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Original Article

Application of *Melastomastrum capitatum* Fern. (Melastomataceae) Loaded-Exosome as Analgesic Drug Carrier in Acetic acid-Induced Swiss Albino Mice

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Exosomes are nanoparticles (carriers) that play vital role in intercellular communication of cells. The present study was aimed at investigating exosomes isolated from biological fluids 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. Then, *Melastomastrum capitatum-exosome* complexes (MCEC) were formed. A total number of 25 Swiss albino mice were divided into five groups of five mice each. MCEC was administered intraperitoneally 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). Results showed that MCEC decreased mean abdominal writhing in mice in a dose dependent fashion with group V having the best mean abdominal contraction value of 12.0± 02 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 ($P< 0.05$) (one-way ANOVA). This study therefore showed that delivery of drugs by nanoparticles offer more therapeutic values than when drug is administered ordinarily. Nanoparticle-delivered drugs are therefore recommended for effective treatment of most resistant disease pathogens.

Keywords: Exosome, nanoparticles, analgesic, *Melastomastrum capitatum*

Exosome are cell-derived vesicles that are present in many and perhaps all biological fluids including blood, urine, and media of cell cultures (1). Exosomes are tiny vesicles secreted by cells in culture. These vesicles which contain specific RNA encoded protein cargos, have many biological properties of which only a small fraction including their role in cell to cell communication

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and signaling within the human body has been discovered. Scientists are also interested in exosomes as diagnostic tools, biomarkers, and therapeutic agents (2-3). Exosomes are generated by budded endosomes which form multi vesicular bodies in the cytoplasm. The exosomes are released into the extracellular space through the secretion of the multi vesicular bodies from the cell surface. A lipidic bilawer forms the nanospherical membrane of exosomes which is also composed of different other lipids and proteins derived from the parental cell. According to exosomes related databases, more than 8000 proteins and 194 lipids have been associated with exosomes. Cholesterol, ceramide and phosphoglycerides as well as saturated fatly acids chains are among exosomal lipids (4). Due to their capacity of inducing *in vitro* and *in vivo* cellular responses, exosomes may be considered as potential therapeutic agents (5-7). Another aspect of research on exosomes correspond to the presence of immunosuppressive molecules in exosomes secreted by specific cells or tissues such as placenta from which ligands for natural killer lymphocytes are secreted in pregnant women's blood circulation. These specific components may play an important role in mother's tolerance to the fetus (8).

Depending on the state of exosomes present in their bronco alveolar fluid, hosts can also bear tolerating molecules such as allergens. Exosomes can also affect other physiological functions as they are secreted by many cellular types including stem cells. Through stem cells, they mediate regenerative outcome in injury and disease. Mesenchymal stem cell exosomes were reported to be involved in activation of signaling pathways important for wound healing and induction of different growth factors expression. The presence of exosomes in body's endogenous system and body's high tolerance to them, make exosomes a carrier of choice for efficient delivery of small interfering RNA (siRNA) (9). Another important feature of exosomes is the composition of their membrane and the presence of multiple adhesive proteins on their surface which make them potentially effective drugs carriers. Accordingly, their involvement in specific cell-cell communications also provides 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 evergreen herbs, shrubs, small trees or lianas with simple opposite leaves and characteristic leaf vein traits. The main veins are usually 5-9 palmate at the leaf base and the secondary veins 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 a dose dependent fashion (12). A large part of the plant has sweet to sour taste. The leaves are used as anti-rheumatic agent. They are also used to cure stomach aches, purify blood, alleviate diuresis, and cause sedation. The leaf methanol extract possesses analgesic and anti-inflammatory activities in mice and the plant was very safe as an ethnomedicinal prescription in traditional medicine. The leaf sap is used to correct pulmonary and intestinal problems (12-13). This study was carried out to investigate the biological application of exosomes as analgesic drug carriers in pain situation.

Materials and methods

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 were 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.

Preparation and extraction of plant material

The leaves of *Melastomastrum capitatum*, were air-dried at room temperature for two weeks and were reduced 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 order: 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.

Isolation of exosomes

Serum was obtained from a freshly procured liver by squeezing the liver to release blood. The blood was subjected to ultracentrifugation at 1000xg overnight at 4 ^{o}C to obtain serum containing exosomes. The cell suspension was transferred to conical tubes and centrifuged at 300xg for l0 min. The supernatant was then transferred to ultracentrifuge tubes and if not completely full, phosphate buffer saline (PBS), was added. The sample was further centrifuged at 3000xg for 10 min at 4 ^{o}C to further remove cell debris. It was then filtered through a 0.2 µm filter to remove particles larger than 200 nm and then transferred into new ultracentrifuge tubes and sealed before ultracentrifugation at 3000xg for 10 min 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 (3x 50 µL) of an appropriate buffer. This buffer depended on the downstream experimental planned following the exosomes isolation. Accordingly, lysis buffer was used for protein and RNA isolation, PBS was used for electron microscopy and flow cytometry and for functional study media (14).

Animals

The total number of 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, 400, and 500 mg/kg of exosome-delivered drug (i.e. exosome + *M. capitatum* methanol extract; MCME), respectively. "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

Analgesic study of exosomes as drug carriers in acetic acid induced writhing in mice

Pain was induced in mice by administering 10 mL 0.6% acetic acid (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 groups III, IV and V

received 300, 400, and 500 mg/kg body weight of *Melastomastrum capitatum* exosome complexes (MCEC), respectively. All groups were observed for the contraction of abdominal muscles by viewing the mice on the abdomen using hand lens for 10 min after a stimulation period of 5 min. Percentage inhibition of abdominal writhing was calculated using the following formula: % inhibition = (MnWc-MnWt / MnWc) x100.

Where MnWc= mean number of writhing in negative control; MnWt= number of writhing in any treated group (15).

Statistical analysis

The data obtained were expressed as mean ± standard error mean (SEM) 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 as statistically significant.

Results

In Table 1, exosome-complex formulation showed percentage entrapment efficiency in a dose dependent manner. This showed that the higher the percentage entrapment efficiency of exosome, the better the therapeutic measures in the cell.

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 weight = 352 g.

Table 2 and figure 1 represent the analgesic effects of MCE-exosome complex assessed through evaluation of abdominal contraction reduction and percentage of pain inhibition. Plant loaded exosomes showed higher percentage of inhibition than the standard drug ibuprofen.

Results 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; No: number.

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 II	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]

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

Group		Dose (mg/kg)	Mean Noabd 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

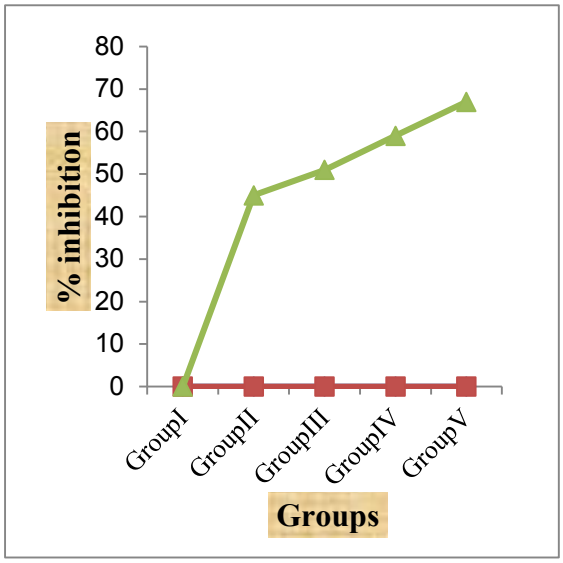

Figure 1. % pain inhibition in different experimental groups.

Discussion

Exosomes may be considered as potential therapeutic agents as they may elicit cellular responses both *in vitro* and *in vivo* (16-18). Interestingly, they may be used to deliver certain antigens to the brain, due to their ability to break down brain-blood barriers (18). Along the same line, the plant *M. capitatum* has been shown by Ukwubile and Agabila to possess analgesic property in *in vivo* mice model (19). The present study demonstrated that MCE-exosome complex exhibited higher analgesic effect than the standard drug ibuprofen. This means that the use of exosome as a drug delivery vehicle is more effective than the use of drug ordinarily. This is in accordance with the report of Batrakova et al. (20) which concluded that exosomes offer distinct advantages that uniquely positioned them as high effective drug carriers, partly due to the fact that they are composed of cell membrane components and various surface adhesive proteins.

In conclusion, the 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 indicated that exosomes are effective tools for delivering 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 gastrointestinal tract that normally occurs in ordinary therapy.

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Conflict of interest

The authors declared no conflict of interest.

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