Available online at www.sciencerepository.org

Science Repository

Research Article

Acute Toxicity and the Effects of *Mangifera indica* on Serum IL-6, and IFN-γ in Breast Cancer-Induced Albino Rats

Hussaini Mohammed Alhassan[1*], Mohammed Haruna Yeldu[2], Usman Musa[3], Isiyaku Adamu[1], Ahmad Hamidu Marafa[4] and Hamisu Abdullahi[1]

[1]*Department of Immunology, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria*
[2]*Department of Chemical Pathology, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria*
[3]*Faculty of Veterinary Medicine, Department of Veterinary Pathology, Usmanu Danfodiyo University, Sokoto, Nigeria*
[4]*Department of Haematology, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria*

ARTICLE INFO

Article history:
Received: 1 April, 2021
Accepted: 5 May, 2021
Published: 25 May, 2021

Keywords:
Mangifera indica
IL-6
INF-γ
DMBA
Breast cancer

ABSTRACT

Background: Breast cancer is an uncontrolled growth of breast tissue, which develops in cells lining the milk ducts and lobules, it's the most common neoplasm in the female. Breast cancer has been declared a universal disaster as it is expected to nearly triple between 2020 and 2030, as most available drugs have not shown any desirable outcome.

Aims/Objective: This research aimed to evaluate the acute toxicity and effects of *M. indica* on serum IL-6, and IFN-γ in cancer-induced albino rats.

Materials/Methods: *Mangifera indica* was subjected to plant identification/authentication and extractions, the acute toxicity was determined using Lorke's method. They are 6 groups of 4 rats each. The groups are normal, positive controls, Ascorbic acids, 500mg, 1000mg and 1500mg *M. indica* groups. All the groups were induced with 65 mg/kg-1 b.w. of 7,12 Dimethylbenzene-(α) anthracene (DMBA), except Group I and observed for 14 days, before treatment with 100mg of AA (Group III), and 500mg, 1000mg, and 1500mg of *extracts* (Groups IV- VI) respectively. The rats were sacrificed, 24 hours. after the last treatment.

Results: The results of acute toxicity study of the extracts in both phase 1 and 2, has shown no signs of behavioural changes and mortality in all the experimental animals. This has proven that methanolic extracts of *M. indica* is safe. There was a significant down-regulation of serum IL-6, and INF-γ expressions (P>0.005).

Conclusion: This research indicated that *M. indica* extract is safe and possesses anti-tumor, and immunomodulatory effects, it may be used for breast cancer management.

Research Highlights

i. This research assessed the effects of *Mangifera indica* in the treatment of breast cancer.
ii. There was a significant downregulation of serum IL-6 and IFN-γ after treatment with extract of *Mangifera indica*.
iii. The results signified that *M. indica* possessed anti-cancer, immunosuppressive and immunomodulatory potentials.
iv. *M. indica* maybe used as an alternative treatment options in the breast cancer management.
v. Clinical trials should be embarked on to evaluate if the results obtained can replicated in human.

Introduction

A breast tumor is a neoplasm originating in the mammary gland [1]. Signs of breast cancer may include a lump in the breast, a change in breast shape, dimpling of the skin, fluid coming from the nipple, an inverted nipple, and a red scaly patch of skin [2]. In those with a distant spread of the disease, there may be bone pain, swollen lymph nodes,

Correspondence to: Dr. Hussaini Mohammed Alhassan, Ph.D., Department of Immunology, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria; Tel: +2348039705336; GSM: +2348039705336; E-mail: halhassanmohd@gmail.com, hussaini.alhasan@udusok.edu.ng

http://dx.doi.org/10.31487/j.COR.2021.05.01

shortness of breath, or yellow skin [3]. World breast cancer congress has currently declared breast cancer a universal disaster as the global burden of breast cancer doubled between 1975 and 2000 and is expected to nearly triple between 2020 and 2030 [4]. The International Agency for Research on breast cancer estimates that the incidence of mortality and prevalence arising from about 184 countries of the world revealed that there were 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with breast cancer [5]. Risk factors for developing breast cancer include being female, obesity, lack of physical exercise, drinking alcohol, hormone replacement therapy during menopause, ionizing radiation, early age at first menstruation, having children late or not at all, older age, prior history of breast cancer, and family history [6]. Breast cancer most commonly develops in cells from the lining of milk ducts and the lobules that supply the ducts with milk [6].

In those who have been diagnosed with cancer, a number of treatments may be used, including surgery, radiation therapy, chemotherapy, hormonal therapy and targeted therapy [6]. In those whom breast cancer has spread to other parts of the body, treatments are mostly aimed at improving the quality of life and comfort [7]. There are reports which indicate increases in systemic markers of inflammation in breast cancers showing a potential relationship between breast cancer and inflammation [8]. Cytokines are a broad and loose category of a small protein (5-20 kDa) that are important in cell signaling, their release has an effect on the behaviour of cells around them [9]. Cytokines are involved in autocrine, paracrine and endocrine signaling as immune-modulating agents [9]. The balance of cytokines is critical for a normal immune response. Irregular cytokines level can shift the immune response from being beneficial to be harmful [10]. An increase in inflammatory cytokines such as IL-1, IL-6, IL-10, IL-18, TNF-α, INF-γ, has been observed in the blood of patients with breast cancer [10]. Overexpression of inflammatory cytokines plays a vital role in the pathogenesis of breast cancer complication [11].

IL-6 is a mediator with a pleiotropic effect on inflammation, immune response, and hematopoiesis. It is an important cytokine in acute inflammatory responses that has both local and systemic effects [12]. It reduces the synthesis of a variety of other inflammatory mediators in the liver, stimulates neutrophil production in the bone marrow, and promotes the differentiation of IL-17 producing helper T cells [12]. IL-6 is synthesized by mononuclear phagocytes, vascular endothelial cells, fibroblasts, and other cells in response to PAMPs and in response to IL-1 and TNF [13]. It is produced during the inflammatory process of breast cancer and a principal marker of chronic inflammation commonly detected in breast tumor [14]. IFN-γ, or type II interferon, it is an important activator of macrophages and inducer of Class II major histocompatibility complex (MHC) molecule expression. Aberrant IFN-γ expression is associated with a number of autoinflammatory and autoimmune diseases. The importance of IFN-γ in the immune system stems in part, from its ability to inhibit viral replication directly and most importantly from its immunostimulatory and immunomodulatory effects [15]. IFN-γ is produced predominantly by natural killer (NK) and natural killer T (NKT) cells as part of the innate immune response, and by CD4, Th1 and CD8 cytotoxic T lymphocyte (CTL) effector T cells and also plays a major role in establishing cellular immunity in mammary cancer [15].

Approximately 60% of drugs currently in use for mammary cancer treatment have been isolated from natural products. the use of a medicinal plant could be an alternative means to improve health care globally, particularly in poor resource countries [16]. The medicinal plant is locally available, easily accessible regardless of social status [17]. Phytochemicals that emanate from plants offer a notable prospect for the exploration of new varieties of therapeutics [11]. As a result, an effort is being geared globally toward the exploitation of these medicinal plants which possess a significant amount of phytochemicals with a beneficial effect in tackling mammary cancer and associated complications [18].

Mangifera indica known as mango tree is a plant that is locally abundant in Nigeria in the Savanna zones, traditionally, *Mangifera indica* stem bark has been employed in the treatment of several ailments like skin irritation, abnormal lumps or mast tissue (tumor), vaginal warts, neck and breast. It is also used in the treatment of pyrexia of unknown origin. Extract of leaves and unripe fruit have demonstrated anti-bacterial, anti-inflammatory and in treatment for ulcers and anti-snake venom [19]. The increasing prevalence of breast tumor and its complication in rapid succession has become a major concern worldwide [10]. Various mammary anti-cancer medications have been formulated, but have not yielded the desired outcome, this has stimulated the need to explore the potential anti-tumor, anti-inflammatory potentials of *Mangifera indica* stem bark [18]. Hence, this research was design to evaluate the acute toxicity and effects of *M. indica* on serum IL-6, and IFN-γ in 7,12 dimethylbenzene (α) anthracene (DMBA)-induced breast tumor in female albino rats.

Materials and Methods

I Plant Collection

The *Mangifera indica* stem bark was collected from Kafanchan, Jema'a Local Government, Kaduna State and identified by botanists in the herbarium of Faculty of Pharmaceutical Sciences of the Usmanu Danfodiyo University, Sokoto, Nigeria and allocated with the voucher number PCG/UDUS/ANAC/0001.

II Plant Extraction

Mangifera indica stem bark methanol extract was prepared by the maceration method. 300g of *M. indica* stem bark powder was macerated in 1500ml methanol for 72 hours with continuous shaking kept at room temperature (22-25°C). The supernatant was filtered using Whatman number 1 filter paper and then the filtrate was concentrated in an oven at 48°C to obtain 32g brown powder extract. The dried crude extract was stored in a refrigerator at low temperature (4°C) in sterile plastic bottles, at the Faculty of Pharmaceutical Sciences, UDUS, until required for use.

III Experimental Animals

Forty (40) adult healthy female Albino rats (weight 120-140g) were purchased from the Faculty of Pharmaceutical Sciences Ahmadu Bello University, Zaria, Nigeria. They were allowed to acclimatize at the animal house of the Faculty of Pharmaceutical Sciences of the Usmanu Danfodiyo University, Sokoto, Nigeria for a week before the

commencement of the experiment. Rats were kept in the cages at room temperature (22-25℃) under a normal 12h light/dark cycle with free access to food and water. These conditions were maintained constant throughout the experiments. The animals were housed according to regulations for experimental animals' welfare.

IV Determination of Acute Toxicity (Lethal Dose, LD50)

LD50 was determined in accordance with the procedure outlined by Lorkes [20]. The method has two (2) phase, 1 and 2 respectively. Sixteen (16) Female albino rats were used for this study. In phase, I, twelve (12) rats were randomly divided into four (4) groups of three (3) animals each with the first group as a control. The extract was administered to rats in groups II-IV in single oral doses of 10mg/kg, 100mg/kg and 1000mg/kg body weight respectively, using an oral cannula. The control group (group I) received an equal volume of distilled water. In phase II, four (4) rats were randomly divided into four (4) groups of One (1) animal each, with the first group as a control. The extract was administered to rat in groups 2-4 in single oral doses of 1600mg/kg, 2900mg/kg and 5000mg/kg body weight respectively, using an oral cannula. The control group (group I) received an equal volume of distilled water. Observation of toxic symptoms was made and recorded within the first hour, four hours, twelve hours and subsequently for 24 hours after the administration of the extract. Behavioural parameters and mortality were monitored.

V Experimental Design

Twenty-four (24) albino rats were randomly allocated to five experimental groups of 4 rats per group as follows:

Groups (n =4 per group) and Treatment Options

Group I, (Normal control): Rats will be fed orally with normal saline, throughout the whole period of the experiment.
Group II. (Positive control): Rats will be inducted with a single dose of 65 mg/kg body weight of DMBA and fed orally with normal saline throughout the whole period of the experiment.
Group III, Ascorbic acid (AA): Rats will be inducted with a single dose of 65mg/kg-1 body weight of DMBA, observe for 14 days, followed by a daily subcutaneous injection of Ascorbic acid (100 mg/kg/day) for another 14 days.
Group IV 500mg treated group: Rats will be inducted with a single dose of 6mg/kg body weight of DMBA and observe for 14 days and then intervene with 500mg/kg/day of methanolic extracts of *M. indica* administered once orally per day for 14 days.
Group V, 1000mg treated group: Rats will be treated with a single dose of 65 mg/kg body weight of DMBA and be observe for 14 days and intervene with 1000 mg/kg/day of methanolic extract of *M. indica* administered once orally per day for 14 days.
Group VI, 1500mg treated group: Rats will be treated with a single dose of 65mg/kg body weight of DMBA and be observe for 14 days and intervene with 1500 mg/kg/day of ethanolic extract of *M. indica* administered once orally per day for 14 days.

VI Induction of Mammary Tumor

A single dose of dimethylbenzyl (α) anthracene (DMBA), 65 mg/kg/b.w.t was administered to albino rats subcutaneously. The rats were observed for the development of breast tumor after 14 days of induction. A week after the induction, Polyuria, low activities, the appearance of tumor noodles as observed by palpation with the scratching of itching abdomen. Only rats with visible mammary noodles were included in the study.

VII Laboratory Analysis

i Blood Sample Collection and Processing

The blood samples were collected 24 hours after the last treatments, in a sterile plain and EDTA bottles. The samples collected into a plain tube was allowed to clot at room temperature and later centrifuged at 3000g for 5 minutes. The clear unhaemolysed sera were transferred into labelled sterile serum bottles tightly capped and stored at -20℃ until use.

ii Estimation of Serum Cytokines Concentrations

Serum cytokines concentrations of IL-6 and IFN-γ were measured with Sandwich-ELISA technique using kit procured from E-lab Science Technology (USA), Cat. Numbers: Rat IL-6 Cat No. SEKR-0005, and Rat IFN-γ Cat No. SEKR-0008. The procedure was carried out with strict adherence to the manufacturer's instructional manual. Serum cytokine levels were estimated using the mean absorbance for each set of standard control and sample using standard calibration curves with absorbance on the (y-axis) against the concentration on the (x-axis) and unknown concentration determined by interpolation method.

VIII Data Analysis and Presentation

Multiple comparisons of the mean value were carried out using one-way analysis of variance (ANOVA) and SPSS software version 20. The result was analysed and presented using tables and graphs.

Results

I Acute Toxicity Study (LD50)

The results of acute toxicity study of *M. indica* stem bark extract (MISBE) in both phase 1 and 2, taken within the first hour, four hours, twelve hours and subsequently, 24 hours after the administration of the extract has shown no signs of behavioural changes and mortality in all the experimental animals. This has proven that methanolic extracts of *M. indica* is safe and non-toxic (Tables 1 & 2).

II Effect of MISBE on Serum IL-6

Serum level IL-6 was significantly higher in the inducted group (positive control) when compared with not inducted (negative control). Serum level IL-6 was significantly lower in DMBA+100 mg A.A, DMBA+500 mg MISBE, DMBA+1000 mg MISBE and DMBA+1500 mg MISBE groups compared with positive control groups (DMBA+ 65 mg).

Statistical difference (P-Value = 0.00) in all the groups was obtained with $P \leq 0.05$. (Table 3).

III Effect of MISBE on Serum INF-γ

Serum level INF-γ was significantly higher in the positive control group (65 mg DMBA) when compared with the negative control. However, INF-γ significantly decreases in DMBA+100 mg ascorbic acid, DMBA+500 mg MISBE, DMBA+1000 mg MISBE and DMBA+1500 mg MISBE. However, there was a statistical difference (P≤0.05) in DMBA+1000 mg MISBE and DMBA+1500 mg MISBE when compared with DMBA+100 mg Ascorbic Acid. Moreover, there was no statistical difference in the positive group, DMBA+100 mg Ascorbic acid, DMBA+ 500 mg MISBE, when compared with negative control (Table 4).

Table 1: Phase 1 of Acute toxicity (LD50) Study of Methanol extract stem bark of *Mangifera indica* in Albino rats.

Groups	No. of animals	Dosage/kg body weight	Volume of extract	Observational period	Behavioural change	Mortality
1	3	Distilled water	1ml	Up to 24hrs	None	None
2	3	10mg	1ml	Up to 24hrs	None	None
3	3	100mg	1ml	Up to 24hrs	None	None
4	3	1000mg	1ml	Up to 24hrs	None	None

Phase 1 acute toxicity study has 4 groups of 3 rats each, the first group is the control, feed with distilled water, the second to fourth group are given 10mg, 100mg,1000mg. kg/bw respectively. They were observed for 24 hours no sign of behavioural change or mortality weas recorded.

Table 2: Phase 2 of Acute toxicity (LD50) Study of Methanol extract stem bark of *Mangifera indica* in Albino rats.

Groups	No. of animals	Dosage/kg body weight	Volume of extract	Observational period	Behavioural change	Mortality
1	1	Distilled water	1ml	Up to 24hrs	None	None
2	1	1600mg	1ml	Up to 24hrs	None	None
3	1	2900mg	1ml	Up to 24hrs	None	None
4	1	5000mg	1ml	Up to 24hrs	None	None

Phase 2 acute toxicity study has 4 groups of 1 rat each, the first group is the control, fed with distilled water, the second to fourth group are given 1600mg, 2900mg, 5000mg/kg/bw respectively. They were observed for 24 hours no sign of behavioural change or mortality weas recorded.

Table 3: Serum IL-6 in breast tumor induced Albino Rats.

Group (n=4)	IL-6 (pg/ml)	F- Value	P- Value
I (Non DMBA)	39.07±0.69		
II + 65 mg DMBA/Single dose	44.64±0.81[a]		
III +65 mg DMBA+100 mg AA/10days	32.01±1.04[ab]	45.18	0.00
IV +65 mg DMBA+500 mg MISBE/10days	28.64±0.80[ab]		
V +65 mg DMBA +1000 mg MISBE/10days	23.68±2.15[abc]		
VI +65 mg DMBA +1500 mg MISBE/10days	39.83±0.86[cde]		

Data in the table above are expressed as Mean ± SEM, [a]P≤0.05 compare with non DMBA, [b]P≤0.05when compare with 65 mg DMBA/single dose, [c]P≤0.05 when compare with 65 mg DMBA +100 mg AA/10 days, [d]P≤0.05 when compare with 65 mg DMBA + 500 mg MISBE/10 days, [e]P≤0.05 when compare with 65 mg DMBA +1000 MISBE/10 days, IL-6: Interleukin-6; DMBA: 7,12 Dimethylbenzyl (α) Anthracene; MISBE: *Mangifera indica* Stem Bark Extracts; AA: Ascorbic Acid. Values differ significantly at P≤0.05. (One-way ANOVA) by instant graph pad prism VI followed by Turkey Post-Hoc Test.

Table 4: Serum IFN-γ in breast tumor induced Albino Rats.

Group (n=4)	IFN-γ (pg/ml)	F- Value	P-Value
I (Non DMBA)	60.56±0.91		
II + 65 mg DMBA/Single dose	72.41±2.35		
III +65 mg DMBA+100 mg AA/10days	72.64±2.53	5.14	0.004
IV +65 mg DMBA+500 mg MISBE/10days	65.60±2.70		
V +65 mg DMBA +1000 mg MISBE/10days	54.59±7.02[b, c]		
VI +65 mg DMBA +1500 mg MISBE/10days	53.50±3.67[b, c]		

Data in the table above are expressed as Mean ± SEM, [b]P≤0.05 when compare with 65 mg DMBA/Single dose, [c]P≤0.05 when compare with 65 mg DMBA + 100 AA/10 days, IL-6: Interleukin-6, DMBA: 7,12 Dimethylbenzyl (α) Anthracene, MISBE: *Mangifera indica* Stem Bark Extracts; AA: Ascorbic Acid. Values differ significantly at P≤0.05. (One-way ANOVA) by instant graph pad prism VI followed by Turkey Post-Hoc Test.

Discussion

The acute toxicity (LD50) study shows no death or behavioural changes in the entire groups in the two phases. This indicates that *M. indica* stem bark extract (MISBE) is safe, these findings are in agreement with the belief of the users that it is safe, and no harmful effects have been observed among them. The organization for economic cooperation and development (OECD, Paris and France) recommended chemical labelling and classification of acute systemic toxicity based on oral LD50 values as very toxic, <5mg/kg; toxic >5<50mg/kg; harmful, >50<500mg/kg and not toxic or harmful, >500mg/kg. Furthermore, in a more recent development, Mainasara *et al.*, in their published report on hepatoxicity in Albino rats exposed to MILE, concluded that MILE was relatively safe and is not likely to produce a toxic effect when albino rats were infused with 500 mg/kg/b.w.t [21]. Our result is in agreement with the report by Ramadan *et al.*, [22]. Bashir *et al.*, also reported similar findings on *Allium sativum* [23].

Treatment of inducted rats with MISBE and ascorbic acid was commenced 14 days after induction which lasted for 14 days. The research study reveals that there is a significant decrease in IL-6 concentration with the ascorbic acid-treated group, with a corresponding decrease in MISBE treated group with $p \leq 0.05$, when compared with the positive control. Based on this finding, it can be inferred that MISBE decreases the concentration of IL-6 at higher doses (i.e., 1500 mg). IL-6 can induce the differentiation of activated B-cell into antibody-producing plasma cells [13]. Our findings are in agreement with the study of Xiao *et al.*, which reported that aqueous extract of MILE down-regulate IL-6 production [24]. However, the study of Liu *et al.*, on aqueous extract of MILE disagree with these findings [25].

In our present study, MISBE, have been proven to possess potent immunostimulating effect by significantly decreasing IFN-γ production in Albino rat. The level of serum IFN-γ was drastically reduced after MISBE and AA treatments across the group, the higher doses of MISBE was seen to be more effective when compared with the standard treatment of ascorbic acid ($p \leq 0.005$). This shows that treatment with MISBE and ascorbic acid was significant. It is assumed that the observed decreased in the level of IFN-γ may slow or show retardation in the onset of breast cancer in the albino rat, and this could lead to increased receptor sensitivity and repairing of mammary cells. Our findings suggest that an increased concentration of MISBE over a prolonged period could lead to a significant level of cell repair [26]. The study findings show that there is a significant decrease in IFN-γ concentration which corresponds with an increased dose of MISBE across the treatment group ($p \leq 0.05$). Since there is a general decrease in IFN-γ secretion across the group, these findings suggest stimulative activity of MISBE on immune cells.

Conclusion

The methanolic Stem bark extract of *Mangifera indica* has shown anti-tumor and immunomodulatory effects. The extract was able to down-regulates IFN- γ and IL-6. Therefore, *Mangifera indica* may serve as a better therapeutic option in the management of breast cancer patients.

Recommendation

In view of the potentials effects of MISBE as revealed in this study, there is a need to carry out clinical trials to see, if the same result will be replicated in human. Moreso, research studies should be initiated, to actually establish the mechanisms by which MISBE exerts its anti-tumor and immunomodulatory activity in the body.

Acknowledgement

We wish to acknowledge the efforts of the Immunology Department and Faculty of Pharmaceutical Sciences, for providing the enabling environment and the facilities necessary to carry out this research.

Ethical Approval

The ethical approval for the research was obtained from the UDUS Research and ethical committee and the study was conducted in accordance with the Helsinki guide for the care and use of laboratory animals.

Conflicts of Interest

None.

Funding

None.

REFERENCES

1. Kirpenstein Jones B, Regan MM, Bouzyk M (2018) Genotype among Postmenopausal Women with Endocrine, Invasive Breast Cancer. *Cancer Res* 3: 101-106.
2. Giordano SH, Hortobagyi GN (2003) Inflammatory breast cancer: clinical progress and the main problems that must be addressed. *Breast Cancer Res* 5: 284-288. [Crossref]
3. Albert LH (2018) American Cancer Society guidelines on nutrition and physical activity for cancer prevention. *Cancer J Clin* 20: 30-62.
4. Stones P, Aljada A (2017) Phamacological uses of Mangifera indica extract in treatment of cancer. *Trends Immunol* 25: 4-7.
5. Andreu GLP, Maurmann N, Reolon GK, De Farias CB, Schwartsmann G et al. (2010) Mangiferin, a naturally occurring glucoxilxanthone improves long-term object recognition memory in rats. *Eur J Pharmacol* 635: 124-128. [Crossref]
6. Lo SS, Mumby PB, Norton J, Rychlik K, Smerage J et al. (2010) Prospective multicenter study of the impact of the 21-gene recurrence score assay on medical oncologist and patient adjuvant breast cancer treatment selection. *J Clin Oncol* 28: 1671-1676. [Crossref]
7. WHO (2018) Global Status Report on communicable diseases. World Health Organization, Geneva 1-176.
8. Luqman S, Srivastava S, Kumar R, Maurya AK, Chanda D (2012) Experimental Assessment of Moringa oleifera Leaf and Fruit for Its Antistress, Antioxidant, and Scavenging Potential Using In Vitro and In Vivo Assays. *Evid Based Complement Alternat Med* 2012: 519084. [Crossref]

9. D'Anello L, Sansone P, Storci G, Mitrugno V, D'Uva G et al. (2010) Epigenetic control of the basal-like gene expression profile via Interleukin-6 in breast cancer cells. *Mol Cancer* 9: 300. [Crossref]
10. Stumvoll M, Goldstein BJ, Van Haeften TW (2018) Mammary Cancer principles of pathogenesis and therapy. *Lancet* 365: 1333-46.
11. Navarro González JF, Mora Fernández C (2017) The role of inflammatory cytokines in mammary cancer. *J Am Society Nephrol* 19: 433-442.
12. Latz E, Xiao TS, Stutz A (2013) Activation and regulation of the inflammasomes. *Nat Rev Immunol* 13: 397-411. [Crossref]
13. Tanaka T, Narazaki M, Kishimoto T (2014) IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol* 6: a016295. [Crossref]
14. Willerson JT, Ridker PM (2004) Inflammation as a cardiovascular risk factor. *Circulation* 109: 2-10. [Crossref]
15. Sunder J, Sujatha T, Kundu A (2016) Effect of Morinda citrifolia in growth, production and immunomodulatory properties in livestock and poultry: a review. *J Exp Biol Agricultural Sciences* 4: 249-265.
16. Maviya N, Jain S, Malviya S (2010) Antidiabetic potential of medicinal plants. *Acta Pol Pharm* 67: 113-118. [Crossref]
17. Maghrani M, Zeggwagh NA, Michel JB, Eddouks M (2005) Antihypertensive effect of Lepidium sativum L. in spontaneously hypertensive rats. *J Ethnopharmacol* 100: 193-197. [Crossref]
18. Atmakuri LR, Dathi S (2016) Current trends in herbal medicines. *J Pharm Res* 3: 109-113.
19. Shah JI, Zhao W, Zhang M, Usary J, Murrow G et al. (2011) Comparative oncogenomics identifies breast tumors enriched in functional tumor-initiating cells. *Proc Natl Acad Sci U S A* 109: 2778-2783. [Crossref]
20. Lorke D (1983) A new approach to practical acute toxicity testing. *Arch Toxicol* 54: 275-287. [Crossref]
21. Mainasara PM, Siminoff LA, Davis G1J (2016) Adjuvant therapy for women with early breast cancer. *J Clin Oncol* 19: 980-991.
22. Ramadan A, Harraz F, El Mougy S (1994) Anti-inflammatory, analgesic and antipyretic effects of the fruit pulp of Adansonia digitata. *Fitoterapia (Milano)* 65: 418-422.
23. Lawal B, Shittu OK, Oibiokpa FI, Mohammed H, Umar SI et al. (2016) Antimicrobial evaluation, acute and sub-acute toxicity studies of Allium sativum. *J Acute Dis* 5: 296-201.
24. Xiao Y, Chen D, Yan J, Yokoyama Y (2002) Mechanism of action of Tripterygium Wilfordii polyglycoside on experimental endometriosis. *Eur J Gynaecol Oncol* 23: 63-67. [Crossref]
25. Liu J, Shen JX, Hu JL, Huang WH, Zhang GJ (2014) Significance of interleukin-33 and its related cytokines in patients with breast cancers. *Front Immunol* 5: 141. [Crossref]
26. Cho JY, Kim AR, Yoo ES, Baik KY, Park MH (2012) Ginsenosides from panax ginseng differently regulate lymphocyte proliferation. *Planta Med* 68: 497-500. [Crossref]