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Research Article

ANTI-INFLAMMATORY PROPERTIES OF OIL PALM LEAF (*ELAEIS GUINEENSIS JACQ.*) EXTRACT IN AGED RATS

VICTOR UCHENNA ANYANJI[a*], SUHAILA MOHAMED [a,b], JAMES A. ZOKTI[a,c] AND MUHAMMAD ABUBAKAR ADO[a]

[a]Faculty of Food Science and Technology Universiti Putra Malaysia, UPM 43400 Serdang, Selangor, Malaysia,, [b]Institute of Bioscience Universiti Putra Malaysia, UPM, 43400, Serdang, Selangor, Malaysia, [c]Faculty of Food Science and Technology Federal Polytechnic Kaura-namoda, Zamfara State, Nigeria. Email: vikki_003@yahoo.com

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ABSTRACT

Most chronic conditions (aging, cancer, diabetes, cardiovascular disease, allergies, AIDS) are linked to hyper or hypo-active immune functions and therefore the need to look for new anti-inflammatory functional food.

Objectives: This research aims at investigating the anti-inflammatory properties of oil palm leaf (*Elaeis guineensis Jacq.*) ethanol extract in aged Sprague dawley rats.

Methods: Delayed type hypersensitivity, induced by intraperitoneal injection of sheep red blood cells, was measured by footpad inflammatory response, and used as an indicator of cell mediated immunity.

Results: Oil palm leaf extract (OPLE) at 150 mg/kg body weight bw showed significant pro-inflammatory with enhanced 46% late phase inflammation recovery effects. While at high dose, inflammation was significantly suppressed prior to the sixth hour compared to other groups, and did not require much inflammation suppression between the 18th and 48th hour. OPLE 150 mg/kg bw decreased lymphocyte counts, but was not as severely as dexamethasone treatment.

Conclusion: This result suggests that OPLE extract possess strong *in-vivo* inflammatory-regulatory effects.

Keywords: *Elaeis guineensis*; Delayed type hypersensitivity; Inflammation.

Most chronic conditions (aging, cancer, diabetes, cardiovascular disease, allergies, AIDS) are linked to hyper or hypo-active immune functions and therefore the need to look for new anti-inflammatory functional food. The host defense mechanism activation by an agent in an immune suppressive condition can provide supportive therapy to conventional medications. Old age has been associated with decreased naive T cells and a corresponding increase in memory T cells, which however, affects T cell responses, proportion and population. Oil palm (*Elaeis guineensis* Jacq. belongs to Arecaceae family) leaves are the major waste products of the palm oil industry in tropical countries. The methanolic extract of Oil palm leaves was demonstrated to contain 24.35 mg Gallic acid equivalent (GAE)/g dry weight total polyphenol, which was higher than green tea 22.5 mg GAE/g dry weight [1]. The phenolic compounds of OPLE were demonstrated to contain different amounts of flavonoids, epicatechingallate (0.05%), epicatechin (0.01%), catechin (0.30%), epigallocatechin gallate (0.28) [2]. The antioxidant content of oil palm fronds is reported to be higher than papaya shoot; green chili and lemongrass *in-vitro* [3]. It up-regulated the low density lipoprotein receptors (LDL) *in-vitro* [4], and showed cancer chemo-preventive effects on tumour growth [5]. This however, represents their potential use in disease prevention and management and therefore, could stand the basis for the assessment of OPLE as a medicinal agent on cell mediated immunity in aged Sprague-Dawley rats.

This research aims at investigating the anti-inflammatory properties of ethanol extract of oil palm leaf (OPLE) in aged Sprague dawley rats. Oil palm leaves were harvested from within the Universiti Putra Malaysia farm and the dried leaves were extracted with ethanol [6].

Male Sprague-Dawley rats were acclimatized and maintained at the animal house under controlled environmental conditions, temperature 23±2^{0}C and 12 hours light/darkness, with free access to standard rat pellets (Goldcoin SDN BHD, Malaysia) and water *ad libitum*. Fresh sheep red blood cells (SRBCs) were obtained from the animal farm of the Veterinary Faculty Universiti Putra Malaysia, in heparinised tubes. The red cells were centrifuged and washed three times in phosphate buffer solution (PBS) pH 7.2. The cells were diluted to 98%, 2% washed and was counted and thereafter kept under refrigeration at 0-4 ^{0}C until use.

Animals were divided into four groups' viz., control, dexamethasone (0.3 mg/ml), OPLE 150 mg/kg and OPLE 300 mg/kg. On pre-study day, animals were challenged with 0.25 mL of intraperitoneal injection of SRBCs. Then the animals were treated, vehicle (water), dexamethasone or OPLE for next 7 days. All the drugs were dissolved in water and administered per orally. Seven days post immunization; the left hind footpad of all animals was re-challenged with 0.25 mL of SRBC. Footpad thickness (FPT) was measured with a Vernier caliper at 6, 18 and 48 hours. The difference in footpad thickness between the left and right hind footpad was chosen as the measure of delayed type hypersensitivity (DTH) from which % inflammation or recovery was determined. At the end of experiment, blood samples were collected through retro-orbital sinus under light ether anesthesia and were used for differential count [7]. All data are expressed as mean ± SD and statistically analyzed by one way Analysis of Variance (ANOVA). Differences at $P<0.05$ were considered significant.

Results of [Table 1] showed that high dose 300 mg OPLE/kg, significantly reduced paw inflammation prior to the hour 6, indicated by decrease in footpad thickness of rats compared to the control groups. Therefore requiring little responses at both phases of paw inflammation recovery (0 and 17%) in the later phases, while 150 mg OPLE/kg showed significant pro-inflammatory with enhanced 46% late phase inflammatory recovery effects.

Table 1: Effect of oil palm leaf (OPLE) on delayed type hypersensitivity (DTH), challenged by SRBC in rats, at 6, 18 and 48 h

Treatment	Pre-study day paw thickness	Post immunization paw thickness		
		6h	18h	48h
Vehicle	0.15±0.3	2.5 ± 0.2	1.7 ± 0.4	1.6 ± 0.9
Dexamethasone	0.15±0.3	2.1 ± 0.3	1.0 ± 0.5*	1.2 ± 0.5
OPLE 150 mg/kg	0.16±0.4	2.8 ± 0.1*	2.2 ± 0.7*	1.5 ± 0.5
OPLE 300 mg/kg	0.17±0.2	1.2 ± 0.5*	1.2 ± 0.5*	1.0 ± 0.5*

Results are mean ± SD of 6 rats. *P<0.05 significant (Duncan multiple range tests) compared to control

Lymphocyte counts [Table 2] was significantly reduced (P<0.05) at 150 mg OPLE/kg bw, but was not as severely as dexamethasone treatment compared to the vehicle and other treated groups.

Table 2: Effect of OPLE on total and differential leukocyte counts after challenge by SRBC in rats

Treatment	Total WBC count ($\times10^9$/l)	Lymphocytes ($\times10^9$/l)	Neutrophil ($\times10^9$/l)	Monocytes ($\times10^9$/l)	Eosinophil ($\times10^9$/l)	Basophil ($\times10^9$/l)
Vehicle	13.8 ± 2.8	10.0 ± 1.4	2.5 ± 1.6	0.5 ± 0.1	0.4 ± 0.5	0.1 ± 0.1
Dexamethasone	4.5 ± 0.2*	2.1 ± 0.4*	2.0 ± 0.6	0.3 ± 0.1	0.04 ± 0.0	0.1 ± 0.1
OPLE 150mg/kg	11.0 ± 0.6	6.7 ± 0.6*	3.1 ± 0.8	0.6 ± 0.1	0.1 ± 0.0	0.2 ± 0.1
OPLE 300mg/kg	13.2 ± 0.4	9.0 ± 0.5	2.9 ± 0.4	0.7 ± 0.1	0.2 ± 0.1	0.2 ± 0.1

Results are mean ±SD of 4 rats. * P<0.05. Significant (Duncan multiple range tests) compared to the control

Old age has been linked to decreased natural killer cell and T cell proliferation and response to mitogens. However, T cell lymphocytes play significant roles on immune system regulations such as DTH. In the present study, OPLE extract exhibited significant suppression of paw oedema against SRBC induced DTH, a case probably attributed to the ability of the active components of the extract action as antioxidant, have suppressed prostaglandin synthesis from arachidonic acid metabolism, as well as blockage of histamine release from pro-inflammatory mediators [8]. The suppression of paw inflammation is an indication that the extract active components may have polarized cytokine activities towards helper 1 T cells (Th1). As Th1 and Th2 antagonize each other in reciprocal patterns, this may however, assist in intracellular pathogens elimination, leading to an effect on DTH. The 150 mg/kg bw OPLE initially potentiated DTH as similarly reported for other herbs [9]. This was acclaimed to be due to the recruitment of immnocytes, and macrophages into the inflammatory locus, during immune system activation. It later enhanced paw inflammation recovery, explained by the compound activities at both ends of T cell activation and or suppression required to combat diseases [10]. The oil palm leaf contains various flavonoids [2], which may be the reason for the paw inflammation suppression in the high dose of 300 mg/kg bw [Table 1]. Green tea catechins reportedly produced similar effects on inflammation and immunomodulation [11]. Moreover, researches using animal studies have suggested that, a shift to a Th1 cellular immune response is adaptive in actions against infections, and therefore, may increase susceptibility to chronic inflammation and autoimmune diseases. This may be the case for normalization of the white blood cell and lymphocyte counts at the higher doses of 300 mg OPLE [Table 2]. Reduction of lymphocyte counts by the 2 g OPLE /kg bw dose, further indicates the immunosuppression properties by OPLE compounds. This may result to lymphocytopenia [12]· and may be subject to intercurrent viral, bacterial, parasitic and or fungal infections.

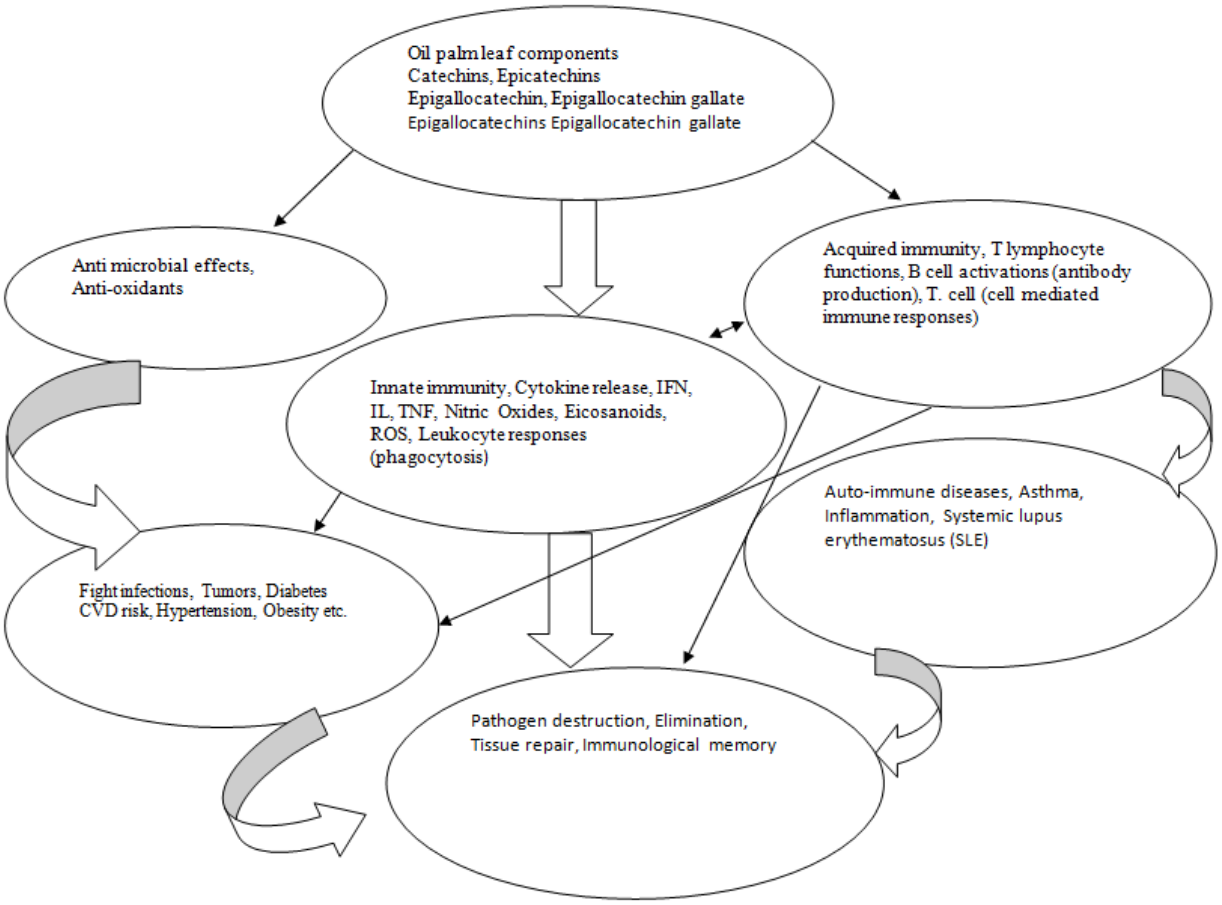

Fig. 1: Probable Mechanism of Oil Palm Leaf Extract (OPLE) Anti-inflammatory Effects.

Mechanisms for the OPLE phenolic compounds immune system modulation may possibly include (i) innate immunity: cytokines release (IFN, IL, TNF, eicosanoids, nitric oxides, ROS), leukocyte responses (phagocytosis) or (ii) acquired immunity: T lymphocyte

functions, B cell activations (antibody production), T cell (cell mediated immune responses) Figure 1. This result suggests that OPLE extract possess strong in-vivo anti-inflammatory effects and therefore, may be potentially useful for various disorders related to aging and cell mediated immune responses.

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