



Body Weight and Serum IgE Levels in Wistar Albino Rats Exposed to Chili Pepper (*Capsicum annuum* L.)

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Abstract

A nano-biotechnology investigation of the body weight changes and serum IgE levels of wistar Albino rats exposed to Chili pepper (*Capsicum annuum*) particles was carried out, in an attempt to proffer a safe nano-organic rat repellent to eradicate or control the Lassa fever outbreak in Nigeria and other endemic West African countries. Body weights of rats were measured before and after exposure to graded doses of *C. annuum* aroma. The wistar Albino rats were randomly placed into four different groups consisting of the control (0%) (A) and exposed groups of 10% (B), 50% (C) and 100% (D) pepper particles in solution. The rats were exposed to cotton balls soaked in the Chili pepper nano-solution for 10 days. At the end of which blood samples of the exposed rats and control were collected for the Radioimmunoassay (RIA). Mean body weights of the exposed rats were significantly ($p < 0.05$) different from that of the control rats. Mean body weights (in grams) before treatment were as follows: groups A (34.75 ± 8.421), B (282.25 ± 5.852), C (304.75 ± 9.179) and D (332.00 ± 13.115) while mean body weights (in grams) after treatment were A (275.50 ± 18.193), B (256.00 ± 6.481), C (279.25 ± 10.404) and D (306.00 ± 7.257). Serum IgE levels after exposure were $13.200 \text{ mg/ml} \pm 0.56 \text{ mg/ml}$, $16.1500 \text{ mg/ml} \pm 0.35 \text{ mg/ml}$ and $19.500 \text{ mg/ml} \pm 0.42 \text{ mg/ml}$ for exposed groups B, C and D respectively, values significantly ($p < 0.05$) higher than the mean value of serum IgE in the control rats ($9.90 \text{ mg/ml} \pm 0.20 \text{ mg/ml}$). The loss of body weight and raised serum IgE levels after exposure to Chili pepper nanoparticles, are indications that Chili pepper may have triggered allergic reactions in the Albino rats, and could be harnessed in part or wholly as a control measure in the fight against the Lassa fever endemic ravaging communities in Nigeria and other West African countries.

Keywords

Nano biotechnology; Lassa fever; Chili pepper; Serum IgE levels; Radioimmunoassay

Introduction

As at March 28, 2020, 185 deaths due to Lassa fever were confirmed in Nigeria alone, with 4,194 suspected cases recorded [1]. Lassa fever is a zoonotic acute viral illness, endemic in parts of West Africa, including Nigeria (Figure 1) and distributed by the multimammate

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rat (*Mastomys natalensis*) [2]. *M. natalensis* has been reported to be found frequently inside houses, where they have both intentional and unintentional contact with human beings [3]. Household rats can be a menace and difficult to get rid of. Rodents have gained the reputation as being one of the most persistent and ubiquitous vertebrate pests affecting human populations. They cause economic problems because of the damage they inflict on agricultural systems, environmental problems due to the chemicals used for their control, social problems associated with their close proximity to human habitation and health problems as carriers of zoonosis [4,5]. In the quest to get rid of these domestic pests, conventional pesticides have been widely employed. But, conventional pesticides possess inherent toxicities that endanger the health of families, farm operators, consumers, and the environment. Hence, the need for a nano-biotechnological solution because of its minimal cost and fewer ecological side effects. It is believed that the nano-botanicals will have advantages over broad-spectrum conventional pesticides. They affect only target pest and closely related organisms, are effective in very small quantities, decompose quickly, and provide the residue-free food and a safe environment to live [6]. Natural products represent one of the most important alternatives to control pests and diseases that affect plants and animals without deleteriously affecting environmental safety [6,7]. There is an age-long belief that the pungent aroma of Pepper (*Capsicum annuum*) is repellent to house rats [8]. The pepper plant in our locality was originated from south and Central America where it is still under cultivation [9]. Peppers are considered the first spice to have been used by human beings and there is an archaeological evidence of pepper and other fossil foods being used from as early as 6000 years ago [10]. The genus *Capsicum* has five domesticated species (*C. annuum*, *C. frutescens*, *C. chinense*, *C. pubescens* and *C. baccatum*) of which *C. annuum* L is the most widely cultivated species worldwide [11]. *Capsicum* can be distinguished by its pungency which varies with cultivar but generally higher in smaller fruit types than larger thick fleshed types. Pepper grows relatively quick with a maturity period of 3-4 months. In Nigeria, it is now grown as a monocrop on a large scale by both peasant and commercial farmers; according to Norman 1992, the derived savanna and northern savanna agro-ecologies are best suited for hot pepper production with an annual rainfall of 600 mm-1250 mm. Pepper particles are also used by the security agencies in the preparation of tear gas. The leaves are simple and alternate, elliptical to laccolite with smooth margins. They are high in vitamins A and C.

The phytochemical components of *C. annuum* L. have been extensively reported on. The phenolic compounds present in the chili pepper are attributed to many medicinally important properties such as anti-diarrheal, antimicrobial, antioxidant, antihyperglycemic, anti-lithogenic and antimutagenic activities [12]. Chili pepper fruits contain phenolic compounds such as flavonoids, β -catenin, capsaicinoids, carotenoids (capsanthin, capsorubin and cryptocapsin), vitamins A, B, C and E, glycolipids, glycerolipids and esters [11]. The principal pungent ingredient present in *Capsicum annuum* L. is the phenolic substance named capsaicin (trans-8-methyl-N-vanillyl-6-non-enamide). The capsaicin content of hot peppers varies from 0.1% to 1% [13].

Immunoglobulin E (IgE) is a type of antibody (or immunoglobulin (Ig) "isotype") that has only been found in mammals, [14]. IgE's main

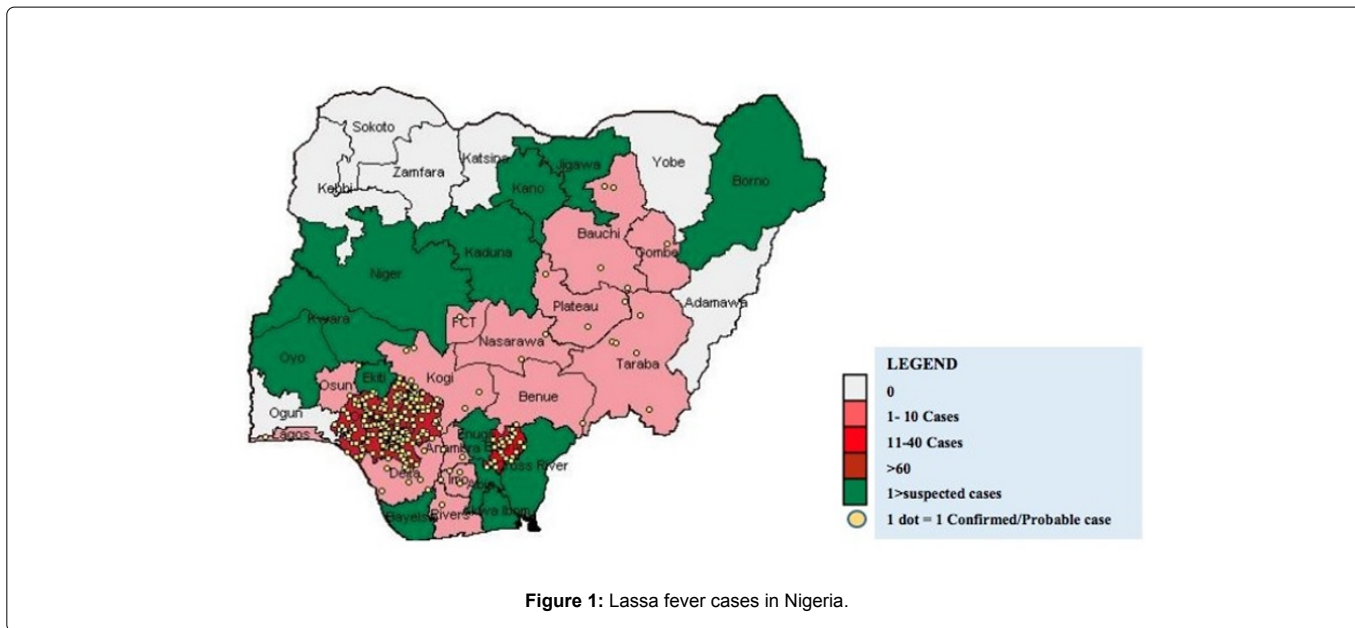


Figure 1: Lassa fever cases in Nigeria.

function is to provide immunity to parasites such as helminths [15] like *Schistosoma mansoni*, *Trichinella spiralis*, and *Fasciola hepatica* [16]. IgE is utilized during immune defense against certain protozoan parasites such as *Plasmodium falciparum* [17]. IgE also plays a pivotal role in responses to allergens, such as: anaphylactic drugs, bee stings, and antigen preparations used in desensitization immunotherapy.

Although IgE is typically the least abundant isotype-blood serum IgE levels in a normal ("non-atopic") individual are only 0.05% of the Ig concentration [18] compared to 75% for the IgGs at 10 mg/ml, which are the isotypes responsible for most of the classical adaptive immune response-it is capable of triggering the most powerful inflammatory reactions. A Radioimmunoassay (RIA) was used for the analyses. It is an immunoassay that uses radiolabelled molecules in a stepwise formation of immune complexes. A RIA is a very sensitive *in vitro* assay technique used to measure concentrations of substances, usually measuring antigen concentrations used by the antibodies.

Nanoparticles can be classified into different types according to their size, morphology, physical and chemical properties. Nanobiotechnology is a fusion of nanotechnology and biology and deals with nanoscale phenomena that occurs within the discipline of nanotechnology. The *C. annum* extract employed in this investigation is a typical nano-biotechnological tool [19].

Materials and Methods

All experimentations were conducted in accordance with the animal's scientific procedures acceptable at the Faculty of Biological Sciences, Cross River University of Technology, Calabar Campus. Thirty-two (32) wistar Albino rats (*Rattus norvegicus*) were used for this research and the rats were maintained in a hygienic environment with constant light and good ventilation, in good and well prepared rats' cages. They had access to rodent growers feed and water ad libitum. After 5 days of acclimatization, the rats were divided into four groups of eight rats each. The groups were group A (Control), Group B (10%), Group C (50%) and Group D (100%). The initial body weights of the rats were measured and subsequently throughout the duration of the research.

Preparation of *Capsicum annum* solution

To prepare the *Capsicum annum* solution, 100 g of pepper was dried at room temperature for two days before being pulverized into nanoparticles. 20 g of the pepper nano-powder was added into 100 ml of distilled water and stirred for 20 minutes using an LBC 15 electric blender, as the stock solution. Three concentrations of the pepper solution were prepared from the stock solution, 10% (0.02 g/ml), 50% (0.1 g/ml) and 100% (1 g/ml). Cotton balls were soaked in the pepper extract and dropped in the rats cages. The rats were exposed to the cotton balls for 10 days. New pepper soaked cotton balls were replaced every day. At the expiration of 10 days, the rats were anesthetized by chloroform inhalation and immediately blood samples were collected from them by cardiac puncture, and emptied into non-EDTA bottles for further analyses.

Radioimmunoassay

For the Radioimmunoassay (RIA), all materials were equilibrated and reagents were prepared at room temperature prior to use. Standard protocol for RIA [20] was followed at the end of which the absorbance of the solutions were determined.

Results

Mean body weights of rats before and after exposure to Chili pepper nanoparticles is presented in Figure 2. Before exposure, body weights (in grams) were 234.75 ± 8.42 for the control and 282.25 ± 5.852 , 304.75 ± 9.179 and 332.00 ± 13.115 for the groups B, C and D respectively. After exposure, the mean body weight (in grams) of the exposed rats dropped to 256.00 ± 6.481 , 279.25 ± 10.404 and 306 ± 7.25 for the exposed rats in groups B, C and D respectively, while for the control rats the body weight increased to 275.50 ± 18.19 .

Table 1 shows mean serum IgE concentrations of wistar Albino rats exposed to Chili pepper nanoparticles. The result for IgE levels for the control (A), 10% extract concentration (B), 50% extract concentration (C) and 100% extract concentration (D) were $9.90 \text{ mg/ml} \pm 0.20 \text{ mg/ml}$, $13.20 \text{ mg/ml} \pm 0.40 \text{ mg/ml}$, $16.15 \text{ mg/ml} \pm 0.25 \text{ mg/ml}$ and $19.50 \text{ mg/ml} \pm 0.30 \text{ mg/ml}$ respectively. The result shows that

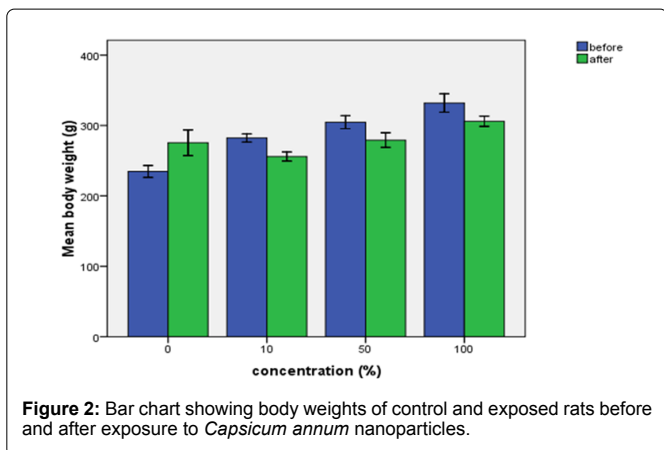


Figure 2: Bar chart showing body weights of control and exposed rats before and after exposure to *Capsicum annum* nanoparticles.

Table 1: Mean serum IgE level in both the control and exposed rats.

Group (%)	Serum IgE	SD	SE
A (0)	9.9	0.28284	0.2
B (10)	13.2	0.56569	0.4
C (50)	16.15	0.35355	0.25
D (100)	19.5	0.42426	0.3

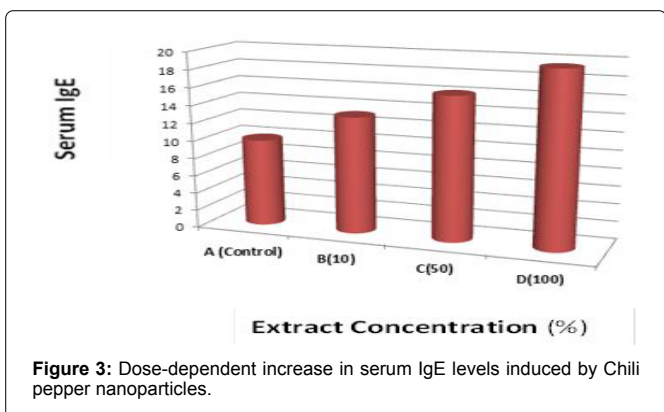


Figure 3: Dose-dependent increase in serum IgE levels induced by Chili pepper nanoparticles.

there was a significant increase ($p \leq 0.05$) in IgE level in rats exposed to 10% extracts concentration of *Capsicum annum* nanoparticles when compared to control; similarly there was a significant ($p \leq 0.01$) increase of IgE level in rats exposed to 50% and 100% extracts concentration of *Capsicum annum* nanoparticles when compared to control.

There was also a dose-dependent increase in serum IgE concentration levels among rats exposed to 10%, 50% and 100% *C. annum* nanoparticles respectively (Figure 3).

Discussion

Body weights of rats exposed to Chili pepper nanoparticles were significantly ($p \leq 0.05$) reduced across the three exposure groups. When compared with the control rats, the body weights of the exposed rats were affected in a dose-dependent manner (Figure 2). This corroborates report by Yoshioki et al. [21] that *Capsicum annum* aids in weight loss as demonstrated by them, that Capsaicin, the component that makes pepper hot, cause weight loss and fight fat build up by triggering certain beneficial protein changes in the body. Body weights have also been reported to be affected positively

or negatively by consumption of herbs singularly or in synergy with several others. Loss of body weight, was also recorded by Adelaja et al. [22], when they tested the anti-spermatogenic and antifertility effects of an anti-malarial drug, Artemether (a chemical modification of *Artemisinin annua*) on the body weights of adult wistar rats; There were dose-dependent increments in body and organ weights, when ethanolic extracts of Sesame seeds were fed to rats to test their pro-fertility effects [23]. A herbal tea mixture containing gypnoside as the main constituent was shown to increase the body weights of rats [24]. When body weight is lost, it is inferred that food consumption too must have reduced, thus in this investigation, it can be said that a component of the pepper nanoparticles may have affected the food intake of the subjects hence the loss of bodyweight in the exposed rats, that was not observed in the control group. It was also observed that the weight loss in the rats were dose-dependent. Yoshioka et al. [21] reported that the ingestion of red pepper decreased appetite and subsequent protein and fat intake in Japanese women, subsequently reducing their body weight. A 2014 study has implicated Capsaicin in Chili pepper as an appetite suppressant that leads to decrease caloric intake and subsequently increased weight loss [25]. Therefore it can be deduced that Capsaicin, which is the major phenolic compound in Chili pepper nanoparticle, may have suppressed appetite in the rats, reducing their feed intake, resulting in their loss of body weight over the exposure period as indicated in Figure 2. The serum IgE level showed significant ($p < 0.05$) difference between the 10% treated group (13.2 ± 0.4 mg/ml) and the 100% treated group (19.5 ± 0.3 mg/ml) in the IgE production. This result too was dose-dependent. There was also significant ($p < 0.05$) difference between the exposed groups and the control in serum IgE levels statistically. The significant IgE levels indicates that a component of the chili nano-extract did trigger allergic response in the exposed rats, in a dose dependent way (Figure 3) causing the rats' body to over react as IgE is known to play a pivotal role in responses to allergens, such as: anaphylactic drugs, bee stings, and antigen preparations used in desensitization immunotherapy. Although IgE is typically the least abundant isotype, blood serum IgE levels in a normal ("non-atopic") individual are only 0.05% of the Ig concentration [18]. Warbick et al. [26] induced elevated serum IgE levels in experimental rats when they exposed them to Trimellitic Anhydride (TMA). The elevated concentrations of IgE induced by topical exposure to TMA were persistent; the results they reported demonstrated that induced changes in IgE are maximal or near maximal at approximately 35 days, with a significant increase in IgE demonstrable for at least 42 days following the initiation of exposure. Hence, *C. annum* nanoparticles supposedly elicited the same effect as TMA in the rats. Karlsson et al. [27] had earlier proven that the serum IgE level is not sex-dependent. They showed that environmental factors, in this case, the Chili pepper nanoparticles, have a pronounced influence on levels of serum IgE with great fluctuations. Hybridization experiments suggest a multigenic control, with low IgE strains having a dominating suppressor effect [27].

Conclusion

C. annum L. nanoparticles at different concentrations, triggered allergic reactions in wistar Albino rats by raising the serum IgE levels, abnormally. It also lowered food consumption of the rats, thus reducing their body weight significantly ($p < 0.05$). Hence, in the search for a way to control or eradicate rats that are the chief culprits in the Lassa fever infection prevalent in Nigeria and other West African states, Chili pepper particles may be employed wholly or incorporated

as a component of a nano-biotechnological repellent.

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