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EFFECTS OF ETHANOL EXTRACTS OF GYNURA PROCUMBENS ON IN-VIVO PHAGOCYTOSIS OF WISTAR ALBINO RATS

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Abstract

Gynura procumbens is a traditional medicinal plant that is commonly used in Southeast Asia for treatment of various ailments. Nonetheless, there is very limited information on the effect of Gynura procumbens on the immune system. This preliminary study evaluates Gynura procumbens immunomodulatory properties of ethanol extracts at four dose levels ranging from 100 to 400 mg/kg body weight by focusing on the effects on phagocytic activity of macrophages on healthy female Wistar albino rat models. Assessments on phagocytosis enhancement were evaluated using carbon clearance test together with the examination on the mortality, behavioral changes, blood cells count, and serum biochemistry. The result from carbon clearance test showed significant increase of phagocytic index ($P<0.05$) on Gynura procumbens extract treated group of all doses. Furthermore, this study also demonstrates that there were no significant changes in the general condition, haematological counts, and biochemistry values from the Gynura procumbens treated groups in comparison to the control group. These findings demonstrate the potential of Gynura procumbens as an immunomodulatory agent.

Keywords: Gynura procumbens, wistar albino rat, immunomodulatory, phagocytic activity, haematological analysis, biochemical analysis

1.0 INTRODUCTION

Several plants are believed to improve resistance against infection by stimulating natural and adaptive immune system. The immune-modulating potential of these plants are being investigated extensively with more interest because of the increasing awareness on the therapeutic powers of plants as an agent of immunotherapy [1]. Gynura procumbens, a composite, is an evergreen shrub that grows abundantly in Southeast Asia, especially in Malaysia, Thailand, and Indonesia. Traditionally, Gynura procumbens is used for treatment of many ailments such as rashes, fever, rheumatism, inflammation, viral infection, constipation and cancer [2]. Previous studies show that *Gynura procumbens* contain compounds that exhibit certain biological properties which include enhanced lymphocyte proliferation, anti-hyperglycaemic, anti-cancer properties, wound-healing potential, anti-ulcerogenic activity, and non-toxic [3, 4, 5, 6, 7, 8]. Several possible active phytochemical components were also isolated and identified from the polysaccharides of the plant, that consist of flavonoids, saponins, tannins, terpenoids and sterol glycosides [9]. The medicinal value of this plant lies in bioactive phytochemical constituents that produce definite physiological action on the human body [10].There is very limited data available on immune-modulating activity of *Gynura procumbens* leaf extract. This study was carried out to investigate the effects of *Gynura procumbens* ethanolic extracts on phagocytic activity in healthy female Wistar rats.

2.0 MATERIALS AND METHOD

2.1 Plant Materials

Fresh leaves of *Gynura procumbens* were acquired from Penang, Malaysia (Herbagus Sdn. Bhd.) and identified by the herbarium unit, Forest Research Institute Malaysia (FRIM). A voucher specimen (PID 180413-09) was deposited in the herbarium unit for future reference. Leaves were washed twice with tap water then rinsed using distilled water.

2.2 Preparation Of *Gynura Procumbens* Ethanolic Extracts

The method of extraction follows Mahmood *et al*. (2010) with a slight modification [7]. Dried leaves were powdered using an electrical blender. Exactly 50 grams of the fine powder were then soaked in 250 mL of absolute ethanol in a schott bottle for 3 days. The ethanol extracts were then stirred mechanically for 4 hours, pooled, filtered with Whatman No. 1filter paper, and then evaporated under reduced pressure at 90°C using a rotary evaporator. The concentrated extracts were freeze dried and then stored at -40°C until further use.

2.3 Experimental Animals

Healthy female Wistar albino rats 8 weeks of age (150-200 gm) were used for this study. The animals were obtained from Laboratory Animals Facility and Management (LAFAM), Faculty of Pharmacy, Universiti Teknologi MARA. The selected animals were divided into 5 groups containing six rats each and were maintained under 26 ± 3°C under a light/dark cycle of 12 hours with free access to food (normal laboratory chow, altromin). All procedures in this study compiled with the Animal Ethics Guidelines of Universiti Teknologi MARA (approval No. 07/2013).

2.4 Administration Of Extracts And Animal Grouping

The animals were randomly divided into five groups of six rats each. Group I (control) was given 1.0% carboxymethyl cellulose (CMC) in water (2 mL/rat) for 7 days. Groups II to V (treated) the *Gynura procumbens* extract was administered orally for 7 days at different doses (100, 200, 300, and 400 mg/kg body weight [b.w.]).

2.5 Preparation Of Blood Samples And Serum

After the treatment period, each animal was starved into 18 hours fasting state. The animals were then anesthetized with diethyl ether and blood was drawn from cardiac puncture and collected into ethelene diamine tetra acetic acid (EDTA) containing tubes for haematological analysis and in plain tubes for blood biochemical analysis. All blood samples were preserved at 3-5°C prior testing.

2.6 Acute Toxicity Study

In order to observe the possible adverse effects of Gynura procumbens ethanol extracts, the behavior and toxicological signs of the five (control, 100, 200, 300 and 400mg/kg b.w.) allocated groups of healthy female Wistar albino rats (n=6) were monitored continuously for 30 min, 2, 4, 8, and 24 hours after dosing. Animals were observed daily for 7 days.

2.7 Haematological Analysis

The haematological analyses were determined on a fully automated Beckman Coulter LH 500 (Beckman Coulter, U.S.A.) [11]. The blood samples collected in EDTA containing tubes were analyzed not more than 6 hours after blood was drawn from each rat. Full blood cell counts were performed which included total red blood cell count (RBC), total white blood cell count (WBC), platelet count (PLT). Haemoglobin concentration (HB), mean corpuscular volume (MCV), Hematocrit level (HT), and mean corpuscular haemoglobin (MCH).

2.8 Biochemical Tests

No more than 6 hours after blood collection from the rats, the blood samples were centrifuged at 3000 rpm at 4°C and the serum separated from the plain tubes, transferred into bullet tubes, and then analyzed for alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin and globulin by using COBAS INTEGRA 400/800 (Roche, Switzerland).

2.9 Carbon Clearance Test

Around 48 hours after the end of the 7-day treatment, the rats were injected via the tail vein with carbon ink suspension (10 µL/gm b.w.) (Pelikan, Germany). Blood samples were then drawn from the retro-orbital vein at intervals of 15 minutes and then collected into EDTA containing tubes. Each 25 µL blood samples were mixed with 0.1% sodium carbonate solution (2 mL) and its absorbance was measured at 660 nm [12]. The phagocytic index K was calculated using the following equation: $K= (Log_e\ OD_1-Log_e\ OD_2)/15$, where OD_1 and OD_2 are the optical densities at 0 and 15 min, accordingly. Results were expressed as the arithmetic mean ± S.D of 6 rats.

3.0 RESULTS AND DISCUSSIONS

3.1 Acute Toxicity Study

Acute toxicity for this study was done by observing the healthy female Wistar albino rats' behavioral changes or even death. The animals were fed orally with ethanol extracts of *Gynura procumbens* for 7 days. The animals were grouped (n=6) according to the different doses of extracts treatments (100, 200, 300, and 400 mg/ kg b.w.) No fatality and no other signs of toxicity were observed with doses up to 400 mg/kg b.w. No behavior changes and no other adverse effects preceding death were observed during the 7 days of treatment. This finding coincide with another study conducted on acute toxicity of *Gynura procumbens* on animals by A. N. Shwter *et al.* (2014) and Rosidah *et al.* (2009) which showed no mortality or significant changes in the behavior of rats in dose up to 5000 mg/kg b.w. [8, 13]. This showed that the acute oral toxicity of the *Gynura procumbens* extracts on the animals was not observed in this study.

3.2 Haematological Analysis

Full blood count which included RBC, WBC, PLT, HB, MCV, HT, and MCH were performed on a fully automated Beckman Coulter LH 500 (Beckman Coulter, U.S.A). No abnormalities were observed in all of the values of the haematological parameters for all the animals treated with *Gynura procumbens* ethanol extracts (100, 200, 300, and 400 mg/kg b.w.) with no significant difference when compared to the control animals (Table 1). The haematological parameters serve as an important indicator of the pathophysiological status for both humans and animals [14].These results are in agreement with identical studies on *Gynura procumbens* 3 months treatment-related changes on haematological analysis [8]. The non-significant decrease or increase in the haematological values is a sign that the orally administered doses of *Gynura procumbens* ethanol extracts used in this study do not affect the animals' haematopoietic system.

Table 1 Effect of Gynura procumbens ethanol extracts on haematological analysis of healthy female Wistar albino rats (mg/kg b.w.)

Dose (mg/kg)	Mean ±S.E.M.						
	RBC	**WBC**	**PLT**	**HB**	**MCV**	**HT**	**MCH**
Control	6.88±0.37	6.72±1.58	820.83±282.58	12.43±0.57	57.85±1.29	39.73±1.93	19.67±0.66
100	6.37±0.67	9.07±0.72	789.83±216.47	13.13±1.17	59.97±0.49	36.20±4.62	20.40±0.35
200	6.59±0.43	6.58±1.64	676.33±268.75	13.70±0.83	58.03±1.34	38.82±2.90	19.88±0.35
300	6.86±0.17	7.17±1.15	1058.00±175.59	13.75±0.36	58.40±0.84	40.05±0.96	19.95±0.30
400	7.09±0.15	6.62±0.38	1200.67±110.79	13.25±0.57	56.63±1.75	40.18±1.55	19.40±0.67

Results were expressed as ± S.E.M One Way ANOVA followed by Dunnett's test. Significant values at $P<0.05$. There are no significant differences between the groups. RBC: total red blood cell count, WBC, total white blood cell count, PLT: platelet count, MCV: mean corpuscular volume, HT: haematocrit, MCH: mean corpuscular haemoglobin

Table 2 Effect of *Gynura procumbens* ethanol extracts on biochemical analysis of heathy female Wistar albino rats (mg/kg b.w.)

	Mean ±S.E.M.					
Group (mg/kg)	**ALP (μ/L)**	**ALT (μ/L)**	**AST (μ/L)**	**Total protein (g/L)**	**Albumin (g/L)**	**Globulin (g/L)**
Control	68.75±9.59	54.68±9.35	176.23±18.94	63.82±3.10	47.60±2.67	16.22±0.90
100	85.40±6.67	50.10±3.60	180.37±11.61	61.80±2.67	46.35±1.85	15.45±1.56
200	83.85±12.44	73.82±17.73	183.52±5.44	63.20±1.68	48.64±1.41	14.56±0.78
300	83.32±12.64	56.77±7.33	156.90±16.71	62.82±1.69	48.30±1.08	14.52±0.75
400	80.18±9.87	55.28±6.07	134.55±7.85	64.78±2.33	48.68±2.31	16.10±1.24

Results were expressed as ± S.E.M. One Way ANOVA followed by Dunnett's test. Significant values at $P<0.05$. There are no significant differences between the groups. ALP: alkaline phosphatase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, Total Protein, Albumin, Globulin

3.3 Biochemical Analysis of Blood

Serum level of ALP, ALT, AST, total protein, albumin, and globulin were analyzed using COBAS INTEGRA 400/800 (Roche, Switzerland). The assessment of biochemical analytes play an important role in the diagnosis of liver toxicity [15].The biochemical analysis indicated with the lowest dose (100 mg/kg), highest dose (400 mg/kg) showed no significant differences for the values of AST, ALP, ALT, Total protein, Albumin and Globulin for the animals treated with ethanol extracts of *Gynura procumbens* compared to animals in the control group (Table 2).

3.4 In-vivo Analysis of Phagocytic Index

Effect of *Gynura procumbens* ethanol extracts on the phagocytic function of reticuloendothelial system (RES) was evaluated by the carbon clearance test [16, 17]. Administration of ethanol extract of *Gynura procumbens* showed significant increase in clearance of carbon particle from blood with significant phagocytic index ($P<0.05$) for all treated groups (Table 3). The significant increase in the phagocytic activity of ethanolic extracts of *Gynura procumbens* may be associated with the high content of total phenolics and flavonoids that can be found in its leaves [18, 19, 20]. Phagocytosis play an important role in the removal of pathogens, foreign materials and dead cells [21]. The findings suggest that *Gynura procumbens* may stimulate macrophages in the clearance of carbon particles from the animals' bloodstream hence stimulate cell mediated immunity.

Table 3 Effect of *Gynura procumbens* ethanol extracts on phagocytic index (K) (carbon clearance test) of healthy female Wistar albino rats (mg/kg b.w.)

Dose (mg/kg)	**Control**	**100**	**200**	**300**	**400**
Phagocytic Index (K)	0.033 ± 0.004	0.041 ± 0.005*	0.041 ± 0.005*	0.045 ± 0.004*	0.042 ± 0.003*

Results were expressed as ± S.E.M One Way ANOVA followed by Dunnett's test. Significant values at $P<0.05$. There are significant differences between all of the treatment groups (*)

4.0 CONCLUSIONS

According to the results of the present study, it can be suggested that ethanolic extracts of Gynura procumbens may have immunomodulatory properties with no toxicological concerns. Gynura procumbens ethanolic leaf extract orally administered to healthy female Wistar albino rats was safe and there were no drug-related toxicity detected with non-significant differences in the behavior and mortality, haematological analysis, or biochemical analytes values for the treatment groups in comparison to the control group. The increase in carbon clearance test phagocytosis index indicates Gynura procumbens may be beneficial in the treatment of immune-related diseases.

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