous extracts was found to be 8.7 and 13.3%. The LD_{50} determination of *C. crista* seed extract was reported by Kshirsagar (2011).

Experimental animals

Wistar albino rats weighing 180 to 200 g of either sex maintained under standard husbandry conditions at temperature 23 ± 2°C, relative humidity 55 ± 10% and 12 h light dark cycle. Animals were fed with standard laboratory food and water *ad libitum*. Experiments on animals were performed after taking the approval of experimental protocols by the institutional animal ethics committee under the registration no.IAEC/NIMS/PH/JPR/12/2011.

Active phytochemical constituents

Crude extracts (ethanolic and aqueous) of the plant *C. crista* contains phytosterols, alkaloids, triterpenoids, saponins, flavonoids, tannins, carbohydrates, reducing sugars, proteins, etc. (Harborne, 1973).

Experimental design

Hepatoprotective activity against CCl_4 induced hepatotoxicity

Animals were randomly divided into seven groups (n=6 animals in each group). The first group served as vehicle control (that is, olive oil - 1.5 ml/kg of rat b.w). The second group served as carbon tetrachloride (CCl_4) intoxicated control and received single oral administration of CCl_4 mixed with olive oil as vehicle in 1:1 ratio (3 ml/kg of rat body weight). The third group was given standard drug silymarin at a dose of 100 mg/kg and remaining groups were given two different extracts of both (ethanolic and aqueous) of *C. crista* at a dose of 100 and 200 mg/kg, respectively (group IV to VII) (Mir et al., 2011).

Hepatoprotective activity against paracetamol induced hepatotoxicity

Animals were divided into seven groups (n=6 animals in each group). The animals in group I served as vehicle control. Group II rats served as control and were administered with distilled water by oral administration of paracetamol (PCM) at a dose of 3 g/kg body weight, 1 h after distilled water administration. Group III served as standard and given silymarin at a dose of 100 mg/kg. The animals in group IV, V, VI and VII served as test groups and were treated orally with ethanol and aqueous extract of *C. crista* of 100 mg and 200 mg/kg body weight, once in a day for 10 days followed by a single oral administration of PCM (3 g/kg body weight), respectively, 1 h after extract administration. After 24 h of PCM administration on 10th day, rats of all groups were sacrificed by decapitation and the blood was collected by retro-orbital method (Ranawat et al., 2010).

Blood collection

Each animal was anaesthetized with diethyl ether. Blood was collected by retro-orbital method in a 5 ml disposable syringe and 2

Table 1. Effect of extracts of seeds of *Caesalpinia crista* on CCl_4-induced hepatotoxicity.

	Group	Aspartateamino Transferase (AST) (IU/L)	Alanineamino transferase (ALT) (IU/L)	Alkaline phosphatase (ALP) (IU/L)	Total protein (TP) (g/dl)	S. Bilirubin (mg/dl)
I	Vehicle Control	13.43±0.499	16.06±0.861	43.33±0.433	6.69±0.051	0.67±0.008
II	CCl_4+Vehicle	80.31±0.579$^{\#}$	92.49±1.723$^{\#}$	131.8±1.46$^{\#}$	2.33±0.132$^{\#}$	2.72±0.003$^{\#}$
III	CCl_4+Standard (Silymarin)	19.71±0.310***	23.12±0.469***	50.38±0.382***	6.29±0.154***	0.85±0.003***
IV	CCl_4+CCED (100 mg/kg)	34.57±0.746**	40.47±0.861**	69.4±1.345**	4.65±0.093**	1.68±0.003**
V	CCl_4+CCED (200 mg/kg)	27.64±0.348***	32.12±0.574***	58.73±4.076***	5.82±0.079***	1.32±0.003***
VI	CCl_4+CCAD (100 mg/kg)	55.71±0.809*	64.87±0.861*	74.87±6.418*	3.47±0.062*	2.29±0.036*
VII	CCl_4+CCAD (200 mg/kg)	36.5±0.635***	42.39±0.574***	62.1±1.408***	3.61±0.135***	1.79±0.005***

Values are expressed as mean ± SEM (n = 6); ***p ≤ 0.001when compared with CCl_4 control group, **p ≤ 0.01when compared with CCl_4 control group, *p ≤ 0.05when compared with CCl_4 control group, $^{\#}$p≤0.001when compared with vehicle control group, CCED (100 mg/kg))- *Caesalpinia crista* Ethanolic extract at a dose of 100 mg/kg, CCED (200 mg/kg)- *Caesalpinia crista* ethanolic extract at a dose of 200 mg/kg. CCAD (100 mg/kg) – *Caesalpinia crista*, aqueous extract at a dose of 100 mg/kg, CCAD (200 mg/kg) - *Caesalpinia crista* aqueous extract at a dose of 200 mg/kg.

ml blood was drawn very gently and slowly. The blood collected was immediately shifted to dried clean centrifugation tubes and allowed to clot then serum was separated by centrifugation at 3,000 rpm for 15 min. Serum was separated and then preserved in the cuvettes at -20°C in the freezer until analysis. Biochemical estimations were made the following day.

Assessment of liver function

Biochemical parameters like alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), serum bilirubin and total protein were analysed from the serum collected from the different animals of different groups.

Histopathology

The liver tissue was dissected out and fixed in 10% formalin solution, dehydrated in ethanol (50 to 100%), cleared in xylene and embedded in paraffin wax. 5 to 6 mm thick sections were prepared and then stained with hematoxylin and eosin dye for microscopic observations.

Statistical analysis

The significance of difference among the control group and various treated groups were analyzed by means of analysis of variance (ANOVA) with least significant difference (LSD) post hoc test used to compare the group means and $P < 0.05$ was considered statistically significant. The experimental results are represented as mean ± standard error mean (SEM). Statistical package for social sciences (SPSS) for windows (version 15.0, Chicago, IL, USA) was used for statistical analysis.

RESULTS AND DISCUSSION

Carbon tetrachloride is one of the most commonly used hepatotoxin in the experimental study of liver diseases and the hepatotoxic effects of it are largely due to its active metabolite, trichloromethyl radical (Ranawat et al., 2010). The activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids, which leads to the formation of lipid peroxides. This lipid peroxidative degradation of bio-membranes is one of the principle causes of hepatotoxicity of CCl_4 (Kaplowitz et al., 1986).

In the present study, it was observed that the administration of CCl_4 increased serum bilirubin decreased the levels of proteins and increased the levels of serum marker enzymes such as AST, ALT, ALP significantly ($P < 0.001$) which is an evidence of existence of liver toxicity when compared to normal group animals (Table 1). These elevated marker enzymes were brought back and the total protein levels were elevated in case of silymarin treated animals and was found to be highly significant ($P < 0.001$) and the effects of these can be easily seen in histopathology (Figure 1). *C. crista* at a dose of 200 mg/kg produced highly significant ($P < 0.001$) reduction in the elevated marker enzymes like ALT, AST, ALP and

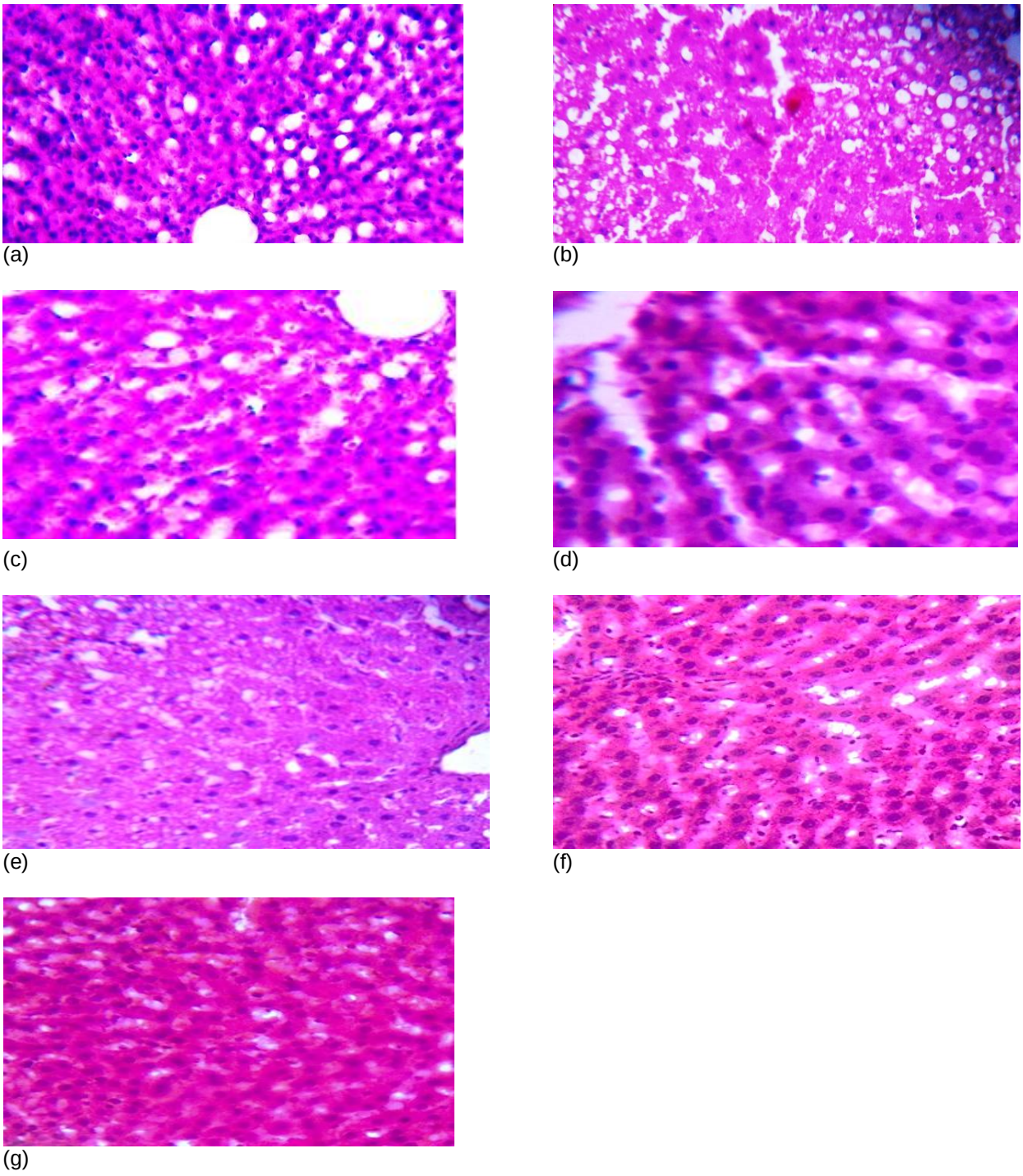

Figure 1. Effect of extracts of Caesalpinia crista Linn. on AST, ALT and ALP in CCl_4 induced hepatotoxicity.
(a) Normal Liver showing a normal portal triad, sinusoids, and arrangement of hepatocytes; (b) CCl_4 treated liver showing marked fatty changes around portal tract & Hepatocytes are laden with fat vacuoles; (c) Liver (CCl_4 + silymarin at the dose of 100mg/kg b.w.) showing normal appearing hepatocytes and no fatty change, or absence of fatty change in hepatocytes, there is also no necrosis; (d) Liver exposed to CCl_4 and pretreated with CCED at the dose 100mg/kg body wt. showing less fatty changes; (e) Liver exposed to CCl_4 and pretreated with CCED at the dose 200 mg/kg b.w, showing almost normal appearing hepatocytes. Fat vacuoles are seen only in some hepatocytes; (f) Liver exposed to CCl_4 and pretreated with CCAD at the dose 100 mg/kg body wt. showing moderate degree of fatty changes; (g) Liver exposed to CCl_4 and pretreated with CCAD at the dose 200 mg/kg body wt. showing mild degree of fatty changes. Histopathology of Liver (CCl_4 hepatotoxicity) (magnification 10×45).

Table 2. Effect of extracts of seeds of *Caesalpinia crista* on PCM-induced Hepatotoxicity.

	Group	Aspartateamino Transferase (AST) (IU/L)	Alanineamino Transferase (ALT) (IU/L)	Alkaline Phosphatase (ALP) (IU/L)	Total protein (TP) (g/dl)	S. Bilirubin (mg/dl)
I	Vehicle Control	13.2±0.407	15.74±0.551	36.21±0.262	6.69±0.012	0.68±0.012
II	PCM+Vehicle	67.1±0.13$^{\#}$	83.2±0.003$^{\#}$	109.9±0.927$^{\#}$	2.69±0.048$^{\#}$	2.63±0.048$^{\#}$
III	PCM+Standard (Silymarin)	22.2±0.006***	19.2±0.003***	41.8±0.195***	6.49±0.015***	0.87±0.015***
IV	PCM+CCED (100 mg/kg)	42.3±0.26**	25.66±0.002**	50.89±3.34**	4.64±0.027**	1.15±0.027**
V	PCM+CCED (200 mg/kg)	41.8±0.22***	23.21±0.002***	57.6±1.155***	5.48±0.021***	1.46±0.021***
VI	PCM+CCAD (100 mg/kg)	61.2±0.792*	31.36±0.003*	51.43±1.163*	3.79±0.037*	1.99±0.037*
VII	PCM+CCAD (200 mg/kg)	53.8±0.008***	28.8±0.003***	61.96±5.167***	4.5±0.029***	1.57±0.029***

Values are expressed as mean ± SEM (n = 6); ***p ≤ 0.0001when compared with PCM control group, **p ≤ 0.001 when compared with PCM control group, *p ≤ 0.01 when compared with PCM control group, $^{\#}$p ≤ 0.0001when compared with vehicle control group, CCED (100 mg/kg))- *Caesalpinia crista* ethanolic extract at a dose of 100 mg/kg, CCED (200 mg/kg)- *Caesalpinia crista* ethanolic extract at a dose of 200 mg/kg. CCAD (100 mg/kg) - *Caesalpinia crista*, aqueous extract at a dose of 100 mg/kg, CCAD (200 mg/kg) - *Caesalpinia crista* aqueous extract at a dose of 200 mg/kg.

bilirubin.

Paracetamol is a commonly and widely used analgesic antipyretic agent, but over doses of paracetamol deplete the normal levels of hepatic glutathione. Cytochrome P450 enzyme system metabolizes paracetamol and forms a minor but significant alkylating metabolite known as NAPQI (*N*-acetyl-*p*-benzo-quinone imine), which in turn is irreversibly conjugated with the sulfhydryl groups of glutathione (Jollow et al., 1973). Production of NAPQI (responsible for the toxic effects of paracetamol) is mainly because of two isoenzymes of cytochrome P450 (CYP2E1 and CYP1A2). Gene of cyp450 is highly polymorphic, however, an individual differences in paracetamol toxicity were believed to be due to a third isoenzyme that is, CYP2D6, which metabolises paracetamol into NAPQI to a lesser extent than other P450 enzymes, its activity may contribute to paracetamol toxicity in ultra rapid metabolizers and when it is taken at high and chronic doses. In the liver, the cytochrome P450 enzymes CYP2E1 and CYP3A4 were primarily responsible for the conversion of paracetamol to NAPQI which undergoes conjugation with glutathione. This in combination with direct cellular injury by NAPQI leads to cell damage and death (Wendel et al., 1979). Excess production of paracetamol metabolite causes the initial hepatic damage and subsequent activation of inflammatory mediator TNF-α which in turn contribute to tissue necrosis (Borne, 1995).

In the present investigation it was observed that the administration of paracetamol increased serum bilirubin, decreased the levels of proteins and increased the levels of serum marker enzymes like AST,ALT,ALP significantly ($P < 0.001$) which may be due to acute hepato-cellular damage and biliary obstruction (Table 2). Ethanolic extract of *C. crista* at a dose of 200 mg/kg produced highly significant ($P < 0.001$) reduction in the elevated marker enzymes like AST, ALP, ALT and serum bilirubin in a dose dependant manner and also by silymarin at a dose of 100 mg/kg. The difference in the effects of induction drug, standard drug and test drug at different dose level can be easily seen in histopathology of livers as shown in Figure 2.

In accordance with these results, it may be stated that tannins, saponins and flavonoids which are present in the seed extracts could be considered responsible for the hepatoprotective activity (Takate et al., 2010). Based on the results observed in this study, it is well evident that *C. crista* is pharmacologically effective for the treatment of liver disorders at a higher dose levels when compared to silymarin.

Conclusion

The present results provide strong evidence that ethanolic extract of seeds of *C. crista* inhibits

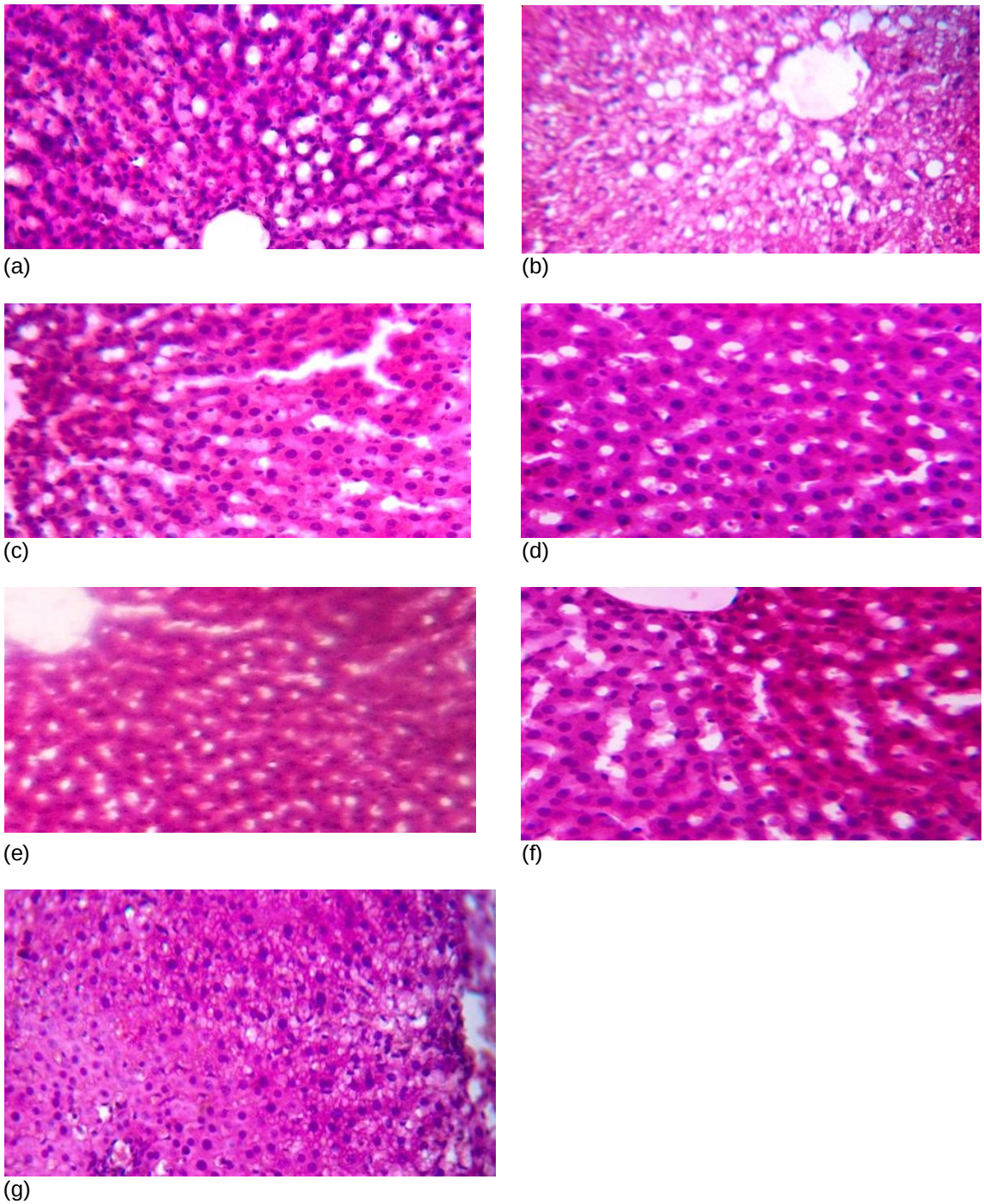

Figure 2. Effect of extracts of Caesalpinia crista Linn on paracetamol induced hepatotoxicity on rat liver (histopathology).
(a) Normal Liver showing a normal portal triad and normal arrangement of hepatocytes; (b) Paracetamol treated liver showing marked fatty changes around portal tract as well as around central vein. Hepatocytes are laden with fat vacuoles; (c) Liver exposed to PCM and pretreated with silymarin at the dose of 100mg/kg body wt, showing normal appearing hepatocytes and no fatty change, or absence of fatty change in hepatocytes; (d) Liver exposed to Paracetamol and pretreated with CCED at the dose 100mg/kg body wt. showing moderate degree of fatty changes; (e) Arrangement of hepatocytes is nearly similar as that in normal at dose of 200 mg/kg b.w; (f) Liver exposed to paracetamol and pre-treated with CCAD at a dose of 100 mg/kg showing very less effect; (g) Liver exposed to paracetamol and pretreated with CCAD at a dose of 200 mg/kg b.w. Histopathology of Paracetamol induced hepatotoxicity.

hepatotoxicity induced by carbon tetrachloride and paracetamol. The hepatoprotective action was much more significant at the dose of 200 mg/kg when compared to 100 mg/kg. So, further research work is required to isolate the compound responsible for this activity.

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