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EFFECT OF AQEUOUS STEM BARK EXTRACT OF *VITELLARIA PARADOXA* ON IFN-γ and IL-4 LEVELS IN ALBINO RATS

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ABSTRACT

Vitellaria paradoxa is a well-known plant of Africa origin whose leaves, roots, and stem bark extract have been used for treatment of various ailments. Cytokines are small, non-structural proteins with low molecular weight and high complex regulatory influence on inflammation and immunity, they are mostly produced by T helper cells. The study was aimed at assessing the effect of aqueous stem bark extract of *V. paradoxa* on IFN-γ and IL-4 in albino rats. A total of 25 Albino rats aged 12-18 weeks old, and weighted between (15g-38g) of either sex was used, they were fed with different concentration of stem bark extract of *Vitellaria paradoxa* for 21 days. Blood sample was collected through the cardiac puncture, serum levels of Interferon gamma (IFN-γ) and Interleukin- 4 (IL-4) cytokines were quantified by quantitative sandwich enzyme Linked immunosorbent assay (ELISA). *Vitellaria paradoxa* was found to have effect on IFN-γ and IL-4 cytokines of Albino rats by increasing their levels. The result shows statistically significant increase. Therefore, since the extracts possesses immunostimulatory activity, it is highly recommended to be use in treatment of different infection but at a control dose.

Key words: *Vitellaria. paradoxa*, IFN-γ, IL-4, Albino rats, immunomodulation

INTRODUCTION

Natural products from plants, animals and minerals are the basis for treating human diseases [1]. Medicinal plants are presently in demand and their acceptance is increasing progressively. In fact, the use of medicinal plants for the treatment of diseases dates back to the history of human life, that is, since human beings have sought a tool in their environment to recover from disease, the use of plants was their only choice of treatment [2]. Different parts of plants extract were used for curing illness ranging from seeds, root, leaf, fruit, skin, flowers or even the whole plant. The active compounds in most parts of the medicinal plants have direct or indirect therapeutic effects and are used as medicinal agents. *V. paradoxa,* commonly known as shea tree, or sheanut tree, shea-butter tree, man ka'dai, or Ka'danya is a tree of the *Sapotaceae* family. It is the only species in genus *Vitellaria* [3,4]. The shea tree grows naturally in the wild in the dry savannah belt, it is abundant in African countries [3]. Shea butter has many uses and traditional applications, it is used extensively as food or for medicinal purposes. The leaves of the shea tree contain saponin which makes it lather in water and hence is used for washing and bathing [5]. The leaves were used for the treatment of stomach ache, fevers, eye problems and headaches especially in children [6]. The shea fruit is made up of a green epicarp, a fleshy pulp or mesocarp and a relatively hard shell or endocarp which encloses a shea kernel or embryo. The fleshy pulp is sweet and is eaten as food. The pulp is also used to make jam [7]. The roots of the shea tree are used by locals in Northern Nigeria as chewing sticks, for treatment of jaundice, diarrhoea and stomach pain [8]. The stem bark of the shea tree is claimed to be able to cure diabetes and destroy intestinal worms. The shea tree produces latex, which when mixed with palm oil produces adhesive glue. *V paradoxa* has been employed in the treatment of several ailments. It promotes wound healing and soothes skin irritation, also used to treat inflammation, rashes in children, dermatitis, chapping and ulcers, as well as rub for rheumatism [9]. Nutritionally, the pulp of the shea fruit is a rich source of micro nutrients and sugars including ascorbic acid, calcium, vitamins, glucose, fructose and sucrose [7]. Shea butter is a good sun screening agent. Sun-screening agents act by absorbing or reflecting some of the ultraviolet (UV) radiation from the sun and prevents it from reaching the skin. This helps to protect the skin from sunburn, preventing erythema and also reducing further risk of skin cancer induced by the sun's rays. According to Situm, during the cold season and summer when the weather is extreme, shea butter is considered as the best skin care during the period because it provides the extra moisture, nutrients and protection needed by the skin. Shea butter has been considered one of the best anti-ageing and moisturising agents for the skin [5,10]. This property of shea butter helps to soften the skin and stimulates cell regeneration hence reduce the aging process [5]. Shea butter is reported to show anti-inflammatory properties attributed to the several derivatives of cinnamic acid that it contains [11] inhibiting Inos, Cox-2, and Cytokines through the Nf-Kb pathway. The ability of shea butter to reduce reaction to skin irritations was also demonstrated in two separate studies [12,13].

Cytokines are large group of soluble extracellular glycoproteins and serve as key intracellular regulators and mobilizers, cells of the immune system communicate with one another by releasing and responding to chemical messengers called cytokines [14,15]. Cytokines are protein molecules secreted by

immune cells and act on the same cell that produces it or on other neighbouring or far away cells in order to coordinate and maintain appropriate immune response [16,17]. The thymus helper 1 (Th1) and thymus helper 2 (Th2) cytokines are required by the body for the production, development and maintenance of T regulatory (Treg) cells and for activation of induced programme cell death, thereby mediating tolerance and controlling unnecessary immune response that may lead to autoimmune diseases or hypersensitivity reaction [18]. IFN-γ is a Th1 cytokine responsible for inducing cell mediated immunity, regulation of inflammation and activation of macrophages against intracellular pathogens, such as viruses and bacteria. Whereas IL-4 is a Th2 cytokine responsible for inducing humoral immunity (regulation and activation of B-cells into plasma), regulation of inflammation and activation of eosinophils and mast cells against extracellular pathogens like parasites and fungi, and regulation of allergic responses [19]. The immunomodulatory activity of aqueous stem back extract *V. paradoxa* (ASBEVP) on IFN-γ and IL-4 cytokines in Albino wistar rats is not well documented. In this study we selected IFN-γ to represent Th1 cytokines and IL-4 to represent Th2 cytokines. The research provide some scientific information on immunomodulatory activity of AVPSBE on the production of IFN-γ and IL-4 in Albino rats.

MATERIALS AND METHODS

Plant collection and identification

V. paradoxa was collected from Rijau town, Rijau local government, Niger State, Nigeria. The stem bark, fruits and flower were identified in Herbarium section in Botany unit, Department of Biological Sciences, Faculty of Science, Usmanu Danfodiyo University, Sokoto, Sokoto state, Nigeria. The Herbarium officer identified the plant and assigned the specimen voucher number as UDUS/ANS/0248.

Preparation of *V. paradoxa* extract

The stem bark was cut into small pieces and air dried under shade at room temperature for 10 days. Subsequently, it was grinded into powder with a pestle and mortar. About 600g of the powdered stem bark was cold macerated with 2500ml of freshly prepared distilled water exhaustively for 24 hours with occasional shaking. The resultant mixture was filtered with local separation rag and then re-filtered with funnel packed with cotton wool at its base. The filtrate was evaporated to dryness in a hot air oven at a temperature of 50°C giving brown residue called 'crude extract' of *V. paradoxa.* The crude extract was stored in a refrigerator until required for use. Solutions of the extract were freshly prepared for each study.

Experimental Animals

A total of twenty five (25) Albino rats of 12-18 weeks old, weight between (15g-38g) of either sex were purchased from Department of Veterinary Pharmacology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. They were allowed to acclimatized for the period of two weeks before commencement of the experiment. They were fed with standard pelletized growers' feed (Vital feed, Jos Plateau) and water *ad libitum* in the animal house of Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto (UDUS). The animals were maintained under standard Laboratory conditions (25±5°C) and the experimental protocol of the animal house was adapted.

Determination of lethal dose (LD_{50}) for aqueous stem back extract of *V. paradoxa*

LD_{50} was determine in accordance with Lorke's method [20]. Twelve (12) Albino rats of either

sex were maintained under controlled conditions of temperature (20 –25°C) and humidity (55%) was used for toxicity study. In both phase 1 and phase 2 no mortality or sign of toxicity was observed.

Table 1: Determination of lethal dose (LD_{50}) for aqueous stem back extract of *V. paradoxa*

Dose (mg/kg)	First Phase	Second Phase
10	3	-
100	3	-
1000	3	-
1600	-	1
2500	-	1
5000	-	1

Study design

This is an experimental study design to determine the effect of aqueous stem bark extract of *V. paradoxa* on Th1 cytokine (IFN-γ) and Th2 cytokine (IL-4) in Albino rats.

Animal grouping and treatment

A total of twenty five (25) Albino rats were randomly divided into five groups and each group contain five rats. Group 1 received 10ml /kg/bwt of normal saline (Normal control), Group 2 received 25mg/kg/bwt of levamisole hydrochloride (Positive control), Group 3 received 250mg/kg/bwt of aqueous stem bark extract of *V. paradoxa* (ASBEVP), Group 4 received 500mg/kg/bwt of ASBEVP, Group 5 received 1000mg/kg/bwt of ASBEVP daily, using oral gavage cannula for a period of 21 days.

Table 2: Summary of Animal grouping and treatment

Groups (n=5)	Dose (mg/kgbwt) + Duration	Route of administration
Group I (Normal control)	Normal saline 10ml + 21 days	Orally
Group 2 (Positive control)	levamisole hydrochloride 25mg/kg + 21 days	Orally
Group 3 (Test)	ASBEVP 250mg/kg + 21 days	Orally
Group 4 (Test)	ASBEVP 500mg/kg + 21 days	Orally
Group 5 (Test)	ASBEVP 1000mg/kg + 21 days	Orally

n = no of animal per group, mg/kgbwt = milligram per kilogram body weight, ASBEVP = Aqueous stem back extract of *V paradoxa*

BLOOD SAMPLE COLLECTION AND PROCESSING

On day 21, 2 mls of blood were aseptically collected from each rat though the cardiac puncture and placed in a plain container. It was allowed to cloth at room temperature (25°C) and spun at 3000rpm for 5 minutes to obtain the serum. The clear, unhaemolyzed serum was collected in serum separation tube and stored at -20^0c until assay in batches.

LABORATORY ANALYSIS

Estimation of IFN-γ and IL-4

Serum level of IFN-γ and IL-4 was estimated using the quantitative sandwich ELISA procured from Beijing Solarbio Science and Technology Co., Ltd China. IFN-γ (Catalog Number PRS-00153hu, storage tem. 2°C to 8°C), IL-4 (Catalog Number PRS-00153hu, storage tem. 2°C to 8°C). The procedures were carried out in accordance with the manufacturer's instructions.

DATA ANALYSIS

The results obtained was entered into SPSS version 22 (IBM, USA) for analysis. Test for normality was carried out to ascertain the distribution of the variables. The data was not normally distributed, Shapiro-Wilk, supported by Q-Q plot. As such non-parametric tests were carried out, and the results obtained was presented in table as median and interquartile (IQR). The P-value of ≤ 0.05 was used to determine the level of statistical significance.

RESULTS

The result presented in Table 1: Shows the median (IQR) of IFN-γ and IL-4 values, the concentration of IFN-γ increased with increase in concentration of ASBEVP with group 5 having the highest median and IQR of 36.67 pg/ml (35.34-38.44), when the groups were subjected to Kruskal Wallis test, a statistically significant (X^2 = 16.67, P = 0.03) difference was observed. The concentration of IL-4 increased with increase in concentration of ASBEVP with group 5 having the highest median and IQR of 7.2 ng/l (6.8-7.9), when the groups were subjected to Kruskal Wallis test, a statistically significant (X^2 = 47.36, P = 0.05) difference was observed. Comparison between the groups on the effect of ASBEVP on IFN-γ and IL-4 in Albino rats (Bonferoni multiple comparison) is shown in Table 2;

Table 1: Effect of Stem back extract of *V. paradoxa* on IFN-γ and IL-4 in Albino rats

Groups (n=5)	Treatment (mg/kg/bwt)	Media (IQR) conc. of IFN-γ (pg/ml)	Media (IQR) conc. of (IL-4) (ng/l)
Group I (NS)	10ml + 21 days	31.72 (28.53-33.00)	6.4 (5.6-7.2)
Group 2 (LEV)	25mg/kg+ 21 days	47.32 (45.60-51.12)	8.5 (7.4-9.3)
Group 3 (SBEVP)	250mg/kg+ 21 days	33.39 (30.45-36.22)	6.4 (6.0-7.7)
Group 4 (SBEVP)	500mg/kg+ 21 days	36.67 (35.34-38.44)	6.5 (6.1-7.8)
Group 5 (SBEVP)	1000mg/kg + 21 days	40.56 (37.56-42.89)	7.2 (6.8-7.9)
Kruskal Wallis test		*X^2 = 16.67, P = 0.03*	*X^2 = 47.36, P = 0.05*

NS = Normal saline, n = no of animal per group, mg/kg bwt = milligram per kilogram body weight, SBEVP = stem back extract of *V paradoxa,* LEV = levamisole hydrochloride, IQR = interquartile range, *X^2* = Kruskal Wallis value, P-value was obtained using Kruskal Wallis test

Table 2: Comparison between the groups on the effect of stem back extract of *V. paradoxa* on IFN-γ and IL-4 in Albino rats

Group (n=5)	IFN-γ p- value	IL-4 p- value
Grp 1 vs Grp 4	0.04	0.001
Grp 1 vs Grp 5	0.001	0.001
Grp 2 vs Grp 3	0.07	0.34
Grp 2 vs Grp 5	0.05	0.23
Grp 3 vs Grp 4	0.98	0.34

Discussion

Aqueous stem back extract of *V paradoxa* are abundantly gaining popularity globally as compared to allopathic medicine for treatment of different types of ailments [21]. Both immunostimulation and immunosuppressant needed to be put in check and balance depending on the interest in treatment regimen in order to regulate the normal immunological function [22]. The findings in this study shows that, the concentration of Th1 cytokine (IFN-γ) increased significantly with increased in dose of Stem back extract of *Vitelaria paradoxa* across the treatment groups (X^2 = 16.67, *P* = 0.03). Similarly, our finding demonstrated a statistically significant increase in the concentration of IL-4, Th2 cytokines in dose dependent manner across the treatment groups. This findings suggest that there is a potential role played by aqueous stem back extract of *Vitelaria paradoxa* on Th1 and Th2 cytokines that leads to the increased release of IFN-γ and IL-2 in albino rats. The group treated with 1000mg/kg tend to compete with standard immunostimulant drug (levamisole) used in this study. This finding is in consonance with an earlier report by Keshavarzi [23], who reported that level of IL-10 increase after treatment of the rats with different concentrations of *Biebersteinia multifida* extract. Similarly, Vahid [24] reported an increased Th1 and Th2 cytokines ration in isolated human PBMCs treated with hydro-thanolic extract of *Portulaca oleracea.* Another Study by Hamid [25] indicates an increased IL-2, IL-4, IL-6 and IFN-γ secretion in vivo following stimulatory effect of polyherbal formulation on Th1 and Th2 subset of lymphocyte in rats. The Th1 and Th2 cytokines are required for the production, development and maintenance of T regulatory (Treg) cells and for activation of induced programme cell death, thereby mediating tolerance and controlling unnecessary immune response that may lead to autoimmune diseases or hypersensitivity reaction [26]. In comparison between standard stimulatory drug and treatment extract, it is clearly shown that 1000mg/kg body weight group was nearly competing with the standard stimulatory drug (levamisole hydrochloride).

Conclusion

This study revealed that the aqueous stem bark extract of *V. paradoxa* induce the secretion of IFN gamma and IL-4 cytokines by T-helper cells in albino rats.

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