Pharmacological Reports
2013, 65, 761–767
ISSN 1734-1140

Short communication

Effect of anhydrosophoradiol-3-acetate of *Calotropis gigantea* (Linn.) flower as antitumoric agent against Ehrlich's ascites carcinoma in mice

Muhammad R. Habib, Muhammad R. Karim

Department of Biochemistry and Molecular Biology, Faculty of Science, Rajshahi University, Rajshahi-6205, Bangladesh.

Correspondence: Muhammad R. Karim, e-mail: rezaplazad@yahoo.com

Abstract:
Background: Over 60% of currently used anti-cancer agents are derived in one-way or another from natural sources, including plants, marine organisms and microorganisms. *Calotropis gigantea* (Linn.) (Family: Asclepiadaceae) is a perennial shrub and it is used as a traditional folk medicine for the treatment of various health complications. But there is no report on isolation of anticancerous chemicals from the flower of *Calotropis gigantea*. The objective of the present study is to explore the antitumor effect of anhydrosophoradiol-3-acetate (A3A), isolated from the flower of *Calotropis gigantea* (Linn.) against Ehrlich's ascites carcinoma (EAC) in Swiss albino mice.
Methods: Antitumoric effect of A3A was assessed by evaluating viable tumor cell count, survival time, body weight gain due to tumor burden, hematological and biochemical (glucose, cholesterol, triglyceride, blood urea, SALP, SGPT and SGOT) parameters of EAC bearing host at doses of 10 and 20 mg/kg body weight.
Results: Treatment with A3A decreased the viable tumor cells and body weight gain thereby increasing the life span of EAC bearing mice. A3A also brought back the altered hematological (Hb, total RBC and total WBC) and biochemical parameters more or less to normal level.
Conclusion: Results of this study conclude that *in vivo* the A3A was effective in inhibiting the growth of EAC with improving in cancer induced complications.

Key words:
anhydrosophoradiol-3-acetate, antitumor, *Calotropis gigantea*, Ehrlich's ascites carcinoma, flower, mice

Introduction

Despite the great development of organic synthesis, currently 25% of prescribed drugs worldwide are derived from plant sources. Plant species are still an important source of new drugs for diseases that continue to lack a cure, such as cancer [21, 31]. *Calotropis gigantea* (Linn.) (Family: Asclepiadaceae) is a moderate to large sized perennial shrub and widely distributed in tropical region and most abundant in Bangladesh, India, Burma and Pakistan [9]. In folklore remedy, this plant was used in the treatment of abdominal tumors, boils, syphilis, tuberculosis, leprosy, skin diseases, piles, wounds, rheumatism and insect bites. Root bark is considered to be purgative, alterative, diaphoretic, digestive and emetic whereas flowers are stomachic, digestive and tonic. Powdered flowers, in small doses, are useful in the treatment of colds, coughs, asthma, catarrh, indigestion and loss of

appetite [23]. In earlier screening, *Calotropis gigantea* was shown to possess antipyretic [5], analgesic [26], anticonvulsant [2], anxiolytic [2], sedative [2], hepatoprotective [20], wound healing [8], antidiabetic [29], larvicidal [27], anti-inflammatory [1] and anti-diarrheal [6] activities. Several phytochemicals have been isolated from *Calotropis gigantea* and they include cytotoxic cardenolides [18, 19, 33], antifeedant nonprotein amino acid [25], naphthalene and terpene derivatives [10], flavonol glycosides [34], pregnanes [17, 35, 39], ursane-type triterpenoids [23] and steroids [12, 37]. However, no investigation is still done on isolation of anticancerous chemicals from the flower of *Calotropis gigantea*. We herein report the *in vivo* antitumor activity of isolated anhydrosophoradiol-3-acetate (A3A) from *Calotropis gigantea* flower against Ehrlich ascites carcinoma (EAC) cells in mice.

Materials and Methods

Plant material

The flowers of *Calotropis gigantea* were collected from the relevant area (Meherchandi) of Rajshahi University Campus, Rajshahi, Bangladesh. The plant specie was authenticated by Professor A.T.M. Naderuzzaman, Department of Botany, Rajshahi University and a voucher specimen (No. 1A.Alam, Collection date 15.08.2004) was kept in the Department of Botany, Rajshahi, University.

Extraction and isolation

The shed-dried powdered flower (1.0 kg) of *Calotropis gigantea* was extracted with ethyl acetate (E. Merck, Germany) at room temperature. The solvent was completely removed by rotary vacuum evaporator from the crude extract to yield a residue of 38 g. Then crude ethyl acetate extract (15 g) was applied on silica gel (60–120 mesh) chromatography using n-hexane (E. Merck, Germany) with a gradient of ethyl acetate up to 100% and followed by chloroform (E. Merck, Germany). Sixty four fractions were collected. Among these fractions, fraction 21–30 afforded compound 1 as white crystals (275 mg) which were preserved in a vacuum desiccator at 25°C. The purity of the isolated compounds was checked on TLC plates.

General methods

High resolution TOF mass spectra were obtained using a Waters LCT Premier mass spectrometer (UK) coupled with a Waters AQUITY HPLC system, with data acquisition achieved using MassLynx software, version 4.0. NMR spectra were recorded on Bruker 400 MHz FT spectrometer (DPX-400, Switzerland). All the spectra were taken in Analytical Research Division, Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratories, Dhaka-1205, Bangladesh.

Experimental animals

Male Swiss albino mice (25–30 g) were procured from the Animal Research Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR,B). The animals were housed in air-conditioned (25 ± 2°C) room with controlled lighting (from 6.00 a.m to 8.00 p.m). The animals were fed on pelleted food and tap water was available *ad libitum*. The animals were acclimatized to the laboratory for at least 5 days before testing. The experiments were carried out after approval of the protocol by the Institutional Ethics Committee for Experimentations on Animal, Human, Microbes and Living Natural Sources (225/320-IAMEBBC/IBSc), Institute of Biological Sciences, University of Rajshahi, Bangladesh.

Tumor cells

EAC cells were obtained by the courtesy of Indian Institute for Chemical Biology (IICB), Kolkata, India and were maintained by weekly intraperitoneal (*ip*) inoculation of 10^5 cells/mouse in the laboratory.

Acute toxicity study

For dose selection, male Swiss albino mice were divided into four groups (n = 6). After 2 h of a single *ip* administration of A3A on the animals of group 1, 2 and 3 at 25, 50 and 100 mg/kg doses did not show any gross changes in behavioral, neurological and autonomic profiles when compared with untreated control (group 4) and no mortality was observed after 24 h. Then to optimize the dose levels, 10 and 20 mg/kg body weight (b.w.) were selected for the evaluation of A3A.

Effect on EAC cell growth

In order to determine the effect of compound 1 on EAC cell growth, 24 mice were randomly divided into four groups (6 animals in each group) and for therapeutic evaluation, the mice of all groups were inoculated with 1.5×10^5 cells/mouse on the day first. After 24 h of tumor inoculation treatment was started and continued for 5 days. The mice in group 1, 2, 3 and 4 received intraperitoneally 2% v/v dimethyl sulfoxide (DMSO), compound 1 (10 and 20 mg/kg b.w.) and standard drug, bleomycin (Biochem Pharmaceutical, India; 0.3 mg/kg b.w.), respectively. On day 6 after tumor transplantation, animals were sacrificed. Tumor cells were collected by repeated washing with 0.9% saline and viable tumor cells per mouse of the treated group were compared with EAC control (i.e., group 1) [13].

Effect on survival time

Mice in four groups (6 animals per group) were inoculated with 1.5×10^5 cells/mouse on the day 0. After 24 h of inoculation, mice in group 1, 2, 3 and 4 were treated (*ip*) with 2% v/v DMSO, compound 1 (10 and 20 mg/kg) and bleomycin (0.3 mg/kg), respectively, and continued for 10 days. The mice in group 1 were considered as EAC control. The average b.w. changes and mean survival time of each group were noted. The mean survival time (MST) of the treated groups was then compared with that of the control group and percentage increase in life span (%ILS) was also calculated [28].

Effect on hematological and biochemical parameters

Swiss Albino mice were divided into five groups (n = 6). All the animals were injected with EAC cells (2×10^5 cells/mouse) intraperitoneally except for the normal group. This was taken as day 0. Group 1 served as the normal control and group 2 served as the EAC control. These two groups received 2% DMSO. Group 3 and 4 were treated with compound 1 at 10 and 20 mg/kg b.w., respectively. Group 5, which served as the positive control, was treated with bleomycin at 0.3 mg/kg b.w. All these treatments were given 24 h after the tumor inoculation, once daily for 10 days. Six mice from each group were sacrificed on 12^{th} day after tumor inoculation for the study of hematological and biochemical parameters. Hematological parameters (hemoglobin, RBC, WBC and differential count of WBC) were measured from freely flowing tail vein blood of each mice of each group [24]. Then, every mouse was sacrificed and blood was collected by cardiac puncture. Serum was separated by centrifugation at 4000 rpm for 10 min and analyzed for glucose, total cholesterol, urea, triglyceride, serum alkaline phosphatase (SALP), serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) in an bioanalyzer (Microlab 200) using commercial kits (Atlas Medica, UK).

Statistical analysis

All values were expressed as the mean ± SEM (standard error of the mean). Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Dunnett's 't' test using SPSS statistical software of 15 version; $p < 0.05$ was considered to be statistically significant.

Results and Discussion

Isolated and purified compound **1** was characterized by mass and NMR spectral data. Its EI-MS showed a molecular ion (M^+) peak at m/z 466.4023. Its ^{1}H-NMR spectrum exhibited three olefinic proton at δ 5.34, δ 5.14 and δ 5.12 ppm and an acetyl methyl proton at δ 2.04 ppm. The ^{13}C-NMR showed an acetyl carbonyl carbon at δ 171.01 ppm, four olefinic carbons at δ 122.5 (C-12), δ 143.75 (C-13), δ 139.67 (C-21), δ 124.37 (C-22) ppm and acetyl methyl carbon at 15.76 ppm.

Compound **1**: White crystals; IR (KBr, cm^{-1}): 2979, 1735, 1456, 1365, 1022. ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 0.86 (3H, s, H-30), 0.87 (3H, s, H-29), 0.91 (3H, s, H-28), 0.97 (3H, s, H-27), 0.98 (3H, s, H-26), 1.06 (3H, s, H-25), 1.13 (3H, s, H-24), 1.25 (3H, s, H-23), 1.65 (1H, s, H-7), 1.66 (2H, s, H-6), 1.67 (2H, s, H-2), 1.67 (1H, s, H-5), 1.89 (2H, m, H-19), 1.90 (2H, m, H-15), 1.92 (2H, m, H-11), 1.97 (2H, d, J = 11.5 Hz, H-16), 2.00 (1H, s, H-9), 2.01 (2H, d, J = 11.5 Hz, H-1), 2.04 (3H, s, -CO--CH_3), 4.50 (1H, t, J = 11.6 Hz, H-3), 5.12 (1H, t, J = 3.5 Hz, H-12), 5.18 (1H, t, J = 3.5 Hz, H-21), 5.34 (1H, t, J = 3.5 Hz, H-22). ^{13}C-NMR (150 MHz, $CDCl_3$, δ, ppm): 15.7 (-CO-CH_3), 16.7 (C-25), 16.9

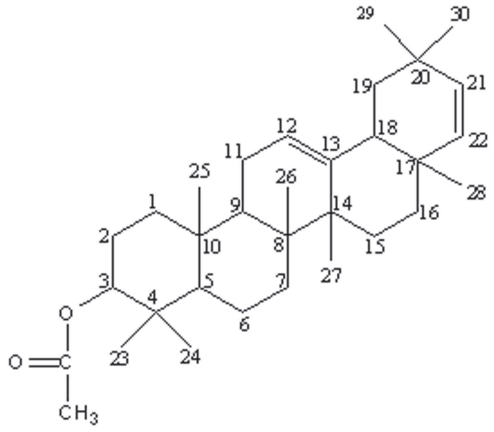

Fig. 1. Chemical structure of anhydrosophoradiol-3-acetate (A3A)

(C-24), 17.5 (C-26), 17.6 (C-27), 18.2 (C-6), 21.3 (C-30), 21.4 (C-29), 23.2 (C-23), 23.4 (C-2), 23.6 (C-11), 23.6 (C-28), 28.1 (C-1), 28.1 (C-4), 28.7 (C-10), 29.7 (C-7), 32.9 (C-15), 36.8 (C-17), 38.5 (C-16), 39.6 (C-8), 40.0 (C-14), 41.5 (C-19), 41.5 (C-20), 42.1 (C-18), 47.7 (C-9), 55.3 (C-5), 81.0 (C-3), 122.5 (C-12), 124.3 (C-22), 139.6 (C-21), 143.7 (C-13), 171.0 (-CO-CH_3). EI-MS (m/z): 466.4 (M^+), 443.3, 409.3, 391.3.

Based on the foregoing observations and a comparison of the above data with the literature [16], compound **1** was determined to be anhydrosophoradiol-3-acetate (A3A) (Fig. 1). Isolation of A3A is reported for the first time from this plant.

The effects of A3A at the doses of 10 and 20 mg/kg on survival time, viable tumor cell count and body weight gain due to tumor burden are shown in Figure 2. The prolongation of life span is a reliable criterion for judging efficacy of anticancer drugs [7] and the A3A isolated from flower of *Calotropis gigantea* were able to meet this criterion through reducing the viable EAC cells. Treatment with A3A at the doses of 10 and 20 mg/kg significantly ($p < 0.05$) reduced viable tumor cell count and b.w. gain in a dose-dependent manner as compared to that of the EAC control group (Fig. 2A and Fig. 2D). In the EAC control group, the mean survival time was 19.8 ± 1.40 days, while it increased to 25.6 ± 1.58 (10 mg/kg), and 31.0 ± 0.96 (20 mg/kg) days ($p < 0.05$), respectively, in the A3A-treated groups. The standard drug bleomycin (0.3 mg/kg)-treated group had a mean survival time of 39.0 ± 0.85 days (Fig. 2B). The percentage increase in the lifespan (%ILS) of tumor-bearing mice treated with A3A (20 mg/kg) was found to be

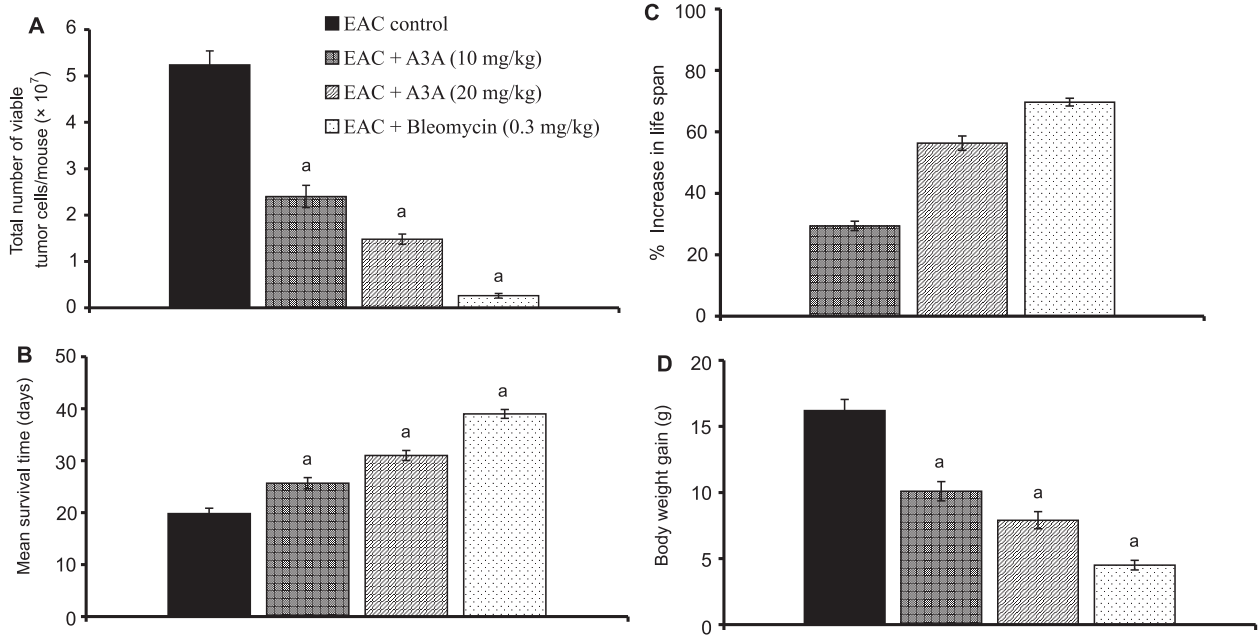

Fig. 2. Effect of A3A on EAC cell bearing mice. **A:** Viable EAC cells on day 6 after tumor cell inoculation, **B:** Mean survival time, **C:** % Increase in life span and **D:** Body weight on day 12. Data are expressed as the mean ± SEM (n = 6); [a] $p < 0.05$: between EAC control and A3A-treated group

56.32% as compared to the control group whereas it was 96.97% for bleomycin (0.3 mg/kg) (Fig. 2C).

Myelosuppression and anemia (reduced hemoglobin) have been frequently observed in ascites carcinoma [14, 22]. Anemia encountered in ascites carcinoma mainly due to iron deficiency, either by hemolytic or myelopathic conditions which finally lead to reduced RBC number [14]. In this study, elevated WBC count, reduced hemoglobin and RBC count were observed in EAC control mice. Administration of A3A at 20 mg/kg significantly ($p < 0.05$) reduced total WBC count in respect to that of EAC control group. RBC count and hemoglobin content were found to be significantly restored to the normal levels in the animals treated with A3A (10 and 20 mg/kg) as well as standard drug bleomycin (0.3 mg/kg) (Tab. 1). The restoring capability of A3A on altered hemoglobin, total RBC and WBC count has indicated its hematopoietic protecting activity without inducing myelotoxicity, the most common side effects of cancer chemotherapy. A3A did not show any significant efficacy to bring the altered differential count of WBC.

Numerous studies on the enzymes of carbohydrate metabolism in cancer showed that actively dividing neoplastic tissues require more energy than normal cells [32]. The consequent display of a high rate of glycolysis in malignant conditions is clinically manifested in the increased activity of several serum enzymes [3, 30]. In our study, twelve days of inoculation with EAC brought the significant elevation in the levels of SGOT and SALP (Tab. 1). Treatment with A3A restored the elevated biochemical parameters more or less to normal range thereby supporting its protective effect on the tumor induced complications.

The development of hypoglycemia and hyperlipidemia in experimental animals with carcinoma has been previously reported [15, 36]. In this experiment, reduced glucose level and elevated cholesterol and triglycerides were returned more or less to normal level in drug treated mice (Tab. 1). Inoculation and progression of EAC proliferation in mice have also an association with some extent of kidney damage and elevation of blood urea [4]. A3A reduced the elevated level of blood urea.

A3A was a pentacyclic terpenoid and *in vitro* it exhibited potent cytotoxicity against A549, SK-OV-3, SK-MEL-2, MES-SA and HCT-15 tumor cell lines [16]. Generally pentacyclic triterpenoids exert their antitumor activity through apoptosis induction. They were associated with early disruption of the endoplasmic reticulum and alterations in calcium homeostasis which are early events in activation of caspases [11, 38]. In our previous study, we reported the potent inhibition of Ehrlich's ascites carcinoma by ethyl ace-

Tab. 1. Effect of A3A on hematological and biochemical parameters of EAC cell bearing mice

Parameters	Normal	EAC control	EAC + A3A (10 mg/kg)	EAC + A3A (20 mg/kg)	EAC + Bleomycin (0.3 mg/kg)
Hgb (g/dl)	12.2 ± 0.55	5.8 ± 0.53[a]	7.9 ± 0.47	9.6 ± 0.58[b]	15.8 ± 0.25[b]
RBC($\times 10^9$ cells/ml)	5.4 ± 0.28	2.5 ± 0.27[a]	3.6 ± 0.27[b]	4.2 ± 0.24[b]	5.16 ± 0.09[b]
WBC($\times 10^6$ cells/ml)	9.1 ± 1.57	36.1 ± 3.89[a]	27.6 ± 3.05	21.3 ± 2.07[b]	8.62 ± 0.59[b]
Lymphocytes (%)	70.0 ± 1.82	30.3 ± 1.83[a]	38.5 ± 1.76	36.3 ± 2.21	69.4 ± 0.90[b]
Neutrophils (%)	25.5 ± 1.64	64.1 ± 1.64[a]	56.3 ± 1.54	58.5 ± 2.32	26.1 ± 0.93[b]
Monocytes (%)	2.6 ± 0.49	4.3 ± 0.80	3.83 ± 0.47	3.83 ± 0.47	2.00 ± 0.27
Glucose(mg/dl)	142.3 ± 2.74	76.9 ± 2.87[a]	103.9 ± 5.02[b]	108.0 ± 4.02[b]	140.5 ± 1.21[b]
Cholesterol (mg/dl)	109.3 ± 2.50	183.7 ± 3.68[a]	155.4 ± 5.87	141.9 ± 4.46[b]	120.4 ± 0.71[b]
Triglyceride (mg/dl)	125.4 ± 1.86	179.6 ± 4.09[a]	173.1 ± 2.92	150.2 ± 3.81[b]	126.7 ± 1.28[b]
Blood Urea (mg/dl)	27.3 ± 1.93	85.1 ± 3.48[a]	75.5 ± 2.08	42.9 ± 1.66[b]	30.5 ± 0.52[b]
ALP (U/l)	122.2 ± 4.28	234.6 ± 2.38[a]	196.0 ± 3.36[b]	180.3 ± 3.75[b]	129.6 ± 0.72[b]
SGPT (U/l)	67.0 ± 2.33	73.3 ± 2.70	68.5 ± 3.12	70.5 ± 2.18	73.3 ± 0.36
SGOT (U/l)	39.3 ± 0.84	229.0 ± 3.75[a]	216.0 ± 4.61	197.3 ± 4.17[b]	94.2 ± 0.78[b]

Data are expressed as the mean ± SEM for six animals in each group. [a] $p < 0.001$: against normal group and [b] $p < 0.05$: against EAC control group

tate extract from the flower of *Calotropis gigantea* in mice [13]. In this study, we demonstrated *in vivo* the remarkable antitumor activity of anhydrosophoradiol-3-acetate of *Calotropis gigantea* flower against EAC but the mechanisms of action should be further addressed.

Conclusion

The overall findings of this study indicate that anhydrosophoradiol-3-acetate (A3A) of *Calotropis gigantea* flower has noteworthy antitumoric effects that might be a source of herbal drugs in respective therapeutic area and it also contributes in the proper use of this medicinal plant for better health care system of common people in Bangladesh.

Acknowledgments:
The authors are thankful to Indian Institute for Chemical Biology (IICB), Kolkata, India for providing Ehrlich ascites carcinoma (EAC) cells to carry out the research. The authors are also grateful to the University Grant Commission (UGC), Bangladesh for their financial support (662-5/52/UGC research grant/science/2010) in this research work.

References:

1. Adak M, Gupta JK: Evaluation of anti-inflammatory activity of *Calotropis gigantea* (AKANDA) in various biological systems. Nepal Med Coll J, 2006, 8, 156–161.
2. Argal A, Pathak AK: CNS activity of *Calotropis gigantea* roots. J Ethnopharmacol, 2006, 106, 142–145.
3. Bacci G, Picci P, Ferrari S: Prognostic significance of serum alkaline phosphatase measurements in patients with osteosarcoma treated with adjuvant or neoadjuvant chemotherapy. Cancer, 1993, 71, 1224–1230.
4. Barar FSK: Essentials of Pharmacotherapeutics, S. Chand and Company Ltd., New Delhi, India, 2004.
5. Chitme HR, Chandra R, Kaushik S: Evaluation of antipyretic activity of *Calotropis gigantea* (Asclepiadaceae) in experimental animals. Phytother Res, 2005, 19, 454–456.
6. Chitme HR, Chandra R, Kaushik S: Studies on antidiarrhoeal activity of *Calotropis gigantea* R.Br. in experimental animals. J Pharm Pharm Sci, 2004, 7, 70–75.
7. Clarkson D, Burchneal JH: Preliminary screening of antineoplastic drugs. Prog Clin Cancer, 1965, 1, 625–629.
8. Deshmukh PT, Fernandes J, Atul A, Toppo E: Wound healing activity of *Calotropis gigantea* root bark in rats. J Ethnopharmacol, 2009, 125, 178–81.
9. Ghani A: Medicinal Plants of Bangladesh, 2nd edn., Asiatic Society of Bangladesh, Dhaka, Bangladesh, 2003.
10. Gupta J, Ali M: Rare chemical constituents from *Calotropis gigantea* roots. Indian J Pharm Sci, 2000, 62, 29–32.
11. Gurfinkel DM, Chow S, Hurren R, Gronda M, Henderson C, Berube C, Hedley DW, Schimmer AD: Disruption of the endoplasmic reticulum and increases in cytoplasmic calcium are early events in cell death induced by the natural triterpenoid Asiatic acid. Apoptosis, 2006, 11, 1463–1471.
12. Habib MR, Nikkon F, Rahman M, Haque ME, Karim MR: Isolation of stimasterol and β-sitosterol from methanolic extract of root bark of *Calotropis gigantea* (Linn). Pak J Biol Sci, 2007, 10, 4174–4176.
13. Habib MR, Aziz AA, Karim MR: Inhibition of Ehrlich's ascites carcinoma by ethyl acetate extract from the flower of *Calotropis gigantea* L. in mice. J Appl Biomed, 2010, 8, 47–54.
14. Hogland HC: Haematological complications of cancer chemotherapy. Semin Oncol, 1982, 9, 95–102.
15. Killington RA, Williams AE, Ratchffe NA, Whitehead TP: Hypoglycemia in rats bearing ascites tumor. Br J Cancer, 1991, 25, 93–102.
16. Kim M, Lee H, Hahm K, Moon Y, Woo E: Pentacyclic triterpenoids and their cytotoxicity from the stem bark of *Styrax japonica* S. et Z. Arch Pharm Res, 2004, 27, 283–286.
17. Kitagawa I, Zhang RS, Park JD, Baek NI, Takeda Y, Yoshikawa M, Shibuya H: Indonesian medicinal plants. I. Chemical structures of calotroposides A and B, two new oxypregnane-oligoglycosides A and B, from the root of *Calotropis gigantea* (Asclepiadaceae). Chem Pharm Bull, 1992, 40, 2007–2013.
18. Kiuchi F, Fukao Y, Maruyama T, Obata T, Tanaka M, Sasaki T, Mikage M et al.: Cytotoxic principles of a Bangladeshi crude drug, akond mul (roots of *Calotropis gigantea* L.). Chem Pharm Bull, 1998, 46, 528–530.
19. Lhinhatrakool T, Sutthivaiyakit S: 19-Nor- and 18, 20-epoxy-cardenolides from the leaves of *Calotropis gigantea*. J Nat Prod, 2006, 69, 1249–1251.
20. Lodhi G, Singh HK, Plant KK, Hussain Z: Hepatoprotective effects of *Calotropis gigantea* extract against carbon tetrachloride induced liver injury in rats. Acta Pharm, 2009, 59, 89–96.
21. Łapiński Ł, Orzechowska-Juzwenko K, Wiela-Hojeńska A, Wołowiec D, Ganczarski G, Głowacka K, Czarnik-Matusewicz H et al.: Influence of anticancer therapy on oxidation phenotype and acetylation phenotype in patients with acute myeloblastic leukemia. Pharmacol Rep, 2011, 63, 149–156.
22. Maseki M, Nishiagaki I, Hagishara M, Tamoda Y, Yagi K: Lipid peroxidation levels and lipid content of serum lipoprotein fractions of pragnent subjects with or without pre-eclampsia. Clin Chim Acta, 1981, 41, 424–26.
23. Mueen AKK, Rana AC, Kixit VK: Calotropis species (Asclepiadaceae)-A comprehensive review. Pharmacogn Mag, 2005, 1, 48–52.

24. Mukherjee KL: Medical Laboratory Technology, Tata McGraw Hill Publishing Company Ltd., New Delhi, India, 1988.
25. Pari K, Rao PJ, Devakumar C, Rastogi JN: A novel insect antifeedant nonprotein amino acid from *Calotropis gigantea*. J Nat Prod, 1998, 61, 102–104.
26. Pathak AK, Argal A: Analgesic activity of *Calotropis gigantea* flower. Fitoterapia, 2007, 78, 40–42.
27. Rahuman AA, Bagavan A, Kamaraj C, Saravanan E, Zahir AA, Elango G: Efficacy of larvicidal botanical extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitol Res, 2009, 104, 1365–1372.
28. Rajkapoor B, Jayakar B, Murugesh N: Antitumor activity of *Bauhinia variegain* against Ehrlich ascites carcinoma induced mice. Pharm Biol, 2003, 41, 604–607.
29. Rathod NR, Raghuveer I, Chitme HR, Chandra R: Free radical scavenging activity of *Calotropis gigantea* on streptozotocin-induced diabetic rats. Indian J Pharm Sci, 2009, 71, 615–21.
30. Sagman U, Maki E, Evans WK: Small cell carcinoma of the lung-derivation of a prognostic staging system. J Clin Oncol, 1991, 9, 1639–1649.
31. Samuel JL: Scholar research library. Der Pharm Lett, 2010, 2, 10–15.
32. Scott SC, Davey M, Hamilton A, Norfolk DR: Serum enzyme concentrations in untreated acute myeloid leukemia. Blut, 1986, 52, 297–304.
33. Seeka C, Sutthivaiyakit S: Cytotoxic cardenolides from the leaves of *Calotropis gigantea*. Chem Pharm Bull, 2010, 58, 725–728.
34. Sen S, Sahu NP, Mahato SB: Flavonol glycosides from *Calotropis gigantea*. Phytochemistry, 1992, 31, 2919–2921.
35. Shibuya H, Zhang R, Park JD, Back NI, Takeda Y, Yoshikawa M, Kitagawa I: Chemical structures of Calotroposides C, D, E, F and G, five additional new oxypregnane-oligoglycosides from the root of *Calotropis gigantea* (Asclepiadaceae). Chem Pharm Bull, 1992, 40, 2647–2653.
36. Silverstein H, Dervot K, Oscar D: Studies on carbohydrate metabolism and different types of tumors bearing animals. Lancet, 1988, 22, 40–45.
37. Singh S, Bharti N, Chugh M, Naqvi F, Azam A: Activity of extracts and procesterol from *Calotropis gigantea* against *Entamoeba histolytica*. Nat Prod Commun, 2010, 5, 867–868.
38. Wang SR, Fang WS: Pentacyclic triterpenoids and their saponins with apoptosis-inducing activity. Curr Top Med Chem, 2009, 9, 1581–1596.
39. Zhu-Nian W, Mao-Yuan W, Wen-Li M, Zhuang H, Hao-Fu D: A new cytotoxic pregnanone from *Calotropis gigantea*. Molecules, 2008, 13, 3033–3039.

Received: August 19, 2012; **in the revised form:** December 28, 2012; **accepted:** January 11, 2013.