UNIVERSITI PUTRA MALAYSIA

ANTI-INFLAMMATORY EFFECT OF SEMI-PURIFIED LEAF EXTRACT OF Ficus deltoidea IN ACUTE AND CHRONIC INFLAMMATION

BY
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ANTI-INFLAMMATORY EFFECT OF SEMI-PURIFIED LEAF EXTRACT OF *Ficus deltoidea* IN ACUTE AND CHRONIC INFLAMMATION

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ABSTRACT

Ficus deltoidea is an epiphytic shrub which is native and widely distributed in several countries of the Southeast Asia. In Malaysia, it is commonly known as Mas cotek, serapat angin, telinga beruk and other names. Different parts of the plant are used traditionally to treat various kinds of ailments. The fruits are chewed to relieve headache,toothache and cold; powdered root and leaves of the plant has been applied externally to wounds and sores, and around the joints for relief of rheumatism. The objective of this study is to evaluate the anti-inflammatory effect of semi-purified leaf extract of *Ficus **d**eltoidea* in acute inflammation and chronic inflammation. Fractionation of ethanol crude extract of *Ficus deltoidea* was carried out with hexane, ethyl acetate and water partition. Hypothesis of this experiment are the 3 partitions have different effectiveness compared to non steroidal anti inflammatory drugs. All of experimental animal used in this study are male Sprague-Dawley(150- 300g). Paw edema test was conducted to evaluate acute inflammation. The negative control group (group 1) received 1 ml of 5% Tween 80, positive control groups received 10 mg/kg aspirin (group2). Meanwhile for treatment groups, the rats received 150 mg/kg of partition extract respectively. For chronic inflammation test, the cotton pellet granuloma test was conducted containing 5 groups as above. Based on the result**,** Ethyl acetate partition showed the most significant reduction in acute inflammation whereas hexane partition showed the most significant reduction in chronic inflammation. All treatments showed significant difference ($P<0.05$) in reduction of paw volume when compared to the negative control group (5 % Tween 80). 150 mg/kg EAPT showed the best inhibitor with the progressing inhibition. Secondly is 150 mg/kg WPT which is better that positive control drug (10 mg/kg Aspirin). The least inhibition was by HPT. The statistical result showed that all the treatments are significant compared to negative control 5 % Tween 80. Based on result, 150 mg/kg HPT is the highest percentage of inhibition of both anti-transudative and anti-proliferative and 150 mg/kg WPT is second. Then, it is followed by 10 mg/kg Aspirin and lastly 150 mg/kg EAPT. In conclusion, all partitions of *Ficus deltoidea* ethanol leaf extract have different effectiveness compared to non steroidal anti-inflammatory drugs (NSAIDs).

KESAN EKSTRAK DAUN Ficus deltoidea (SEMI-PURIFIED) DALAM INFLAMASI AKUT DAN INFLAMASI KRONIK

Mohd Hafiz Razali

ABSTRACT

Ficus deltoidea adalah satu tumbuhan semula jadi yang membiak dengan meluas di beberapa negara Asia Tenggara. Di Malaysia, ia dikenali umum sebagai Mas cotek, serapat angin, telinga beruk dan nama-nama lain. Bahagian-bahagian berlainan pada tumbuhan digunakan secara tradisi untuk merawat pelbagai jenis penyakit. Buahnya dikunyah bagi melegakan sakit kepala ,sakit gigi dan demam; akar dan daun-daun tumbuhan itu dijadikan dalam bentuk serbuk dan disapu secara luaran untuk luka-luka dan sakit, dan sekitar sendi-sendi untuk melegakan penyakit sendi. Objektif kajian ini adalah untuk menilai kesan anti-inflamasi ekstrak daun Ficus deltoidea (semi-purified) dalam inflamasi akut dan inflamasi kronik. Penyaringan ekstrak etanol Ficus deltoidea telah dijalankan dengan heksana, etil asetat dan air. Hipotesis eksperimen ini adalah 3 ekstrak tersebut (partition) mempunyai keberkesanan yang berbeza berbanding dengan ubat anti-inflamasi bukan steroid (NSAIDs). Haiwan ujikaji digunakan dalam kajian ini adalah tikus jantan,Sprague-Dawley(150- 300g). Untuk mengukur inflamasi akut, eksperimen yang dijalankan adalah ujian edema pada tapak kaki tikus. Kumpulan kawalan negatif (kumpulan 1) disuntik 1 ml 5% Tween 80, kumpulan kawalan positif disuntik 10 mg/kg aspirin (group2). Sementara itu untuk kumpulan-kumpulan rawatan, tikus-tikus itu menerima 150 mg/kg ekstrak masing-masing. Dalam ujikaji inflamasi kronik pula, ujian granuloma pelet kapas dijalankan ke atas lima kumpulan seperti diatas. Keputusan menunjukkan etil asetat ekstrak (partition) menunjukkan pengurangan paling signifikan dalam inflammasi akut manakala heksana ekstrak (partition) menunjukkan pengurangan paling signifikan dalam inflammasi kronik . Semua rawatan menunjukkan perbezaan yang signifikan apabila dibandingkan dengan kawalan negative. Keputusan menunjukkan bahawa 150mg/kg etil asetat ekstrak mempunyai peratus perencatan edema tertiinggi. Yang kedua adalah 150mg/kg akuas ekstrak dan dikuti dengan 10 mg/kg aspirin. Yang terakhir adalah heksana ekstrak. Untuk peratus perencatan anti-transudatif dan anti-proliferatif, akuas ekstrak alah yang tertinggi dan ikuti oleh heksana ekstrak. Kesimpulannya, ekstrak-ekstrak (partition) daripada daun Ficus deltoidea etanol ekstrak mempunyai keberkesan yang berbeza berbanding dengan ubat anti-inflamasi bukan steroid (NSAIDs).

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Last but not least, I would like to thanks my parents, siblings and cousin for their endless support throughout the entire period of this project and for my education.

APPROVAL

It is hereby certified that I have read this project paper entitled **Anti-Inflammatory Effect of Semi-Purified Leaf Extract of *Ficus Deltoidea* In Acute Inflammation and Chronic Inflammation** by Mohd Hafiz Bin Razali, and in my opinion it is satisfactory in terms of scope, quality and presentation as fulfilment of the requirements for the course SBP 3999.

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged.

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LIST OF ABBREVIATIONS

%	percent
^{o}C	degree Celsius
±	plus minus (varied)
HPT	Hexane partition
EAPT	Ethyl acetate partition
WPT	Water partition
ANOVA	Analysis of Variance
COX	cyclooxygenase
g	gram
kg	kilogram
m	meter
mg	milligram
mg/kg	milligram per kilogram
ml	millilitre
NSAIDs	nonsteroidal anti-inflammatory drugs
S.E.M	Standard Error of Mean

CHAPTER 1

INTRODUCTION

Inflammation is a complex reaction of the innate immune system in vascularised tissues that involves the accumulation and activation of leukocytes and plasma proteins at a site of infection, or cell injury (Abbas and Litchman, 2005). In simple word Inflammation is a response of body tissues to injury or irritation. It is a protective attempt by the organism to remove the injurious stimuli as well as to initiate the healing process for the tissue.

There are two types of inflammation; are acute and chronic inflammations. Acute inflammation is comparatively short duration, lasting from a few minutes up to hours or several days. Acute inflammation is characterized by exudation of fluid and plasma protein and influx of inflammatory cells into the site of injection. It is the immediate and early response that intended to deliver leukocytes to the site of infection (Kumar *et al.*, 2007). Meanwhile chronic inflammation is a longer duration that it may take weeks,

months or even years. Chronic inflammation is a pathological condition characterized by prolonged active inflammation, tissue destruction, and attempts at repair.

Nowadays, the usage of non-steroidal anti inflammatory is well known as the treatment for inflammation disease to relief of mild to moderate pain or inflammation however there are still some side effect come along with the treatment. The utilization of non-aspirin NSAIDs has increased dramatically over the past 30 years, and along with the benefits, these drugs showed the increase in the associated adverse drug reactions (Singh *et al.,* 1994).

Due to this problem with usage of NSAIDs, the herbal plants are used alternatively to prevent the prolong usage of this drug. This step will avoid the side effect or complication from using the drug. One of plants that are being used is mistletoe fig or the scientific name, *Ficus deltoidea*. The plant is native to Southeast Asia, Borneo, and the Philippines. In Malaysia, *F. deltoidea* is known in various names such as "cotek mas", "mas secotek", "telinga beruk", "serapat angina" and "Agolauran" (Fasihuddin and Hasmah, 1993*)*.

Ficus deltoidea is an evergreen shrub or small tree used traditionally to treat cardiovascular diseases and diabetes. Besides that, *F. deltoidea* also can be used as aphrodisiac, specifically to increase male virility. Almost all of the parts of *F. deltoidea* plant including the roots, bark, leaves and fruits are believed to have medicinal properties (Adam *et al*., 2007). However, there are limited studies conducted to claim these attributions. Therefore, the benefit of this plant is not proven scientifically yet. *F. deltoidea* is still under ongoing research in order to evaluate its potentials.

In this study, the semi-purified leaves extract of *F. deltoidea* variety *angustifolia* ethanol extract were evaluated to show its effect in curing acute inflammation and chronic inflammation. The tests were paw edema test for acute inflammation and cotton pellet granuloma test for chronic inflammation.

Specific objective:

To evaluate the anti-inflammatory effect of semi-purified leaf extract of *Ficus deltoidea* in acute inflammation and chronic inflammation.

Hypothesis:

The 3 partition (hexane, ethyl acetate and water partition) have different effectiveness of inhibition of inflammation compared to non steroidal anti inflammatory drugs.

CHAPTER 2

LITERATURE REVIEW

2.1 *Ficus deltoidea*

Ficus deltoidea or Mas Cotek, also known as "mistletoe fig", has been scientifically researched by many local institutions, Universiti putra Malaysia, Universiti Sains Malaysia, Universiti Kebangsaan Malaysia, Universiti Malaya, Forest Research Institute Malaysia (FRIM), and Malaysian Agricultural Research and Development Institute (MARDI). The scientific name, *deltoidea,* means delta or triangle-shaped is cites to its leaves (Riffle, 1998).It comes from the habit of the plant as an epiphyte that often growing on larger trees. The plant is around 2 m tall. The leaves are broadly spoon-shaped, obovate, around 2.5-12 cm long, bright green above and rust-red to olive brown beneath. The plant has spherical to round figs, around 1.5 cm, pink in color when ripe (Kamarudin and Abdul Latiff, 2002).

Phytochemical screening of the FDA had been performed to detect the presence of different classes of constituents, such as alkaloids, flavonoids, saponins, steroids, terpenes and tannins (Sulaiman *et al*., 2008). There are about six varieties of *F.*

deltoidea; *F. deltoidea* var. *angustifolia, F. deltoidea* var. *bilobata*, *F. deltoidea* var. *trengganuensis*, *F. deltoidea* var. *kunstleri, F. deltoidea* var. *intermedia,* and *F. deltoidea* var. *motleyana* (Kamarudin and Abdul Latiff, 2002). In this study, the variety that is evaluated is *F. deltoidea* Jack var. *angustifolia.*

Figure 2.1: *Ficus deltoidea* Jack var *angustifolia*

2.2 Inflammation

Inflammation is the most important of the body's defence mechanisms. Inflammation is the local response to injury in living, vascularised tissues. Its purpose is to localize and eliminate injurious agent and then restore the tissue to normal structure and function. However it also may detrimental and cause extensive tissue damage. Inflamed tissues are named with the suffix 'itis'; thus appendicitis is inflammation of appendix and hepatitis is inflammation of liver (Paul and Clair, 1997).

Inflammation can be categorized into two types based on time frame which are acute inflammation and chronic inflammation. Acute inflammation is rapid onset and of short duration, lasting from a few minutes to as long as few days, and is characterized by fluid and plasma exudation and predominantly neutrophilic leukocyte accumulation. Chronic inflammation may be insidious, is of longer duration (days to years), and is typified by influx of lymphocytes and macrophages with associates with vascular proliferation and fibrosis (scarring). However these two types can be overlapping and many variables modify their course and histological appearance (Kumar *et al*., 2007).

2.2.1 Acute Inflammation

Acute inflammation is defined as the early inflammatory response to an injurious agent involving the neutrophil and later on macrophages. Acute inflammation usually last for a few hours and then resolves, leaving little or no permanent damage. It is characterized by the presence of oedema fluid, fibrin and neutrophil polymorphs in the extracellular space of injured tissue. These changes occur due to arteriolar dilatation and opening up of capillary channels leading to increase blood flow into the damaged tissues, increased vascular permeability allowing fluid and protein to leak into the extracellular tissues and lastly emigration of neutrophils from vessels via several stages(Paul and Clair, 1997).

The stages occur in process: (1) margination of neurophils reduced blood flow and increased viscosity in the dilated capillaries cause neutrophils to flow close to the vessel wall, (2) neutrophils adhere to the endothelium, (3) emigration of neutrophils to pass through endothelial gap in amoeboid movement and (4) a process called diapesis (passive leakage of red blood cells through leaky vessel wall) also occurs (Paul and Clair, 1997).

2.2.2 Chronic Inflammation

It is characterized by the infiltration with mononuclear cells, including macrophages, lymphocytes and plasma cells. It also involve tissue destruction which mostly due to product of inflammatory cells. In repairing process, new vessels proliferation (angiogenesis) and fibrosis formation (scar formation). Chronic inflammation maybe caused by prolongs acute inflammation. This transition occurs when the acute response cannot be resolved, either because of the persistence of injurious agent or because of interference with the normal process of healing (Kumar *et al*., 2007).

2.3 Nonsteroidal Anti-inflammatory Drugs (NSAIDs)

All the NSAIDs are thought to exert their clinical effects by inhibiting prostaglandin synthesis. In this experimentation, I used Aspirin as a control drug to inhibit or reduce inflammation. The primary site of action is the cyclooxygenase enzyme that catalyses the conversion of arachidonic acid to prostaglandin and endoperoxide. Prostaglandins modulate components of inflammation, control of body temperature, pain

transmission, platelets aggregation, and other effects. They are not stored by cells, but are synthesized and released on demand. Their half-life is not more than minutes. Therefore, if you control the enzyme that makes prostaglandins, then you control the prostaglandins themselves. Aspirin are used in the treatment of moderate pain, fever, tendinitis, sunburn, rheumatoid arthritis, and osteoarthritis (Janet *et al.*, 1996).

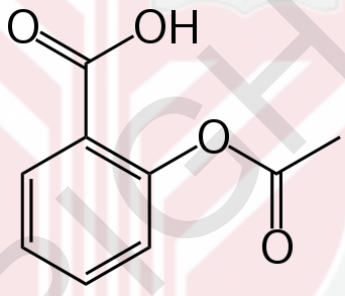

Figure 2.3: Aspirin structure

Aspirin is metabolized to salicylic acid by removal of acetate group. If the acetate is taken by cyclooxygenase enzyme, the ezyme is inactivated. In this way aspirin causes an irreversible inbition of cyclooxygenase. This will inhibit inflammation (Janet *et al.*, 1996).

CHAPTER 3

METHODOLOGY

3.1 Preparation of *Ficus deltoidea* Ethanol Extract

Throughout of the study, leaves of *F. deltoidea* var. *angustifolia* were used. The plant was taken in Sepang, Selangor. The leaves were separated from its stalks, cleaned, washed and rinsed with distilled water and left to be dried in laboratory for about two weeks. Then, the dried leaves were grinded into powder form. The powder form then was soaked and mixed in 95% ethanol in ratio of 1:5 for 2 days. The objective of this procedure is to dissolve polar compounds in ethanol extract. Then the mixture was filtered using filter paper (Whatman Paper No. 1) in order to remove the insoluble particles. After that, the filtrate was evaporated to get *Ficus deltoidea* leaf ethanol extract paste form.

3.2 Preparation of Hexane, Ethyl Acetate and Water Partition.

Ethanol extract was soaked in hexane in ratio of 1:10 for 1 day. Then, it was filtered using Whatman filter paper in order to remove the insoluble particles. The process was repeated till the solution gets colourless and evaporated to get hexane partition. The same steps repeated for ethyl acetate and water partition. For the administration of the treatment, the partition extract was dissolved in 5% Tween 80 according to the respective dose.

3.3 Experimental Animals

These experiments involved about 100 male Sprauge-Dawley rats. 50 rats were used in paw edema test and the remaining 50 rats of the same strain were used in cotton pellet granuloma test. All animals were maintained in the Animal House of FPSK, UPM for at least 1 week before used. The appropriate weight standardised about 150-300g for the experiment. They were housed in cages under normal laboratory conditions of humidity, temperature (25 ± 4ºC) and light (12/12 hour light dark cycle), and allowed to free access of food and water. The ethical clearance had been approved by Animal Care and Use Comittee, Faculty of Medicine and Health Sciences, UPM. The reference number is UPM/FPSK/PADS/UUH/F01.

3.4 Drugs and Chemicals

The drugs and chemicals used during the experimentation period were from analytical grade. Aspirin, Carrageenan, Commercial Grade, Type 1 and Tween 80 (FLUKA) were purchased from Sigma Company. 95% Hexane 86.18 g/mol and 95% ethyl acetate 88.10 g/mol were purchased from Systerm Chemical company and lastly 99.7% denatured ethyl alcohol was purchased from R&M chemical company..

Preparation of solutions:

a) 1% carrageenan: About 1ml of 1% carrageenan was needed for 10 animals. So, 10 mg of carrageenan powder was diluted in 1 ml distilled water to get 1% carrageenan. The same technique used to prepare for another groups of animals.

b) 5% Tween 80 prepared from 100% Tween 80 by using M1V1=M2V2 formula. For example, volume needed is 10mL (V1) for each group, substituted in formula:

5% X 10L = 100% X (V2)

V2 = (5% X 10m L)/ 100%

= 0.5 mL of 100% Tween 80

Therefore, approximate 9.5 mL distilled water was added to 0.5 mL of 100% Tween 80 to get 10 ml 5% Tween 80.

c) 95% ethyl alcohol prepared from 99.7% denatured ethyl alcohol by using M1V1=M2V2 formula. For example, volume needed is 1L (V1), substituted in formula:

95% X 1L = 99.7% X (V2)

V2 = (95% X 1 L)/ 99.7%

= 0.953 L of 99.7% ethyl alcohol

Therefore, approximate 0.047 L distilled water was added to 0.953 L of 99.7% ethyl alcohol to get 1 L of 95% ethyl alcohol.

d) 95% hexane and 95% ethyl acetate were stock solution, so no further preparation process needed.

3.5 The evaluation of acute inflammation

For evaluation of acute inflammation, the mediator-induced paw edema test was conducted according to according to the method of Winter *et al*., (1962). The edema was induced by injecting 0.1 ml of 10^{-3}g/mL of chemical mediators; 1% carregenan into respective 5 groups of ten animals. All treatments were given at 1.0 ml intraperitoneally, 30 minutes prior to chemical mediators' injection into the sub-plantar region of the right hind paw. The paw was measured using plethysmometer for each 30 minutes till 5 hours total. The negative control group (group 1) received 1 ml of 5% Tween 80, positive control groups received aspirin (group2). Meanwhile for treatment groups, the rats received 150 mg/kg of each partition extract respectively. The percentage of edema inhibition was calculated using the following formula as described by Garcia *et al.,* (1995).

Percentage inhibition =

$$\frac{(V_t - V_0)\ \text{control} - (V_t - V_0)\ \text{treated}}{(V_t - V_0)\ \text{control}} \times 100$$

where:

V_t = time (0.5, 1, 1.5, 2, 2.5, 3,3.5, 4, 4.5, 5 hour)

V_0 = initial time (0 hour)

3.6 The evaluation of chronic inflammation test

For chronic inflammation test, the cotton pellet granuloma test was conducted containing 5 groups as above. The sterile cotton pellets were implanted into subcutaneous tissue of the dorsal of the thoracic vertebrae region of the rats. Two hours after the implantation and for the next seven days, the rats were administered with the treatments intraperitoneally. On the eighth day, the rats were sacrificed and the pellets were removed to obtain the wet weight. The pellets then were dried in the oven to obtain the dry weight (Olajide *et al*., 1999).

Percentage of inhibition of anti transudative and anti proliferative were calculated as described below:

Percentage inhibition of anti transudative

$$= \frac{\text{Weight of wet pellet (-ve) control group - Weight of wet pellet treated group}}{\text{Weight wet pellet (-ve) control group}} \times 100$$

Percentage inhibition of anti proliferative

$$= \frac{\text{Weight of dry pellet (-ve) control group - Weight of dry pellet treated group}}{\text{Weight of dry pellet (-ve) control group}} \times 100$$

3.7 Statistical Analysis

The results were expressed as mean ± S.E.M. The data was analyzed statistically using the Univariate Analysis of Variance (ANOVA) done by SPSS 16.0. The statistical differences between treatment groups and control were evaluated using Duncan post hoc test. Results were considered significant if $P < 0.05$.

CHAPTER 4

RESULT

4.1 Carrageenan-Induced Paw Edema Test

Results of Carrageenan-induced paw edema test were displayed in Table 4.1, 4.2 and 4.3. The result was further illustrated in Figure 4.1, 4.2 and 4.3. All partitions of *F. deltoidea* ethanol extract used were hexane partition, ethyl acetate and water partition at the same dose (150 mg/kg). The parameter used to determine anti-inflammatory effects were volume of edema, percentage of swelling and lastly percentage of inhibition of edema in right paw of rats. The data were presented in table 1, table 2 and table 3 and were expressed as mean ± S.E.M for volume of edema, percentage of swelling and lastly percentage of inhibition of edema respectively. Meanwhile, Figure 4.1, 4.2 and 4.3 showed progression of edema in 5 hours period involving volume of edema, percentage of swelling and lastly percentage of inhibition of edema respectively.

For carrageenan paw edema test, there were two peaks of sudden increase in edema and slowly subsided after that. The first peak located about in 0.5-1st hour of time period however the second peak located at 3-4th hour of time period. All treatments

showed significant difference ($P<0.05$) in reduction of paw volume when compared to the negative control group (5 % Tween 80). HPT and EAPT showed significant difference ($P<0.05$) when compared to positive control 10 mg/kg aspirin as well as when compared to other treatment groups.

Based on the result, 150 mg/kg EAPT showed the best inhibitor with the progressing inhibition. Secondly was 150 mg/kg WPT which was better that positive control drug (10 mg/kg Aspirin). The least inhibition was by HPT.

Nevertheless, the statistical showed that all of the treatments were significant compared to negative control that was 5% Tween 80. These results showed that all partitions of *F. deltoidea* ethanol extract may have anti-inflammatory properties in carrageenan-induced paw edema test.

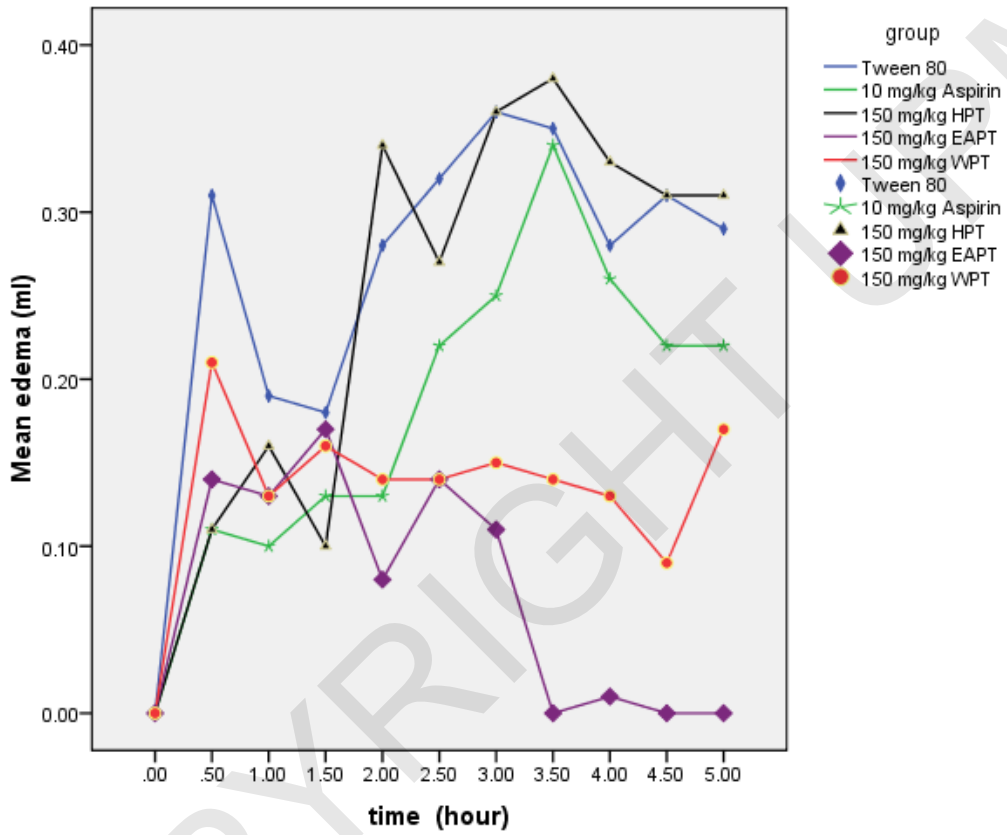

Figure 4.1: Effect of different partitions of *F. deltoidea* ethanol extract at specific time intervals in carrageenan-induced paw edema test.

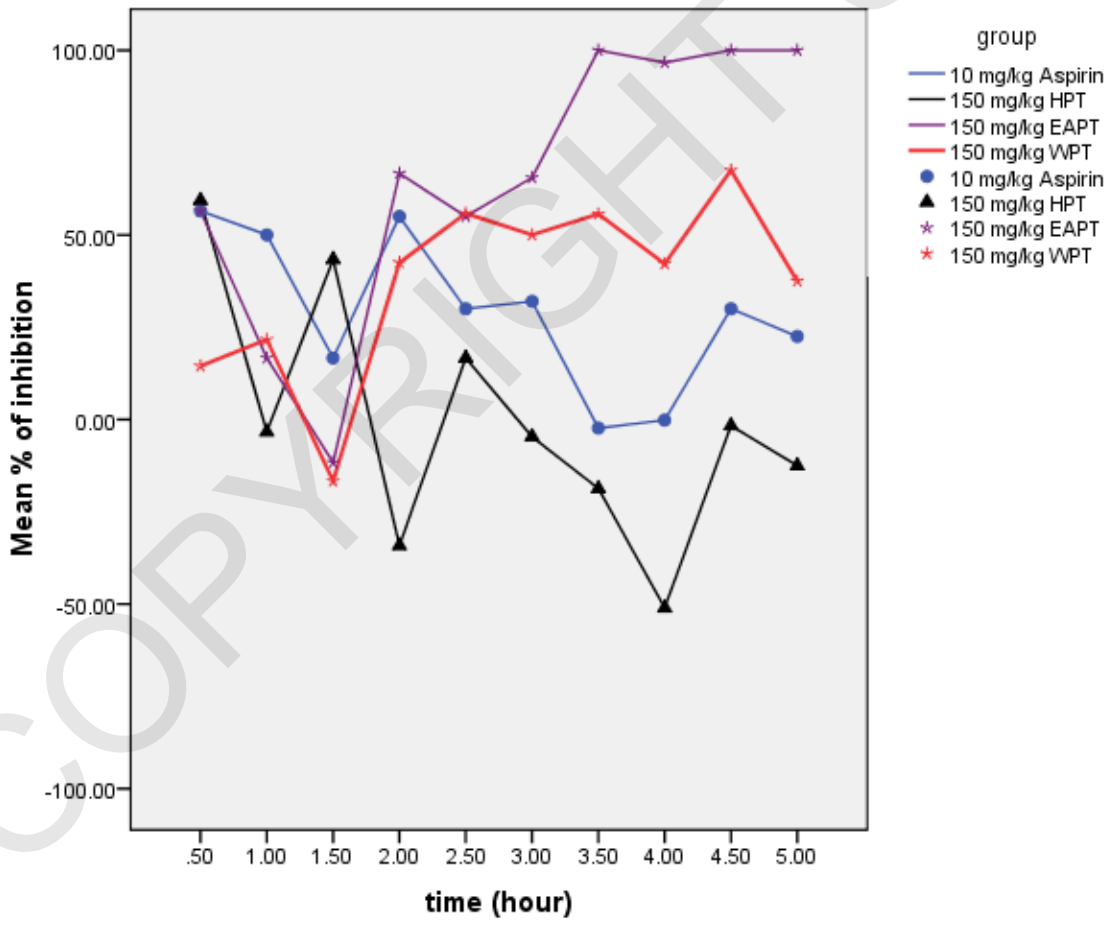

Figure 4.2: Percentage of inhibition of different partitions of *F. deltoidea* ethanol extract at specific time intervals in carrageenan-induced paw edema test.

Table 4.1: Effect of different partitions of *Ficus deltoidea* ethanol extracts in carrageenan-induced paw edema test.

Volume of edema (ml)

Group	Duration (hour)										
	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
5% Tween 80	0	0.31 ± 0.0407[abc]	0.19 ±0.0277	0.18 ±0.0249	0.28 ±0.0291[acd]	0.32 ±0.02[cd]	0.36 ±0.0267[cd]	0.35 ±0.0342[cd]	0.28 ±0.0359[cd]	0.31 ±0.0233[cd]	0.29 ±0.018[cd]
10 mg/kg Aspirin	0	0.11 ±0.018*	0.1 ±0.0298	0.13 ±0.026	0.13 ±0.0335*[b]	0.22 ±0.0291	0.25 ±0.0401[c]	0.34 ±0.034[cd]	0.26 ±0.0371[cd]	0.22 ±0.0327[cd]	0.22 ±0.02[c]
150 mg/kg HPT	0	0.11 ±0.0315*	0.16 ±0.034	0.1 ±0.0394	0.34 ±0.0371[acd]	0.27 ±0.0423[cd]	0.36 ±0.0371[cd]	0.38 ±0.0467[cd]	0.33 ±0.03[c d]	0.31 ±0.0482[cd]	0.31 ±0.0348[cd]
150 mg/kg EAPT	0	0.14 ±0.0306*	0.13 ±0.0213	0.17 ±0.0396	0.08 ±0.0327*[b]	0.14 ±0.0163*[b]	0.11 ±0.0277*[b]	0*[abd]	0.01 ±0.01*[ab]	0*[ab]	0*[abd]
150 mg/kg WPT	0	0.21 ±0.0277	0.13 ±0.0153	0.16 ±0.0163	0.14 ±0.0221*[b]	0.14 ±0.0267*[b]	0.15 ±0.0342*[b]	0.14 ±0.0306*[abc]	0.13 ±0.0335*[ab]	0.09 ±0.0277*[b]	0.17 ±0.026*[bc]

N = 10

Values were presented as Mean ± S.E.M. and are in ml

* indicated significant difference from 5% Tween 80 (negative control) ($p<0.05$)

[a] indicated significant difference from 10 mg/kg Aspirin (positive control) ($p<0.05$)

[b] indicated significant difference from 150 mg/kg HPT ($p<0.05$)

[c] indicated significant difference from 150 mg/kg EAPT ($p<0.05$)

[d] indicated significant difference from 150 mg/kg WPT ($p<0.05$)

Table 4.2: Percentage of inhibition ±S.E.M

Group	Duration (hour)									
	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
10 mg/kg Aspirin	56.5 ±10.2934	50 ±13.1468	16.67 ±22.2222	55 ±12.4969	30 ±8.2589[b]	32 ±8.7975	(-2.33) ±10.2083[cd]	(-0.167) ±14.1649[c]	30 ±9.1456[c]	22.5 ±7.0328[c]
150 mg/kg HPT	59.33 ±11.6831	(-3.33) ±26.8512	43.33 ±20.3670	(-34.167) ±22.3762*	16.67 ±9.8601[cd]	(-4.67) ±12.1914[cd]	(-18.67) ±19.1627[cd]	(-51) ±40.8673[cd]	(-1.67) ±16.3299[cd]	(-12.5) ±17.7104[cd]
150 mg/kg EAPT	56.5 ±10.4422	16.67 ±21.6595	(-11.67) ±30.2306	66.67 ±14.9071	55 ±5.4433[b]	65.5 ±11.3639b	100 ±0*[b]	96.67 ±3.3333*[b]	100 ±0*[b]	100 ±0*[bd]
150 mg/kg WPT	14.5 ±20.2302	21.67 ±9.3128	(-16.67) ±23.8307	42.5 ±12.6351	55.83 ±7.7629[b]	50 ±17.3472b	55.67 ±10.9098*[b]	42.167 ±18.0261[b]	67.5 ±10.8618[b]	37.5 ±10.7762[bcd]

N = 10

Values were presented as Mean ± S.E.M. and are in ml

* indicated significant difference from Aspirin (positive control) ($p<0.05$)
[b] indicated significant difference from 150 mg/kg HPT ($p<0.05$)
[c] indicated significant difference from 150 mg/kg EAPT ($p<0.05$)
[d] indicated significant difference from 150 mg/kg WPT ($p<0.05$)

CHAPTER 5

DISCUSSION

5.1 Carrageenan-Induced Paw Edema Test

In carrageenan-induced paw edema test, all partitions of *F. deltoidea* ethanol extract reduced the paw volume of rats significantly. According to the result, HPT, EAPT and WPT showed significant difference ($P<0.05$) when compared to negative control group (5% Tween 80). HPT and EAPT showed significant difference ($P<0.05$) when compared to positive control 10 mg/kg aspirin as well as when compared to other treatment groups. This proved that semi-purified leaf extract of *Ficus deltoidea* did contain anti-inflammatory bioactive compounds to inhibit acute inflammation.

For acute inflammation test, paw edema test was done to test the anti-inflammatory action of all the partitions. In this study, 0.1 ml of 10^{-3} g/ml of mediators 1 % Carrageenan Type 1 was used to induce inflammation.

The process of edema in the rat's paw was a biphasic event for carrageenan induced mediator. The first phase began immediately after injection and diminished in one hour. It was attributed to the release of histamine and serotonin (Badilla *et al*.,

2003). The second phase in which prostaglandin and bradykinin participation began at one hour and remained through three hour. It had been reported that the second phase of edema was sensitive to both steroidal and non-steroidal anti-inflammatory agents.

According to the result, it showed that the maximum percentage of edema at 2.5-3rd hour. It also demonstrated that the edema started to reduce at 3.5th hour. It was possible that the partitions reduced edema by inhibiting the production or action of chemical mediators in second phase which are bradykinin and prostaglandin. However, the actual mechanism on how the partitions inhibited edema was still not confirmed yet.

It was suggested that early increase in paw volume of carrageenan-induced paw edema was due to the release of histamine and serotonin. On the other hand, the delayed phase of carrageenan-induced paw edema was mostly because of potentiating effect of prostaglandins on mediator release especially for bradykinin.

Previous study done Mamduha, (2008) showed that for histamine mediator induced paw edema, the reference drug piroxicam showed the best inhibitor with the progressing inhibition. In contrast, mefenamic acid showed that the treatment of aqueous extract was better. Between the two doses, inhibition of 10 mg/kg was better than the inhibition of 100 mg/kg.

In bradykinin mediator, both inhibitions of reference drugs were better than inhibition of the treatment of aqueous extracts. Once again, the inhibition of 10 mg/kg was better than the inhibition of 100 mg/kg. (Mamduha, 2008). These results suggested that aqueous extract of *F. deltoidea* may have anti-inflammatory effects in mediator-induced paw edema test.

5.2 Cotton Pellet Granuloma Test

The inflammatory granuloma is a typical feature of an established chronic inflammatory process (Spector, 1961). The cotton pellet granuloma method has been widely employed to evaluate the transudative, exudative and proliferative components of chronic inflammation, because the dried weight of the pellets correlates well with the amount of granulomatous tissue (Swingle and Shideman, 1972).

Based on the result, all partitions showed inhibition of granuloma formation in rats. HPT, EAPT and WPT showed significant difference ($P<0.05$) when compared to negative control group (5% Tween 80). HPT showed the highest percentage of inhibition of anti-transudative and anti-proliferative even compared to aspirin. If we compared to acute inflammation test, EAPT showed the highest inhibition of edema but in chronic

inflammation test, EAPT was least effective to inhibit anti-transudative and anti-proliferative. HPT and WPT were the best treatments to inhibit granuloma formation in chronic inflammation test.

Previous study done by Mamduha, (2008) in cotton pellet granuloma test for wet weight, the results showed that all the doses of *Ficus deltoidea* aqueous extracts were better than both reference drugs (piroxicam and mefenemic acid). Even 50 mg/kg dose was significant compared to mefenamic acid. The statistical result showed that all the treatments were significant compared to negative control normal saline. The inhibition of anti-transudative showed that dose of 50 mg/kg was the higher among all of the treatment groups (25mg/kg and 100mg/kg *Ficus deltoidea* aqueous extracts).

In cotton pellet granuloma test for dry weight, the results showed little progress of doses of *F. deltoidea* aqueous extracts. Nevertheless, the doses were better than mefenamic acid. The statistical result showed that there were no significant differences among the treatment when compared to negative group normal saline. The inhibition of anti-proliferative showed that the doses were better than mefenamic acid even though the highest inhibition was piroxicam (Mamduha, 2008).

Both present and previous study showed correlation in effectiveness of *F. deltoidea* in inhibiting acute and chronic inflammation in rats. These results suggested that aqueous extract of *F. deltoidea* as well as all each partition in ethanol extract of *F.*

deltoidea may have anti-inflammatory properties in paw edema test cotton pellet granuloma test.

In this study, the effectiveness of each partition inhibits acute and chronic inflammation was somehow related to bioactive compounds contained in each partition. WPT, EAPT and HPT contained polar compounds, semi-polar compounds and non-polar compounds respectively. These difference bioactive compounds produced different effectiveness against acute and chronic inflammation. Bioactive compounds on each partition still unknown and further research need to be done to identify these bioactive compounds and the mechanism on how they can inhibit inflammatory process.

CHAPTER 6

CONCLUSION

Ficus deltoidea is one of the potential traditional medicinal plants used to treat inflammation as alternative treatment to chemically synthetic pharmaceutical drugs such as many Nonsteroidal Anti-inflammatory Drugs (NSAIDs) such as aspirin. These NSAIDs inflammatory is well known as the treatment for inflammation disease to relief of mild to moderate pain or inflammation however there are still some side effect come along with the treatment. The number and utilization of non-aspirin NSAIDs has increased dramatically over the past 30 years, and along with the benefits these drugs showed the increase in the associated adverse drug reactions (Singh *et al*, 1994).

It could be suggested that the partitions of *F. deltoidea* ethanol extract possessed anti-inflammatory activities. In carrageenan-induced paw edema activity, it was suggested that EAPT and WPT showed its anti-inflammatory action by inhibiting the edema that was comparable to aspirin whereas in cotton pellet granuloma test all partition except EAPT were more effective compared to aspirin.

6.1 Limitation of Study

Lack of skills in handling animal and improper restraining can influence the result. This might contribute to error during administration of extracts or drugs injected as well as volume calculated might be not accurate. Durability of chemical and extract also concern the study. Chemical and extract should be prepared freshly on the day of experiment and can't be stored too long.

6.2 Recommendations

Several recommendations should be considered for further studies on the plant:

1. Increase sample size of study to get a more reliable result.
2. Access the toxic effect of *F. deltoidea* by determining the LD_{50}.
3. Use another route of administration to compare the effect of the extracts.
4. Use multiple concentrations of the extracts.
5. Include the biochemical analysis for bioactive compounds presents in the extract.

APPENDICES

A) Carrageenan-Induced Paw Edema Test

1) 5% Tween 80 (Negative control)

No	BW	Duration (hour)										
		0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
1	213	1.2	1.7	1.4	1.5	1.6	1.5	1.7	1.7	1.6	1.6	1.6
2	241	1.3	1.6	1.4	1.5	1.5	1.6	1.7	1.6	1.6	1.5	1.5
3	217	1.5	1.8	1.6	1.7	1.7	1.8	1.9	1.7	1.7	1.8	1.8
4	183	1.1	1.6	1.4	1.3	1.5	1.5	1.5	1.6	1.6	1.5	1.4
5	202	1.2	1.5	1.3	1.3	1.5	1.5	1.6	1.7	1.4	1.5	1.5
6	193	1.3	1.6	1.4	1.4	1.6	1.6	1.6	1.6	1.6	1.6	1.6
7	220	1.2	1.6	1.4	1.4	1.4	1.6	1.6	1.5	1.3	1.5	1.4
8	198	1.2	1.4	1.5	1.3	1.4	1.5	1.4	1.5	1.4	1.4	1.5
9	226	1.5	1.6	1.8	1.8	1.9	1.9	1.8	1.8	1.8	1.9	1.8
10	211	1.3	1.5	1.5	1.4	1.5	1.5	1.6	1.6	1.6	1.6	1.6
Mean	210.4	1.28	1.59	1.47	1.46	1.56	1.6	1.64	1.63	1.56	1.59	1.57

2) 10 mg/kg Aspirin

No	Bw	Duration (hour)										
		0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
1	180	1	1.1	1.1	1.2	1.1	1.2	1.4	1.5	1.5	1.3	1.3
2	187	1	1.1	1	1	1	1.2	1.2	1.3	1.2	1.1	1.2
3	173	1.1	1.1	1.2	1.2	1.1	1.2	1.3	1.4	1.3	1.3	1.3
4	173	1.1	1.3	1.2	1.3	1.3	1.5	1.6	1.6	1.4	1.4	1.4
5	165	1	1.1	1	1.2	1.1	1.2	1.2	1.2	1.2	1.3	1.2
6	181	1.3	1.4	1.4	1.4	1.5	1.6	1.6	1.7	1.7	1.7	1.5
7	173	1.1	1.2	1.1	1.2	1.3	1.2	1.4	1.5	1.3	1.3	1.3
8	183	1.2	1.4	1.4	1.4	1.2	1.4	1.3	1.5	1.3	1.3	1.5
9	177	1	1.1	1.3	1.2	1.3	1.3	1.2	1.3	1.3	1.2	1.2
10	163	1	1.1	1.1	1	1.2	1.2	1.1	1.2	1.2	1.1	1.1
Mean	175.5	1.08	1.19	1.18	1.21	1.21	1.3	1.33	1.42	1.34	1.3	1.3

3) 150 mg/kg HPT

No	Bw	Duration (hour)										
		0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
1	206	1.3	1.3	1.4	1.3	1.7	1.6	1.7	1.8	1.7	1.6	1.6
2	183	1	1	1.1	1	1.3	1.2	1.3	1.3	1.2	1.1	1.2
3	189	1	1.3	1.3	1.3	1.4	1.3	1.4	1.4	1.4	1.3	1.3
4	170	0.9	1.1	1.1	0.9	1.3	1.1	1.1	1.2	1.2	1.2	1.1
5	176	1	1.1	1.1	1	1.2	1.2	1.3	1.3	1.3	1.2	1.3
6	207	1.3	1.4	1.4	1.4	1.6	1.5	1.6	1.5	1.6	1.5	1.5
7	162	0.8	1	1.2	1.1	1.4	1.3	1.4	1.5	1.3	1.3	1.3
8	220	1.4	1.4	1.5	1.5	1.7	1.5	1.7	1.7	1.6	1.8	1.7
9	177	1	1.1	1.1	1.2	1.3	1.5	1.5	1.5	1.4	1.6	1.5
10	176	1	1.1	1.1	1	1.2	1.2	1.3	1.3	1.3	1.2	1.3
mean	186.6	1.07	1.18	1.23	1.17	1.41	1.34	1.43	1.45	1.4	1.38	1.38

4) 150 mg/kg EAPT

No	BW	Duration (hour)										
		0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
1	170	1.2	1.4	1.3	1.4	1.2	1.3	1.3	1.2	1.2	1.2	1.2
2	167	1.2	1.4	1.3	1.4	1.3	1.4	1.3	1.2	1.3	1.2	1.2
3	158	1.1	1.2	1.1	1.2	1.2	1.2	1.2	1.1	1.1	1.1	1.1
4	171	1.2	1.3	1.3	1.4	1.4	1.3	1.3	1.2	1.2	1.2	1.2
5	151	1.1	1.2	1.3	1.2	1.1	1.2	1.2	1.1	1.1	1.1	1.1
6	166	1.3	1.5	1.5	1.6	1.4	1.5	1.6	1.3	1.3	1.3	1.3
7	167	1.2	1.5	1.4	1.6	1.2	1.4	1.3	1.2	1.2	1.2	1.2
8	203	1.3	1.5	1.5	1.5	1.6	1.5	1.5	1.3	1.3	1.3	1.3
9	172	1.2	1.2	1.3	1.2	1.2	1.3	1.2	1.2	1.2	1.2	1.2
10	170	1.2	1.2	1.3	1.2	1.2	1.3	1.2	1.2	1.2	1.2	1.2
Mean	169.5	1.2	1.34	1.33	1.37	1.28	1.34	1.31	1.2	1.21	1.2	1.2

5) 150 mg/kg WPT

No	BW	Duration (hour)										
		0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
1	185	1.4	1.6	1.5	1.5	1.5	1.5	1.4	1.5	1.5	1.4	1.5
2	152	1.1	1.3	1.2	1.2	1.2	1.2	1.3	1.3	1.3	1.2	1.3
3	151	1.1	1.2	1.2	1.3	1.2	1.2	1.2	1.2	1.2	1.2	1.2
4	187	1.3	1.5	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
5	164	1.1	1.3	1.2	1.3	1.2	1.2	1.3	1.2	1.2	1.1	1.2
6	161	1.2	1.3	1.3	1.4	1.4	1.3	1.3	1.3	1.2	1.2	1.3
7	150	0.9	1.2	1.1	1.1	1.1	1.2	1.1	1	1	0.9	1.1
8	180	1.1	1.5	1.3	1.3	1.4	1.4	1.5	1.5	1.5	1.3	1.4
9	153	1.2	1.4	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.4	1.4
10	192	1.2	1.4	1.4	1.4	1.3	1.3	1.3	1.3	1.3	1.4	1.5
Mean	167.5	1.16	1.37	1.29	1.32	1.3	1.3	1.31	1.3	1.29	1.25	1.33

B) Cotton Pellet Granuloma Test

1. **5% Tween 80 (Negative Control)**

All animal tested

Rat	Body Weight (g)	Wet Weight (g)	Divide with Body Weight	Dry Weight (g)	Divide with Body Weight
1	140	698	4.99	95	0.68
2	185	662	3.58	96	0.52
3	178	704	3.96	103	0.58
4	199	577	2.90	85	0.43
5	190	700	3.68	102	0.54
6	182	736	4.04	110	0.60
7	175	456	2.61	66	0.38
8	190	731	3.85	108	0.57
9	154	524	3.40	88	0.57

The rats that had been tested statistically

Rat	Body Weight (g)	Wet Weight (g)	Divide with Body Weight	Dry Weight (g)	Divide with Body Weight
1	190	731	3.85	108	0.57
2	182	736	4.04	110	0.60
3	190	700	3.68	102	0.54
4	178	704	3.96	103	0.58
5	140	698	4.99	95	0.68

2.10 mg/kg Aspirin

All animal tested

Rat	Body Weight (g)	Wet Weight (g)	Divide with Body Weight	Dry Weight (g)	Divide with Body Weight
1	241	480	1.99	72	0.3
2	156	439	2.81	69	0.44
3	185	486	2.63	76	0.41
4	184	471	2.56	76	0.41
5	156	439	2.81	69	0.44
6	178	439	2.47	70	0.39
7	183	390	2.13	62	0.34
8	154	416	2.7	55	0.36
9	175	422	2.41	64	0.37

The rats that had been tested statistically

Rat	Body Weight (g)	Wet Weight (g)	Divide with Body Weight	Dry Weight (g)	Divide with Body Weight
1	156	439	2.81	69	0.44
2	156	439	2.81	69	0.44
3	178	439	2.47	70	0.39
4	154	416	2.7	55	0.36
5	175	422	2.41	64	0.37

2. 150 mg/kg HPT

All animal tested

Rat	Body Weight (g)	Wet Weight (g)	Divide with Body Weight	Dry Weight (g)	Divide with Body Weight
1	230	527	2.29	75	0.33
2	155	463	2.99	62	0.4
3	249	502	2.02	80	0.32
4	194	516	2.66	77	0.4
5	172	431	2.51	72	0.42
6	253	527	2.08	75	0.3
7	214	414	1.93	65	0.3
8	198	436	2.2	62	0.31
9	200	370	1.85	58	0.29

The rats that had been tested statistically

Rat	Body Weight (g)	Wet Weight (g)	Divide with Body Weight	Dry Weight (g)	Divide with Body Weight
1	230	527	2.29	75	0.33
2	249	502	2.02	80	0.32
3	194	516	2.66	77	0.4
4	253	527	2.08	75	0.3
5	198	436	2.2	62	0.31

3. 150 mg/kg EAPT

All animal tested

Rat	Body Weight (g)	Wet Weight (g)	Divide with Body Weight	Dry Weight (g)	Divide with Body Weight
1	146	466	3.19	68	0.47
2	197	486	2.47	77	0.39
3	180	590	3.28	91	0.51
4	201	486	2.42	77	0.38
5	187	590	3.16	91	0.49
6	198	592	2.99	90	0.45
7	195	486	2.49	72	0.37
8	171	628	3.67	94	0.55
9	147	472	3.21	82	0.56

The rats that had been tested statistically

Rat	Body Weight (g)	Wet Weight (g)	Divide with Body Weight	Dry Weight (g)	Divide with Body Weight
1	146	466	3.19	68	0.47
3	180	590	3.28	91	0.51
5	187	590	3.16	91	0.49
8	171	628	3.67	94	0.55
9	147	472	3.21	82	0.56

4. 150 mg/kg WPT

All animal tested

Rat	Body Weight (g)	Wet Weight (g)	Divide with Body Weight	Dry Weight (g)	Divide with Body Weight
1	218	524	2.4	88	0.4
2	227	355	1.56	71	0.31
3	225	713	3.17	123	0.55
4	191	522	2.73	75	0.39
5	218	493	2.26	81	0.37
6	231	438	1.9	65	0.28
7	200	483	2.42	75	0.38
8	180	520	2.89	79	0.44
9	196	399	2.04	64	0.33

The rats that had been tested statistically

Rat	Body Weight (g)	Wet Weight (g)	Divide with Body Weight	Dry Weight (g)	Divide with Body Weight
1	218	524	2.4	88	0.4
2	191	522	2.73	75	0.39
3	218	493	2.26	81	0.37
4	200	483	2.42	75	0.38
5	196	399	2.04	64	0.33

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