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Evaluation and Effectiveness of the Anti-Inflammatory and Analgesic Effects of *Garcinia mangostana* Linn. (Mangosteen) on *Rattus norvegicus* (Albino rats) using Carrageenan-Induced Paw Edema and Writhing Test

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Abstract: This study investigated the effectiveness of the anti-inflammatory and analgesic activities of the *G. mangostana* using carrageenan-induced paw edema on *R. norvegicus*, compared it to the standard drug Mefenamic acid, and did post-experimental descriptive histological analysis. The experimental animals were separated into 4 test groups, Group A: Distilled water, Group B: low dosage (500 mg/kg), Group C: high dosage (1000 mg/kg), and Group D: Mefenamic acid. The lyophilized fruit hull of *G. mangostana* (500 mg/kg, 1000 mg/kg) was suspended in an aqueous solution and fed to Group B and C at the required dosage orally 30 minutes before the protocols were done. Although small inhibitory activity is observed this did not significantly inhibit the writhings induced by acetic acid. Additionally, the anti-inflammatory activity of *G. mangostana* extract (500 mg/kg, 1000 mg/kg) was also observed with no statistical significance. Histological analysis shows that leukocyte aggregation were less prominent in rats treated fed with the mangosteen aqueous solution however, this data can still be improved upon. Even though the results show that *G. mangostana* exhibits a small amount of analgesic and anti-inflammatory actions, statistical analysis proves that there has no significant statistical difference from the untreated group.
Key Words: *Garcinia mangostana;* Anti-inflammatory; Analgesic; Carrageeenan Induced Paw Edema; Albino rats

1. INTRODUCTION

Plant products are widely used in in modern medicine for the early development of drug discovery (Shibata et al., 2012) and with the current wide variety of synthetic drugs that are used for treatment of the same diseases and illnesses, medicinal plants could be used in modern medicine to enhance efficacy and can maybe produce minimal side effects and operate in unusual ways compared to the synthetic drugs (Oliveira et al. 2015). Mangosteen (*Garcinia mangostana* Linn.) is regarded as the "Queen of tropical fruits". It is a plant from the family *Guttiferae* genus *Garcinia*. The fruit is native

to Southeast Asia has been widely cultivated both as a source of food and of its medicinal properties (Guo et al. 2016 and Shan et al. 2011). It has an abundant source of Xanthones, Terpenes, Anthocyanins, Tannins, and Phenols (Shan et al. 2011) that attributes observable effects such as anti-inflammatory, antibacterial, antioxidant, and cardioprotective properties (Xie et al. 2014).

There has been research shown that Mangosteen extracts have potential anti-inflammatory properties (Shibata et al. 2013). Its pericarp contains four (4) phenylated xanthones; α-mangostin, ß-mangostin, y-mangostin, and methoxy–ß-mangostin and that these xanthones harvested from the rind of the fruit (Abdul-Rahman 2017) and has been proven by multiple researchers to provide great benefits for human health (Taher et al. 2016, Yoshimura et al. 2015, Johnson et al. 2012, and Ibrahim et al. 2014).

This research was done to assess the anti-inflammatory and analgesic properties of *G. mangostana* by using standard assays and protocols and comparing it to a standard non-steroidal anti-inflammatory drug. Its result and analysis regarding the anti-inflammatory properties is gathered using a Carrageenan induced paw edema and the analgesic properties using Acetic acid writhing test done on male *Rattus norvegicus* (Albino rats) as experimental animals. Its intent is to be a reliable study to either contradict or support several studies that confirm the presence and effectiveness of the pharmacological activities of the *G. mangostana* fruit pericarp extract.

2. METHODOLOGY

2.1 Procurement of Animals

A total of twelve (12) 1-year-old Male Sprague Dawley rats, weighing around 300-400g, were obtained from a registered breeder at Pet Town pet supplies and animal services Quezon City. Each individual were then placed in the Animal House at De La Salle University and were housed in individual standard size cages. Standard commercial rodent food (pellet form) and drinking water were provided. The cages were lined with autoclaved paddy husk and cleaned twice a week. Before the experiment, all mice were acclimatized for 1 week to adapt to an environment with the temperature of 23°C and 55% humidity at a 12 h light: 12 h dark cycle. All succeeding experiments in animals were approved by the Institutional Animal Care and Use Committee of De La Salle University.

2.2 Mangosteen pericarp extract, acetic acid, and carrageenan preparation

The aqueous Mangosteen extract solution was prepared from powdered Mangosteen (*Garcinia mangostana*) pericarp extract that was purchased from Carica herbal health products, Inc. The Mangosteen pericarp powder (20g) was homogenized in distilled water (20ml) by mixing with the use of a hotplate with magnetic stirrer. Homogenization of the Powdered Mangosteen pericarp extract and distilled water produced reddish brown aqueous solution (20ml) that was suitable for administering using a Gavage tube. The concentration of the resulting master mix aqueous solution was computed at 1000 mg/ml based on the weight of the initial Mangosteen pericarp powder. The volume administered to the rats based on its group dosage were also computed based on the weights of the rats. The aqueous solution was stored in a refrigerator at 2-8°C until administering to the experimental animals. 100% pure concentration of acetic acid was obtained from the De La Salle University Biology department laboratory. It was then diluted with distilled water to a concentration of 1% to be used in the acetic acid-induced writhing test. 1% Carrageenan solution was prepared by pouring 500ml of sterile 0.9% saline solution in a 1-liter beaker and while being stirred, 5g of Carrageenan powder was added into the saline solution and the solution was heated to 90°C with stirring until the solution was folly homogenized. The solution was not allowed to boil over during the homogenization process. The solution was then placed in 1-liter glass bottles and was sterilized by autoclaving. The solution was cooled to room temperature and stored on 1-liter bottles at 4°C ready for administering.

2.3 Administration of Treatment

Twelve (12) male Sprague dawley rats were randomly divided into four groups (A-D) of 3 rats per group. The experimental animals were fasted for 24 hours and was treated as follows: Group A rats were given normal saline (negative control), group B rats were given 500 mg/kg *G. mangostana* extract, group C rats were given 1000 mg/kg *G. mangostana*, and group D rats were given with 50 mg/kg mefenamic acid (positive control group). Analgesic activity was evaluated using the writhing test protocol. 30 minutes after administration of drug and extract, 1% acetic acid (10 ml/kg) was injected intraperitoneally to the rats to induce writhing. The number of abdominal constrictions (writhes) was counted in a 30-minute period after the acetic acid injection. The percent protection was computed against abdominal writhing to evaluate and assess the degree of analgesia of the treatments. For the anti-inflammatory activity was done by subplantar injection of 0.1mL of the 1% Carrageenan in saline solution into the left hind paw 30 minutes after dosage intact. Doses were subject to each individual rat's weight for consistency. Measurement of the thickness of the inflamed paw was done hourly starting from the initial thickness after induction and then at the 1st, 2nd, 3rd, 4th, and 5th hour by using a Vernier caliper and using millimeter as the standard unit for the measurements.

2.4 Histological Preparation and Data Analysis

After the evaluation of the anti-inflammatory activity of the Mangosteen in each rat, the left hind paw of each rat was processed in hi-precision diagnostics laboratories for longitudinal sectioning. The sections were then placed in glass slides and analyzed histologically in a descriptive manner. The observable increase in number of polynuclears, such as neutrophils, basophils, and eosinophils is a good indicator of the acute inflammation caused by the induction of carrageenan in the hind footpad of the rat. Histological analysis was performed with a compound light microscope, and photodocumentation was done using a Sony DSC-S930 SteadyShot 10.1 Megapixel digital camera. The values for the edema volume by using paw thickness and the writhing test is expressed as Mean ± SEM, One-way ANOVA with t-test was then used to determine the statistical significance of the data (Jaykaran 2010).

3. RESULTS AND DISCUSSION

3.1 Acetic Acid Induced Writhing List

Table 1 results showed that the extract (500, 1000 mg/kg) does not significantly ($P = 0.14$ and 0.06 respectively; $P < 0.05$) reduce abdominal writhing in rats when compared to the negative control group (normal saline). On the contrary, the reference drug mefenamic acid (50 mg/kg) significantly reduced abdominal writhings (Figure 1). Even though the extract of *G. mangostana* (500 mg/kg, 1000 mg/kg) does not significantly reduce abdominal writhings, it still produced a percentage protection against abdominal writhing of 13.89% and 20.24% respectively while mefenamic acid produced a significant protective effect against the induced writhing by acetic acid by 68.25% inhibition.

Table 1. Analgesic effect of *Garcinia mangostana* pericarp extract on Acetic Acid-Induced Writhing Reflex in experimental albino rats

Group	Treatment mg/kg	Mean number of writhing	% Protection
A	Normal Saline	84 ± 2.89	0
B	GME 500 mg/kg	72.33 ± 5.61	13.89
C	GME 1000 mg/kg	67 ± 5.86	20.24
D	Mefenamic acid 50 mg/kg	26.67 ± 2.40	68.25

Legend: Values shown are Mean ± SEM of each group. Group A: Normal Saline, Group B: 500 mg/kg of G. mangostana extract, Group C: 1000 mg/kg of G. mangostana extract, Group D: Mefenamic acid.

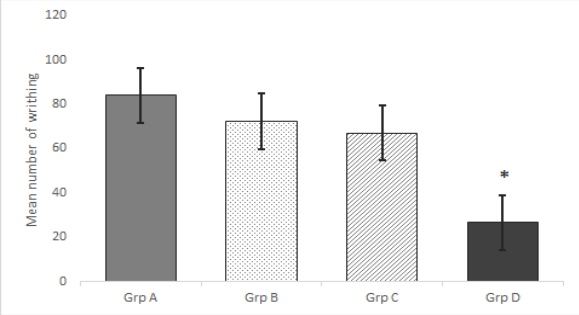

Figure 1. Effects of *G. mangostana* pericarp extract and mefenamic acid on acetic acid-induced writhing in albino rats. Each datum represents the mean ± S.E.M from 3 rats. *p < 0.05 compared to the control group (Dunnett's T- test).

Acetic acid injected intraperitoneally is responsible for secreting endogenous mediators of pain and activating chemical sensitive nociceptors resulting to a writhing reflex in animals. In this experiment, the extract of G. *mangostana* showed no significant analgesic properties even though it was able to produce a small percentage protection from acetic acid writhing response. The percentage protection from both doses of *G. mangostana* was not potent enough to be proven significantly inhibiting nociceptive response from rats. The analgesic effect of the acetic acid-induced writhing model is mainly mediated through peripheral pain mechanism through the prostaglandin pathway being suppressed. Hence, it is suggested that the slight and uncertain antinociceptive activity of the *G. mangostana* pericarp extract is not mainly mediated through peripheral mechanism.

3.2 Carrageenan-Induced Paw Edema

In this experiment, one-way ANOVA was used to determine statistical significance between groups. Significant differences between the mean of treated animals and the control group was considered significant at P < 0.05. Each group contained 3 samples of experimental animal with Group A being the control group which was induced with edema and was then left untreated, Group B being the low dosage group each rat being treated with a 500 mg/kg dosage of Mangosteen extract, 30 minutes before edema induction, Group C being the high dosage being treated with a 1000 mg/kg dosage of Mangosteen extract, 30 minutes before edema induction, and Group D being treated with Mefenamic Acid, 30 minutes before edema induction. Significant differences between groups were observed in the 1st, 2nd, 3rd, and 4th hour with its peak difference at the 2nd hour. A control group of rats was created before the experiment proper and its data was labeled as Group E. As observed from table 1, Group A, B, and C showed an increase in inflammation from initial induction towards the 2nd hour which is the peak inflammation for groups A, B, and C. Afterwards, a steady decline in inflammation is observed towards the 3rd, 4th, and 5th hour after induction. Group B and C shows a similar pattern with an increase in inflammation from initial towards the 2nd hour and then a steady decline afterwards. Group D shows the best inhibition of the edema with a steady decrease in inflammation after the 1st hour. It is worth noting that only Group D did not exhibit a peak inflammation at the 2nd hour but instead showed a decline after initial induction of edema. This further strengthens the use and effectiveness of the standard drug Mefenamic acid.

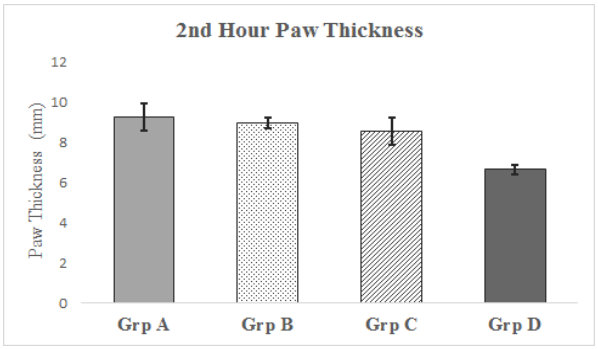

Figure 2. Comparison of 2nd hour paw thickness (Carrageenan-induced paw edema) displayed in Mean ± SEM

Figure 2 shows the peak paw thickness for Groups A, B, and C which were all observed 2 hours after induction. Group D showed a decreased amount of paw inflammation in the 2nd hour of induction compared to the 1st hour of induction. One-way ANOVA was used to determine statistical significance

of data during the 2nd hour and a significant difference was observed. A comparison between the rats fed with NSS (Group A) and the aqueous extracts (Groups B and C) were done by using Dunnett's t-test shows that the difference between the data are not significant. Statistical difference was only observed when comparing the data with Group D.

Table 3 shows the percentage inhibition of each group of rats. The untreated rats or Group A showed the highest inhibition percentage seen at 96.3%, while Group D which was treated with mefenamic acid showed the lowest inhibition percentage at 67.6%. Comparing the aqueous extract groups, low dosage of *Garcinia mangostana* extract showed a lower inhibition percentage compared to high dosage showing 84.2% and 92.4% respectively. Despite the lower inhibitory percentage effectiveness of the standard drug, the effectiveness of Mefenamic acid can be clearly observed in the 5th hour of the investigation by the much smaller paw thickness observed and by being the only group not exhibiting a peak paw thickness at the 2nd hour.

In the histological analysis, the highest degree of inflammation was observed in group A in which severe aggregation of neutrophil with some basophils and eosinophils that densely infiltrates the tissue can be observed. Groups B, and C had an observable increase in number of polynuclears, predominantly neutrophils with basophils and eosinophils with some aggregation indicates the presence of moderate inflammation. However, stromal connective tissues can still be clearly seen in these groups. Group D showed few-low density aggregations of polynuclears with the stromal connective tissue still clearly seen in the areas of aggregation.

The anti-inflammatory and analgesic activities of *G. mangostana* can be accredited to a family of interesting biological compounds known as xanthones. Specifically, α-Mangostin and γ-mangostin are the two dominant biologically active compounds found within its fruit hull. As shown in a study by Nakatani et al. γ-mangostin is capable of binding with cyclooxygenase enzymes which subsequently inhibits its activity. This effect allows *G. mangostana* fruit hull extract to possess a small amount of anti-inflammatory and analgesic activity. All though less

Table 2. Anti-Inflammatory activity of *Garcinia mangostana* pericarp extract on experimental albino rats.

Group	Treatment mg/KG	Initial Paw Thickness	Paw thickness after induction				
			1st hr	2nd hr	3rd hr	4th hr	5th hr
A	Normal Saline	6.55 ± 0.78	8.85 ± 0.77	9.25 ± 0.92	8.4 ± 0.71	7.7 ± 0.28	6.8 ± 0.85
B	GME 500 mg/Kg	7.03 ± 0.55	8.67 ± 0.55	8.93 ± 0.6	8.0 ± 0.61	7.7 7 ± 0.2	6.0 7 ± 0.61
C	GME 1000 mg/Kg	6.67 ± 0.47	7.5 ± 0.89	8.53 ± 1.2	7.73 ± 0.78	7.07 ± 0.90	6.2 ± 1.2
D	Mefenamic Acid	7.37 ± 0.55	6.83 ± 0.59	6.63 ± 0.42	6.27 ± 0.25	5.97 ± 0.05	5.57 ± 0.21

Table 3. Percentage of Inhibition of each group after

Group	Initial Paw Thickness	5th hr(mm)	Difference in Paw Thickness	Inhibition Percentage
A	6.55 ± 0.55	6.8 ± 0.60	0.25	96.3
B	7.03 ± 0.32	6.07 ± 0.35	0.96	84.2
C	6.67 ± 0.27	6.2 ± 0.7	0.47	92.4
D	7.37 ± 0.32	5.57 ± 0.12	1.8	67.6

effective as seen in the tests done in this research, the capability of inhibiting COX enzymes mimics the sought-after effects of Non-steroidal anti-inflammatory drugs or NSAIDs such as Mefenamic acid.

4. CONCLUSIONS

By reference, it can be assumed that the biological activities exhibited by the *G. mangostana* fruit can be attributed to the said active contents found within its fruit pericarp. However, after statistical analysis of the data gathered in this study, it has been proven that the analgesic and anti-inflammatory properties of the *G.. mangostana* pericarp extract are not statistically different from those treated with the negative control. It is recommended by the researchers to perform other tests when screening for the analgesic property of the Mangosteen extract, tests with various doses of the extract to further elucidate the positive effects of the fruit and do biochemical analysis of the fruit pericarp to further analyze the compounds found within the fruit hull and elucidate the mechanisms of xanthones in regards to its capability in inhibiting COX enzymes.

5. ACKNOWLEDGMENTS

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