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Application of *Melastomastrum capitatum* Fern. (Melastomataceae) loaded-exosome as analgesic drug carrier in acetic acid-induced Swiss albino mice

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ABSTRACT

Exosomes are nanoparticles (carriers) that play vital role in intercellular communication of cells. This present study was aimed at investigating exosome isolated from biological fluid for their biological applications in disease treatment especially as analgesic drug carrier. Exosomes were isolated from the kidney of cattle through ultracentrifugation of blood, and characterized by inverted scanning biological microscope. It was followed by the formation of *Melastomastrum capitatum*-exosome complexes (MCEC). A total number of twenty five (25) Swiss albino mice were used divided into five groups of five mice each. MCEC was administered to the mice in dosages of group I (100 mg/kg *M. capitatum* extract), group II (200 mg/kg Ibuprofen; standard drug), group III (300 mg/kg MCEC), group IV (400 mg/kg MCEC), and group V (500 mg/kg MCEC) (i.p). Results showed that MCEC decreased mean abdominal writhing in mice in dose dependent fashion with group V having the best mean abdominal contraction value of 12.0 ± 02^{b} and 67% inhibition of pains in mice. This result was significantly different from the value obtained in the control group I, where the extract was delivered ordinarily at p0.05 (one-way ANOVA). This study therefore showed that delivery of drugs by nanoparticles offer more therapeutic values than when drug is administered ordinarily. It is then recommended that most resistant disease pathogens should be treated with nanoparticle-delivered drugs for effective treatment.

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1. INTRODUCTION

Exosome are cell-derived vesicles that are present in many and perhaps all biological fluids including blood, urine and cultured medium of cell cultures [1]. Exosome are tiny vesicles secreted by cells in culture. These vesicles loaded with unique RNA made protein cargos, have many biological function of which only a small fractions in currently understood for example, they participate in cell to cell communication and signaling within the human body. The spectrum of current scientific interest in exosomes is wide and ranges from understanding diagnostics, as biomarkers, and in the development of therapeutics [2, 3]. Cells continuously secrete a large number of macro molecules complex, micro vesicles and small molecules in to the extracellular space of the secreted micro vesicles. The nanoparticles called “exosomes” are currently undergoing intense scrutiny. Exosomes are distinguished in their genesis by being budded into endosome to form multi vesicular bodies (MVBs) in the cytoplasm. The exosomes are released to extracellular fluid by function of these multi-vesicular bodies with the cell surface resulting in secretion and burst.

The nanospherical membrane type structure of exosomes is formed with a bilayer of lipids. It is also composed of various types of lipids and proteins that are derived from the parent cell from which the exosomes is formed. According to report of exosomes database, there are currently around 8000 proteins and 194 lipids know to be associated with exosomes. Other types of lipids that can form exosomes are cholesterol, ceramide and phosphoglycerides along with saturated fatly acids chains [4].

Exosomes are being recognized as potential therapeutics as they have the ability to elicit potent cellular response *in vitro* and *in vivo* [5,6,7]. Some tissues or cells secrete exosomes bearing immunosuppressive molecules; placenta-derived vesicles which bear ligands for natural killer lymphocytes found in pregnant women's blood circulation. Extensive analysis of component of these vesicles are underway to identify their role in the mother's tolerance to the foetus [8].

Depending on the state of the host exosomes present in their bronco alveolar fluid can bear tolerating molecules for example in mice, form an allergen. In addition to the immune system, exosomes probably affect other physiological functions. Exosomes are secreted by neural epithelial muscle and stem cells and their range of proposal function is extensive. They mediate regenerative outcome in injury and disease that recapitulate observed bioactivity of stem cell population . Mesenchymal stem cell exosomes were found to activate several signaling pathways important in wound healing and induce the expression at a number of growth factors (hepatocyte growth factors, insulin-like growth factors, nerve growth factors). Exosomes can be considered a promising carrier for effective delivery of small interfering RNA (siRNA) due to their existence in body`s endogenous system and high tolerance[9]. Exosomes other distinct advantages that uniquely positioned them as highly effective drugs carriers which include composed of cellular membranes with multiple adhesive protein on their surface, exosomes are known to specialize in cell-cell communication and also provide an exclusive approach for the delivery of various therapeutic agents to targets cells [10].

The plant *Melastomastrum capitatum* is a taxon of dicotyledonous flowering plants found in the tropics. Melastomataceae are annual or perennial herbs, shrubs, small trees or lianas with simple opposite leaves with characteristics variation pattern .The main veins usually 5-9 are palmate at the leaf base and the secondary vain between them are scalariform (i.e. parallel and regularly spaced ladder) [11]. In Nigeria, the plant is found in swampy area of Mambila plateau (Sarduana Local Government Area) Taraba State, where it is locally called “Belko” in Fulani language. The leaf methanol extract have been shown to possess analgesic and anti-inflammatory activity in Swiss albino mice in dose dependent fashion [12]. A large part of the plant has sweet to sour taste. The leaves are used as anti-rheumatic agent, cure stomach aches purification of blood vesicles and blood as well as for alleviating diuresis and as sedatives. The leaf methanol extract possess analgesic and anti-inflammatory activities in mice and the plant was very safe as an ethnomedicinal prescription in traditional medicine. The leaf sap are used to correct pulmonary and intestinal problem [12,13]. This study was carried out to investigate biological application of exosomes as analgesic drug carrier in pain situation.

2. MATERIALS AND METHODS

2.1 Materials

The materials used in this study were: air-dried leaves of *Melastomastrum capitatum*, centrifuges model 800D, oven, buffer solution, 2 mm syringe and needles, twenty-five Swiss albino mice weighing between 20-25 g, methanol (analytical grades), filter paper, scanning biological microscope, and magnetic stirrer.

2.2 Methods

2.2.1 Collection and identification of plant

Fresh leaves of *Melastomastrum capitatum*, were collected in the evening hours from Mambila Plateau Sarduana Local Government Area, Taraba State, and was authenticated by Mr. Cletus A. Ukwubile, of the Department of Science Laboratory Technology. A plant press was prepared and was deposited with voucher number MELA001 in the herbarium of Biology unit of Science Laboratory Technology Department, Federal Polytechnic, Bali, Taraba State, Nigeria.

2.2.2 Preparation and extraction of plant material

The leaves of *Melastomastrum capitatum*, were air-dried at room temperature for two weeks and was reduce into fine powder using electronic blender. 600 g of the powder was defatted in 700 mL petroleum ether and then extracted with 700 mL methanol using Soxhlet apparatus. The extract was then filtered using filter paper. The filtrate was concentrated *in vacuo* at room temperature. After this, the methanol extract was further fractionated successively using solvents in increasing order of polarity from the eluotropic series in this other: carbon tetrachloride, chloroform, acetone, ethyl acetate and methanol. Final weight of the methanol leaf extract was recorded as:

% yield = (final weight of powder /initial weight of powder) x 100

Fractions of extracts were bio-guided by analgesic activity in Swiss albino mice. Fraction with best biological activity (analgesic) was used for the study.

2.2.3 Isolation of exosome

Serum was obtained from a freshly procured liver by squeezing the liver to release blood. The blood was subjected to ultracentrifugation at 1000xg over night at 4^0C to obtain serum containing exosomes. The cell suspension was transferred to conical tubes and centrifuged at 300xg for l0 minutes. The supernatant was then transferred to ultracentrifuge tubes and if not completely full, PBS (phosphate buffer saline), was added. The sample was further centrifuged at 3000xg for 10 minutes at 4^0C to further remove cell debris. It was then filtered through a 0.2µm filter to remove particles larger than

200 nm and then transferred to new ultracentrifuge tubes and sealed before ultracentrifuge at 3000xg for 10 minutes at 4^{o}C to pellet the exosomes. The supernatant was then discarded. For maximal exosomes retrieval, exosomes enriched pellet was re-suspended repeatedly in a small volume (-3x50µL) of an appropriate buffer. This buffer depends on the downstream experimental planned following the exosomes isolated for example, lysis buffer is used for protein and RNA isolation, PBS is used for electron microscopy and flow cytometry and for functional studies medium may be preferred [14].

2.2.4 Animal groupings

A total number of twenty five (25) mice were used. They were divided into five groups of five mice each. Animals in group I (Negative control) were administered plant extract 100 mg/kg, group II (positive control) were administered a standard drug ibuprofen 200 mg/kg, while group III, IV and V were given 300 mg/kg, 400 mg/kg and 500 mg/kg of exosome-delivered drug (i.e. exosome + *M. capitatum* methanol extract; MCME).

2.2.5 Analgesic study of exosome as drug carrier in acetic acid induced writhing in mice

Pain was induced in mice by administering them 10 mL 0.6% (v/v) by intraperitoneal (i.p) route. After 30 min, the mice were administered in this order: group I; 100 mg/kg MCME, group II; 200 mg/kg; ibuprofen, while group III, IV and V received 300 mg/kg, 400 mg/kg and 500 mg/kg body weight of *Melastomastrum capitatum* exosome complexes (MCEC), and observed for the contraction of abdominal muscles by viewing the mice on the abdomen using hand lens for 10 minutes after a stimulation period of 5 minutes. Percentage inhibition of abdominal writhing was calculated using:

% inhibition = (MnWc-MnWt / MnWc) x100

Where; MnWc= mean number of writhing in negative control, MnWt =number of writhing in any treated group [15].

2.3 Statistical analysis

The data obtained were expressed as mean ± SEM (standard error mean) and the results were analyzed by one–way ANOVA followed by Dennett's test (Graph pad prism software version 7, 2016). The value of $p < 0.05$ (at α equal to 95%) was considered statistically significant.

3. RESULTS AND DISCUSSION

Exosomes are being recognized as potential therapeutic as they have the ability to elicit potent cellular response *in vitro and in vivo* [16,17,18] and are used to deliver certain antigen to the brain, since they break down brain-blood barriers [18] . Along the same line, the plant *M. capitatum* has been shown by Ukwubile and Agabila [19] to possess analgesic property in *invivo* mice model (i.p). In table 1, exosome-complex formulation showed percentage entrapment efficiency in dose dependent manner. This showed that the higher the percentage entrapment efficiency of exosome, the better the therapeutic measures in the cell.

In Table 2, plant loaded exosome has higher percentage of inhibition than the standard drug ibuprofen. This means that the use of exosome as a delivery vehicle for drugs is more effective than the use of drug ordinarily. This is in accordance with the report of Batrakova *et al.* [20], that exosome offers distinct advantages that uniquely positioned them as high effective drug carriers composed of cellular membranes with multiple adhesive proteins on their surface, specialized in cell-cell communication and provide an exclusive approach for the delivery of various therapeutic agents to target cells. The role of exosomes in delivery analgesic drug in this study was not different from the above reasons.

4. CONCLUSION

In conclusion, this current investigation showed that exosomes are capable of acting as analgesic drug carriers and are more effective in therapeutic purposes than non-targeted drug-delivery systems. The results of this study

Table 1. Formulation of *M. capitatum*-exosome complexes (MCEC).

Batch code	Drug: Carrier ratio (mL)	Experimental Weight (g)	% EE
ME I	1: 1	161. 5	45.88±0.1^{a}
ME II	1: 2	191. 5	54.40±0.1^{a}
ME III	1: 3	201.5	57.24±0.01^{b}
ME IV	1: 4	211.5	60.1±0.1^{a}
ME V	1: 5	221.5	62.93±0.01^{b}

Results are mean ± SEM, n =2, numbers followed by the same alphabet are statistically significant at p< 0.05(ANOVA), MCEC (*M. capitatum*-exosome complex), EE(entrapment efficiency).
% EE = (Experiment drug weight/Theoretical drug weight) X 100; Theoretical drug weigh = 352 g.

Table 2 . Effect of MCE-exosome complex on writhing in Swiss albino mice.

Group		Dose (mg/kg)	Mean no abd contraction/4min ± SEM	(%) inhibition
1	MCE	100	37±0.04[c]	-
2	Ibuprofen	200	20±0.01[a]	45
3	MCEC	300	18±0.01[a]	51
4	MCEC	400	15±0.01[a]	59
5	MCEC	500	12±0.02[b]	67

Result are mean ±SEM, n=5, number followed by the same alphabets are statistical significant at p< 0.05(ANOVA), MCEC (*M. capitatum*-exosome complexes), MCE (*M. capitatum* extract), abd(abdomen).

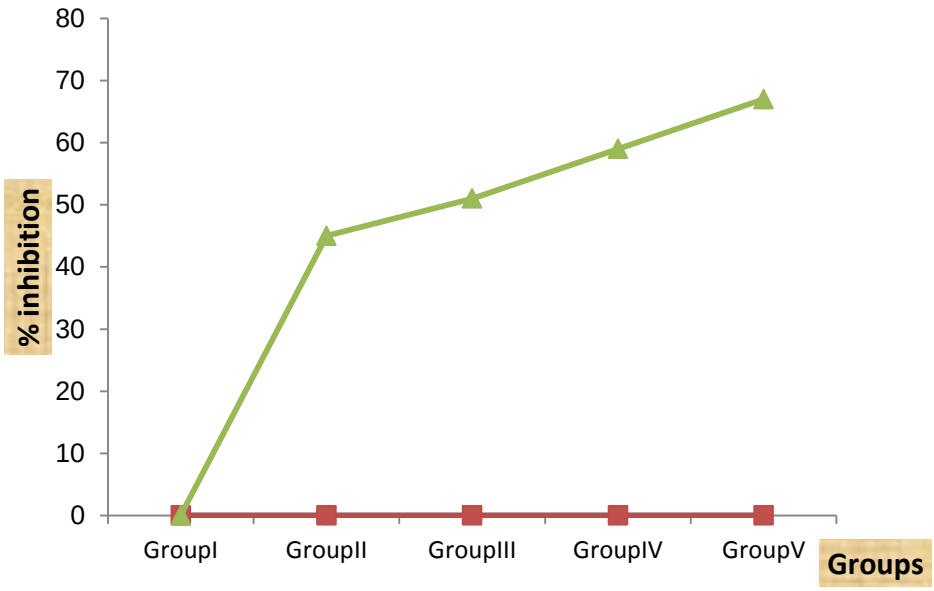

Figure 1: Graph of % inhibition against groups.

indicated that exosomes are effective tools for delivery analgesic drugs. It is therefore recommended that treatment for some diseases such as cancers, inflammations, tumors, liver disorders, etc. should be carried out through exosome-based drug delivery system for better results devoid of enzymatic action of the GIT that normally occurred in ordinary therapy.

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AUTHORS' CONTRIBUTIONS

CAU designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. FMM managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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