Teratogenic Effects of Ethanol Extract of Soursop Leaves (Annona muricata Folium) on Mouse (Mus musculus) Fetus

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Abstract

Background: Soursop leaf ethanol extract (Annona muricata (L) Folium) contains acetogenins which are cytotoxic and have the ability to halt cell growth. This study aimed to understand whether acetogenins have teratogenic effects on mice fetus (Mus musculus).

Methods: This study was performed at the Pharmacology and Therapy Laboratory of the Faculty of Medicine, Universitas Padjadjaran, Bandung, between October and November 2012. The study was an experimental laboratory study utilizing 27 pregnant mice which were divided into 3 groups. The first group was the negative control, the second was given soursop leaf ethanol extract at pre-implantation phase (day 1 to 5) and the third had the extract provided in the organogenesis phase (day 6 to 15). Laparotomy was performed on the 19th day of pregnancy. The parameters used were the number of implantation, the number of live and dead or resorbed fetus, the weight and length of the fetus, as well as the macroscopic external morphology abnormalities. The data gained from test subjects were compared to those of the control group. The statistical test used was the normality tes with the Kolmogorov-Smirnov method which was then followed by T-test or Mann-Whitney statistical tests.

Results: The experiment exhibited significant differences in the weight and length of the fetus (p-value 0.000), proving that soursop leaf ethanol extract could inhibit intrauterine growth. Aside from that, external morphological abnormalities such as hemorrhage on the head, face, neck, back, forelimbs, hindlimbs, and microcephaly were also found.

Conclusion: The soursop leaf ethanol extract (Annona muricata (L) Folium) has a teratogenic effect on mouse (Mus musculus) fetus. [AMJ.2014;1(1):48–53]

Key words: Acetogenins, Soursop leaf ethanol extract, Teratogenic

Efek Teratogenik Ekstrak Etanol Daun Sirsak (Annona muricata Folium) terhadap Fetus Mencit (Mus musculus)

Abstrak

Latar belakang: Ekstrak etanol daun sirsak (Annona muricata Folium) mengandung senyawa aktif Acetogenins yang bersifat sitotoksik atau dapat menghambat pertumbuhan sel. Penelitian ini dilakukan untuk mengetahui efek teratogenik Acetogenins terhadap fetus mencit (Mus musculus).

Metode: Penelitian ini dilakukan di Laboratorium Farmakologi dan Terapi Fakultas Kedokteran Universitas Padjadaran periode Oktober–November 2012. Penelitian bersifat eksperimental laboratorik dengan metode sampel acak menggunakan 27 ekor mencit hamil yang dibagi dalam 3 kelompok. Kelompok 1 sebagai kontrol negatif, kelompok 2 diberikan ekstrak etanol daun sirsak pada masa praimplantasi (hari ke-1 sampai ke-5), dan kelompok 3 menerima ekstrak pada masa organogenesis (hari ke-6 sampai ke-15). Laparotomi dilakukan pada hari ke-19 kehamilan. Parameter yang digunakan berupa jumlah hasil implantasi, jumlah fetus hidup, jumlah fetus mati atau resorbsi, berat dan panjang badan fetus, serta abnormalitas morfologi eksternal fetus yang dinilai secara makroskopis. Data yang didapat pada kelompok perlakuan dibandingkan dengan kelompok kontrol. Uji statistik yang digunakan adalah uji normalitas data dengan uji Kolmogorov-

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Smirnov dan dilanjutkan dengan uji T- atau Mann-Whitney.

Hasil: Hasil penelitian menunjukkan perbedaan signifikan pada berat dan panjang fetus mencit (nilai p 0,000), sehingga ekstrak etanol daun sirsak terbukti menghambat pertumbuhan intrauteri. Selain itu ditemukan juga kelainan morfologi eksternal seperti perdarahan pada kepala, wajah, leher, punggung, tangan, kaki, dan mikrosefali.

Simpulan: Ekstrak etanol daun sirsak (Annona muricata Folium) memiliki efek teratogenik terhadap fetus mencit (Mus musculus).

Kata kunci: Acetogenins, ekstrak etanol daun sirsak, teratogenik

Introduction

The phenomenon of "Back to Nature" trend in the society, in which herbal or traditional drug consumption is increasing, currently draws a lot of attention.[1] The medical professionals cannot turn a blind eye to this trend. The increasing use of herbal traditional medicines is not unrelated to the increasing trend of diseases in the last decade.

The World Health Organization (WHO) recorded that at least 6 million people die each year because of cancer. One of the anti-cancer herbal medicine which is widely used in the society during the last years was Soursop leaf (Annona muricata (L) Folium). The soursop leaf extract contains Acetogenins, which is regarded as an anti-cancer compound as this compound is cytotoxic to cancer cells by inhibiting their proliferation.[2] Acetogenins is highly penetrating. Aside its role as an anti-cancer agent, the soursop leaf has alsobeen empirically used to treat tingling, reduce cholesterol level, ease blood flow on light stroke, treat hypertension, hemorrhoid, urinary tract infection, and dysuria, as well as as a relaxant and treatment for seizure.[3]

However, the soursop leaf ethanol extract has never gone through any pre-clinical examination phase to test its toxicity, including the teratogenic test.[4,5] The purpose of this study was to analyze whether the soursop leaf ethanol extract (Annona muricata (L) Folium) has a teratogenic effect on mouse (Mus musculus) fetus, which was assessed through the number of implantation, live fetus, and intrauterine death (or resorption), as well as the weight and length of fetus and external morphological assessment.

Methods

This study was conducted between October–November 2012 at the Pharmacology and Therapy Laboratory of the Faculty of Medicine, Universitas Padjadjaran. The objects in this study were 27 female mice (Mus musculus) and 12 fertile mice from the Swiss Webster strain, with the average weight of 20–30 grams. All mice were adapted for a week before being paired and given treatment. Female mice used were adult, healthy, actively moving, and pregnant mice which were 8–10 weeks old and already had had a vaginal plug after being paired with male mice. This vaginal plug was the sign of the first day of pregnancy.

This study was a laboratory experimental research with complete random sampling method. All pregnant female mice were divided into three groups, with 9 mice in each group. During pregnancy, the mice

Table 1 Total number and average number of implantation, live fetus, dead fetus or resorbed as well as the weight and length of fetus

Group	Number of mother	Total number			Average number ± SD		Average ±SD	
		Implantation Result	Dead Fetus/ Resorbed	Live Fetus	Implantation Result	Dead Fetus/ Resorbed	Weight (gram)	Length (cm)
1	9	97	40	57	10.78±1.86	4.44+5.79	1.04+0.323a	2.18 + 0.466a
2	9	86	40	46	9.56±4.42	4.44+4.67	0.86+0.225b	1.79 + 0.451b
3	9	82	14	68	9.11±3.62	1.56+2.07	0.73 + 0.359b	1.87 + 0.403b

Note: Differing letter on a column indicates significance (p <0.05)

Table 2 Frequency of Fetus with Morphological Abnormalities

Group	Number of mother	Total Fetus	Frequency of Hemorrhage							Frequency of Microcephaly
			Total	Head	Neck	Face	Back	Forelimb	Hindlimb	
1	9	97	0	0	0	0	0	0	0	0
2	9	86	16	5	1	0	9	0	1	0
3	9	82	54	16	5	2	16	6	9	1

in the first group (negative control) were nurtured and given food, water, and 1 mL of Carboxymethylcellulose (CMC) 1% from the 1st to 18th day of pregnancy. The second and third group had the same treatment regarding food and water, with the addition of 2.34 mg of soursop leaf ethanol extract in 1 mL 1% CMC given per oral. The difference was that the second group had the soursop leaf ethanol extract treatment at the pre-implantation phase, which was between the 1st to 5th day of pregnancy, the third group had the same treatment during the organogenesis phase, which was between the 6th and 15th day of pregnancy. The parameters assessed were the total number of fetus, the number of live fetus, dead fetus or resorbed fetus as well as the weight and length of the fetus and also the morphological abnormalities when fetus was extracted via laparotomy on the 19th day of pregnancy.

All data were then documented and statistically analyzed. The data from the treatment groups (2 and 3) were compared to the negative control group (first group). The data normality test was done using the Kolmogorov-Smirnov test which was followed with the statistical difference test using T-test method if it was normal and Mann-Whitney if it is not normal.[6]

Results

The result showed that the group treated with the soursop leaf ethanol extract had less number of implantation. Yet, there was no significant difference between the total number of fetus and dead or resorbed fetus in these three groups ($p > 0.05$). The average weight and length of fetus had significantly declined ($p < 0.05$) in the treatment groups (2 and 3).

External morphological abnormalities were not found in the control group. However, in the treatment groups (2 and 3) there was a tendency towards external morphological abnormalities. The abnormalities were hemorrhage on the head, face, neck, back, forelimb, hindlimb, and the presence of microcephaly.

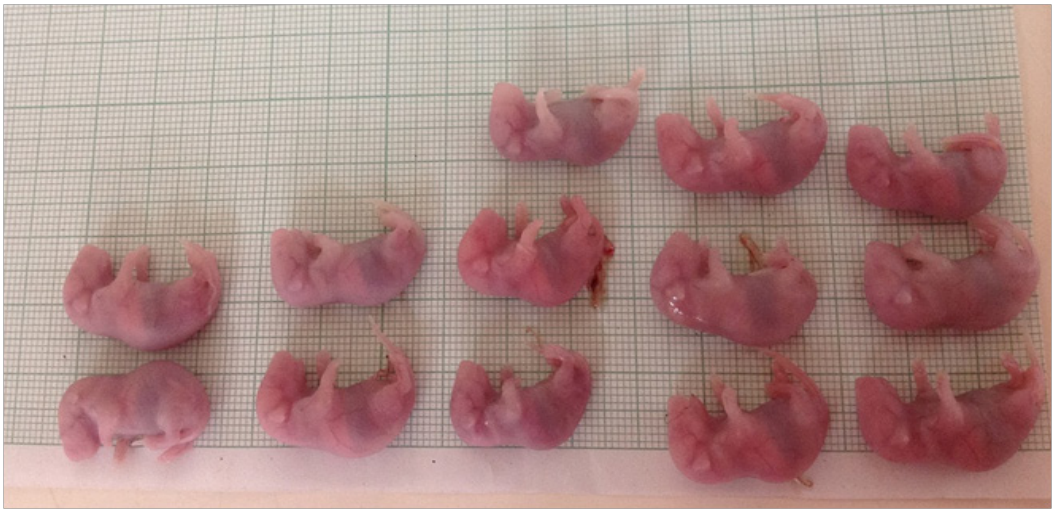

Figure 1 Normal live fetus (without abnormalities) from group 1 (control)

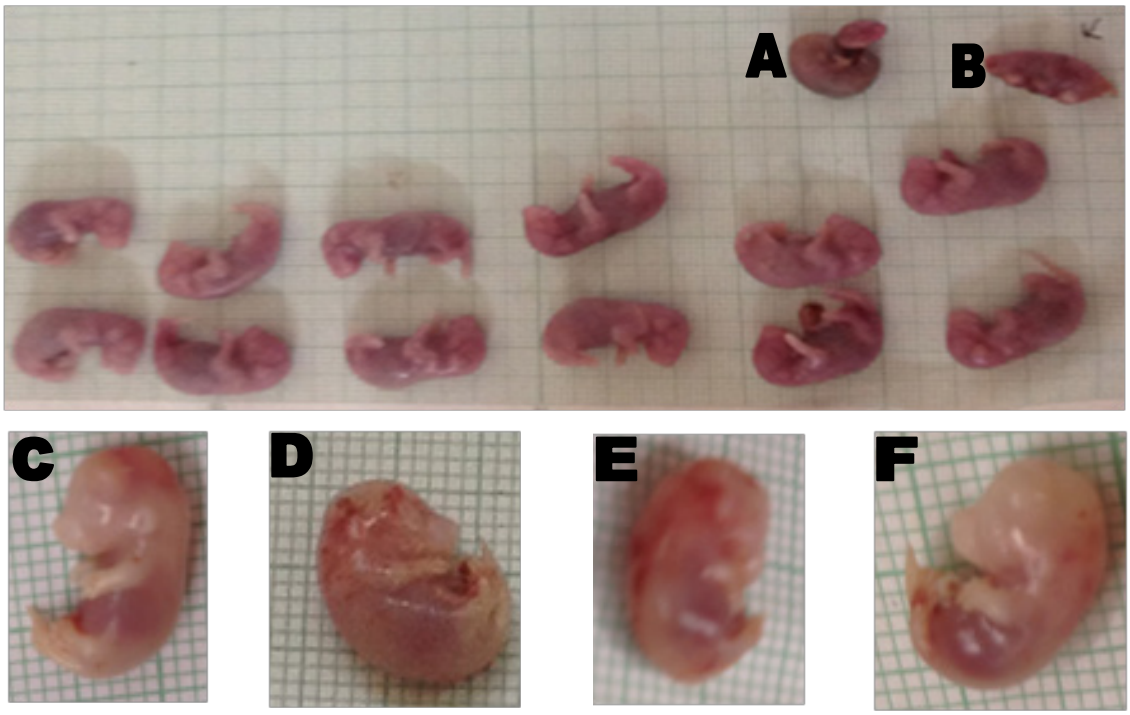

Figure 2 Fetuses of the second group. They have smaller size compared to control. And there are fetuses that experienced intrauterine death, either dead fetus (A) or resorbed fetus (B). Morphological abnormalities were found as hemorrhage on the head (C), neck (D), back (E), and hindlimb (F)

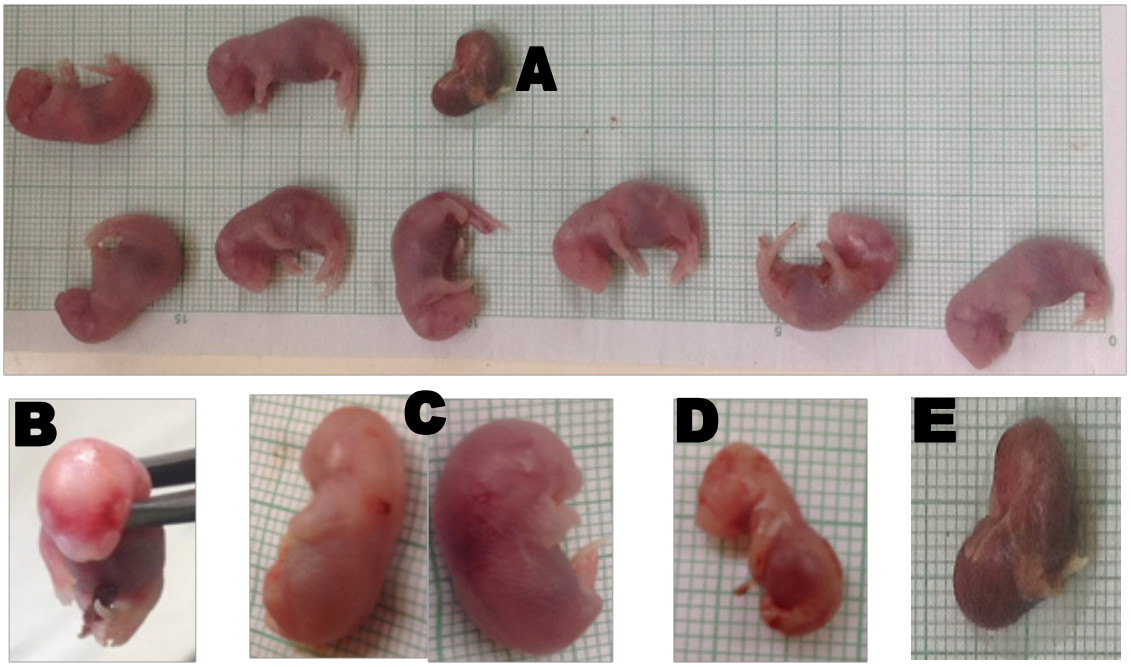

Figure 3 Mice fetus of the third group. Dead fetus was found (A), fetus with hemorrhage on the head and eyes (B), back (C), forelimbs and hindlimbs, and also microcephaly (D), and also fetus which underwent intrauterine death (E)

Discussion

Soursop leaf ethanol extract (Annona muricata (L) Folium) with 2.34 mg/day dosage has a teratogenic effect to mouse fetus which was shown by the length and weight reduction of fetus and presence of hemorrhage of the head, neck, back, forelimbs, and hindlimbs, as well as microcephaly. The soursop leaf ethanol extract can penetrate the placenta and both alkaloid and acetogenin content in the extract can affect fetus intrauterine growth.

The weight and length of fetuses in the second and third group showed significant reductions when compared to those of the first group as the negative control. The cytotoxic nature of acetogenins in the soursop leaf ethanol extract (Annona muricata (L) Folium) acts by inhibiting the energy transport in the form of Adenosine Triphosphate (ATP) which is used in the cellular proliferation. The effect is that the energy production inside the cells is halted, disturbing the growth and proliferation of cells.[3,7] This disturbed cell growth is one of the teratogenic mechanisms which inhibits the metabolic phase for normal growth.[8]

As such, the acetogenins in the soursop leaf ethanol extract (Annona muricata (L) Folium) is proven to be capable of causing Intra Uterine Growth Retardation (IUGR). The mice that received treatment, both in the second and third groups, showed hemorrhage on the head, face, neck, back, forelimbs, and hindlimbs. The hemorrhage or bleeding is an event of the leaking of blood from the cardiovascular system that is indicated by the accumulation of blood inside a cavity or tissue due to viscosity disturbance at different parts of the fetus which differs from the viscosity of plasma blood and the presence of extra-capillary spaces. In normal conditions, the embryo develops inside amnion fluid which is isotonic to the bodily fluid.[9] However, the presence of the alkaloid in the soursop leaf (Annona muricata (L) Folium) which can either be reticuline, coclaurine, coreximine, atherosperminine, stepharine, anomurine, and anomuricine can penetrate the placenta and affect the osmotic pressure disturbance, which then causes viscocity disturbance and hemorrhage in different parts of the body.[9,10]

Microcephaly happened in one of the fetuses in the third group which was treated by soursop leaf extract (Annona muricata (L) Folium) at the organogenesis phase. Microcephaly is a disturbance caused by the failure of the brain to grow, leading to the failure of the skull to expand. Neural crest cells are prone to teratogen because they leave the neuroectoderm from which they originate.[11]

In addition, the cytotoxic nature of acetogenins, which is present in the soursop leaf ethanol extract (Annona muricata (L) Folium),acts to inhibit the metabolic phase which is crucial for a normal brain growth, hence causing microcephaly. As such, it is proven that the soursop leaf ethanol extract (Annona muricata (L) Folium) can have a teratogenic effect on mouse (Mus musculus) fetus.

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