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Research Article

Nano flavonoids might aggravate the abnormal expression of IGF1 R in Rat Brain-An alarming note necessitating careful Dose and Route

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Abstract

Recent decades have a greater attention towards the nanoparticles and their therapeutic aspects in human health. Further, nanoparticles of effective active components of herbal extracts are being investigated widely as an alternative medicine. However, the precise data on such aspects are till scarce. In this study we aimed at elucidating the efficacy and safer dose as well routé of nano flavonoids which have been claimed to have neuroprotective role. We used a potential target namely Insulin like growth factor 1 receptor (IGF 1R) of rat brain tissues to evaluate the efficacy as IGF 1R critically involved in neurological homeostasis. We analyzsed the impact of two different doses (50mg & 100mg/kg bw) of crude extract of orange (rich in flavonoids), isolated fraction of flavonoids and nano flavonoids administered in two different routes [oral and Intravenous IV)] on synaptic plasma membrane and on IGF 1R. We inferred that the oral route of extract and nano flavonoids were not detrimental while an alarming influence of IV mode of flavonoids in nano form showing a significant increase in the expression of IGF 1R which is not a desirable in normal rats as it would result in abnormal conditions such as malignancy. In addition, the isolated flavonoids (IV) also showed a significant increase in the expression of IGF 1R in normal rats at the examined doses which also do require attention. The partial and preliminary study stresses the significance of careful designing of nano medicines using naturally occurring and beneficial flavonoids.

Keywords: Nanoflavanoids, Oral and IV delivery, Synaptic plasma membrane, Insulin like growth factor 1 receptor (IGF 1R)

Introduction

Profound potential of nanoparticles (NP) led these nano dusts to find their way in various fields of science and engineering including human health. Thus, it becomes inevitable to evaluate the efficacy of such high potential NPs molecule to improve the value and suitability of nanoparticle exactly for the purpose where it would be employed. Reports on nanomaterials stress the safety measures in the use of nanoparticles[1] especially in treating different disease conditions of the body. NP are best studied for treating various neuroconditions however it is quite unexplored fact till date whether NP would cause brain injury or not. On the other hand, our traditional practices in India stress the need for natural intervention in challenging various medical ailments. Thus, it conceived to evaluate the NP of naturally occurring flavonoids in influencing the expression of Insulin growth factor 1 receptor in brain tissue which is critical for both normal neurophysiological and neuropathological processes.[2,3] Flavonoids are polyphenol compounds that are ubiquitous in nature and are categorized according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidine and chalcones. Over 4,000 flavonoids have been identified, many of which occur in fruits, vegetables and beverages (tea, coffee, beer, wine and fruit drinks). There has been increasing interest in the

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research of flavonoids from dietary sources, due to growing evidence of the versatile therapeutic benefits of flavonoids through various studies. Many flavonoids are shown to have anti oxidative activity, free-radical scavenging capacity, antiviral, anti-allergic, antiplatelet, anti- inflammatory, antitumor, anticancer activity, and coronary heart disease prevention, while some flavonoids exhibit potential for anti-human immunodeficiency virus functions.[4]

Flavonoids are said to be effective neuromodulators and are also found to cross the blood brain barrier effectively. However, there is a difficulty, still, in understand the therapeutic benefit and the intake of flavonoids, because of the complexity of existence of flavonoids from various food sources, the diversity of dietary culture, and the occurrence of a large amount of flavonoids itself in nature. Accordingly, an appropriate model for a precise assessment of flavonoids needs to be developed. The study aims at elucidating silver nanoparticles which are considered as important players in modern medicine to assess the efficacy of nano flavonoids in normal brain tissue samples with respect to the expression of *IGF1R*.

Materials and Methods

At preclinical level, the study is aimed at elucidating the potency of flavonoids on the expression of insulin growth factor 1 receptors (IGF1R), biochemical assessment of synaptic plasma membrane (SPM) in normal rats. *IGF1R* and SPM were studied as the expression of the former and integrity of the latter are closely related and implicated in many of the nervous system disorders. The study evaluates the effect of flavonoids administered *via* two routes *viz.* oral ingestion, intravenous (IV); both as simple extract and as a nanoparticle.

Experimental Animals

Albino rats of Wistar strain (100-150mg) purchased from the king institute, Guindy, Chennai and Tamil Nadu Animal Science University, Chennai: were received at 5 week of age and housed for acclimatization, at the animal house, Department of Biochemistry, Madras University; until the start of the experiment. The animals were weighted periodically, food pellets and water ad *libitum* provided uniformly, and housed under hygienic conditions. At the end of the treatment period all the experimental animals were euthanized by painless procedures and the tissues used for study for studying the biochemical and marker enzymes.

Experimental Groups

Each group were served with 3 animals

Group I	Served as control.
Group II	100mg/kg body weight of the cold extract was given orally to the normal rats.
Group III	50mg/kg body weight of the crude extract was given intravenously (IV) to the normal rats.
Group IV	100mg/kg body weight of the isolated flavonoids was given orally to the normal rats.
Group V	50mg/kg body weight of theisolated flavonoids was given intravenously (IV) to the normal rats.
Group VI	50mg/kg body weight of the nano flavonoids sample was given intravenously (IV) to the normal rats.
Group VII	100mg/kg body weight of the nano flavonoids tagged sample was given orally to the normal rats.
Group VIII	100mg/kg body weight of the nano tagged cold extract sample was given orally to the normal rats.

Drug Preparation (Crude Sample)

The peel of orange was collected and dried in shade. This was powdered and extraction fraction was mixed with 0.1% DMSO.

Preparation of Orange Peel Extraction

The outer skin of orange was shade dried for 20 days and pulverized to powder. The coarse powder was weighed. 500gm of the dried peel was extracted in methanol using soxhlet apparatus. After 48 hours, the extract obtained was concentrated by open drying. The crude extract was subjected to column chromatography using ethyl acetate, n-butanol, methanol and the fractions were collected. The residue of the fraction was used as drug. The fractions are administered to rats in two dosage forms as 50mg/kg body weight and 100mg/kg body weight.

Cold Extracts

A 50g of dried powdered fruit peel were macerated with a

200ml of pure methanol in a 500 ml flask and left for 8ndays then the extract was filtered andthe residual plant material were collected and evaporated to dryness under reduced pressure using rotary evaporator at a temperature not exceeding 40°C, then the dry extract was weighed and further purified through column chromatography using ethyl acetate, n-butanol and methanol as a mobile phase solvent.

Isolation of Flavonoids

Plants collected were washed in running tap water to remove dust. Aerial part (stems, leaves, flower) of collected plants were separated, shade dried powdered weighed and stored separately for extraction. Each of the dried powdered and weighed samples was soxhlet extracted in 80% methanol for 24 hrs and filtered. The filtrate obtained from each sample was subsequently extracted in petroleum ether, diethyl ether and ethyl acetate following the method reported by Subramanian (1969).[5]

Petroleum ether fraction was discarded due to its being rich in fatty substances. Ether fraction was used for free flavonoids whereas ethyl acetate fraction for bound flavonoids. Ethyl acetate fraction of each sample was hydrolyzed further with 7% H2So4 for 24 hr sand was then re-extracted with ethyl acetate. The fraction obtained was repeatedly washed with distilled water to neutrality, dried and weighed.[6]

Test for Flavonoids

Shinoda's Test

About 0.5 g of each extract was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips was added to the filtrate followed by few drops of concentrated HCl. A pink orange or red to purple coloration was an evidence for the presence of flavonoids.[7]

Ferric Chloride Test for Flavonoids

About 5 mg of the compounds was dissolved in 2ml of ethanol. A few drops of 10%ferric chloride solution were added. A green-blue coloration

indicated the presences of a phenolic hydroxyl group.[8]

Preparation of Nanoparticles from Fruit Extract

Five grams of fresh orange peel was sliced into small pieces and added 100ml of milliQ water and the mixture was boiled for 5min.After cooling the fruit extract was filtered with what mann No.1 filter paper. The filtrate was stored at 4°C for future use.

Synthesis of Silver Nanoparticles of Crude Extract

The synthesis of Ag-NPs from crude extract was done by taking 10 ml of the sample and mixing with 90 ml of 2 mM silver nitrate solution (AgNO3). The mixture was then stirred using a magnetic stirrer in dark at room temperature for 2 hours. The synthesis of NPs was observed by a change in color of the silver nitrate solution. Periodical UV-visible spectroscopic monitoring of the sample mixture was done at 300-750 nm.[9]

Flavonoid Silver Nanoparticle Synthesis

Silver nanoparticles were prepared by chemical reduction of salt solution of silver nitrate using the flavonoid fraction. Aqueous silver nitrate solution was prepared with the strength of 1mM. In a flask, 10mg flavanoid fraction in methanol was subjected to sonication for 5mins in order to ensure that there is no particle matter left in the solution. The resultant strength of the solutions was 100 µgm/mL. About 90ml of aqueous solution of silver nitrate was taken in a flask and kept on a magnetic stirrer into which 10ml of flavanoid solution was added drop by drop using a micropipette.[10]

Table 1.The activities of membrane bound enzymes in control and experimental groups of rats. The results are expressed as mean ± SD. (The activities were expressed as µmoles of phosphorus liberated/min/mg of protein)

Form of Drug administration	Expression of *IGF1R* mRNA (as compared to the control animals)
Extract-oral 100mg/kg body weight	Non-significant
Extract-IV 50mg/kg body weight	0.04
Flavonoids-Oral 100mg/kg body weight	0.05
Flavanoids-IV 50mg/kg body	Non-significant
Nano flavonoids-oral 100mg/kg body weight	0.03
Nano flavonoids = IV 50mg/kg body weight	Non-significant

Results and Discussion

The study had provided an interesting hint about the flavonoids with regard to its form of and route of administration. Table 1 depicts the activities of membrane bound enzymes of SPM. The activity of AChE, 5'Nucleotidase, Na^+/K^+-ATPase (expressed in U/min/mg protein) in synaptosomes isolated

from brains of normal rats differ among the groups tested. The activities of AChE, 5'Nucleotidase were found to be normal in brain synaptosomes isolated from all groups. However, the activity of Na^+/K^+-ATPase showed alarming reduction in groups which received flavonoids both as non nano form and as nano form in either of the routes (oral and iv). As syanpatosomes, the membrane vesicles are suitable for studies which aim to appreciate neurotransmission and also useful for studying structure-function relationship[11,12] the observed decrease in the Na^+/K^+-ATPase represent the impact of the administered neuromodulator and thus necessitates fine tuning of the administration. While the crude extract, nano extract (50mg/kg bw) and nano flavonoids (100mg/kg bw) when administered *via* the oral route did not cause any significant changes in the expression of *IGF 1R* reflecting the safer route, dosage and forms of flavonoids, oral mode of isolated flavonoids resulted, alarmingly, in an over expression of *IGF 1R* ($p<0.03$) (Table 2 and Fig. 1B & 1C) which is said to one of the major feature of CNS diseases such as glioma.[13] Point to be noticed is that the oral route of nanoflavanoids did not cause any abnormal expression of IGF1R in rats. The IV route of administration of isolated flavonoids also could up regulate the expression of *IGF1R* significantly ($p<0.001$) thus highlighting the dosage of 50mg/kg bw (IV) of flavonoids is not ideal for normal rats In the case of nano flavonoids, we did notice an increase in the expression of *IGF1R* ($p<0.001$) when nano flavonoids were administered *via* IV route (50mg/kg bw), in contrast to the oral nanoflavonoids (at double the dosage), thus raising concerns about the effectiveness of the dosage as well as the route of nano flavonoids which would not cause any adverse side effects. IGF1R plays important role in the pathology of degeneration[14,15] and malignancy conditions[16] thus was employed in the current study as a powerful target for the comparative evaluation of the flavanoid treatment. The study analyzed the effectiveness of dose and route of flavonoids (nanoforms) as neuromodulators in normal animals which may be, in long run, beneficial as future therapeutic nano-agent against neurological diseases such as glioma with further and finer pharmaco investigations.

Table 2.Activities of membrane bound enzymes from the synaptic plasma membrane isolated from brain tissues of the control and experimental groups of rats. n=6 for each group. Values are represented by Mean ± SD. *, ** represent p< 0.05, p<0.01 respectively.

Enzymes	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
Na+K+ATPase	0.51±0.02	0.45±0.03*	0.46±0.04*	0.34±0.03**	0.37±0.01**	0.36±0.02**	0.36±0.03**	0.31±0.03**
5'Nucleotidase	0.31±0.01	0.33±0.03	0.32±0.03	0.41±0.04*	0.32±0.07	0.38±0.03	0.31±0.05	0.33±0.03
AchE	0.18±0.02	0.13±0.02	0.17±0.04	0.17±0.06	0.15±0.03	0.15±0.03	0.15±0.03	0.16±0.02

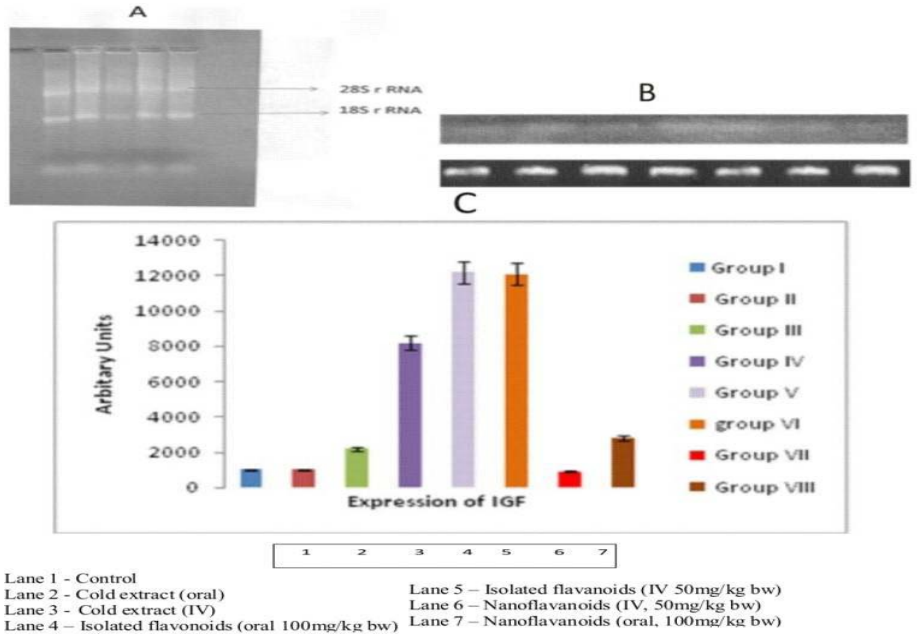

Figure 1.A. Isolation of RNA from rat brain tissue showing 28SrRNA and 18SrRNA. B. Expression of IGF1R in control and experimental groups. The IGF1R expression in Group V&VI was significantly when compared to that of control. C. Densitometric scanning pattern of IGF1R expression. Three independent experiments were performed. Data was represented by Mean ± SD. P value < 0.05 considered as significant.

The simple report recommends a cautious approach in utilizing nano preparations of the beneficial flavonoids in the appropriate route for attaining maximal health benefits with minimal side effects.

References

1. Dupraz S, Grassi D, Karnas D et al. The insulin- like growth factor 1 receptor is essential for axonal regeneration in adult central nervous system neurons. *PLoS One* 2013; 8(1): e54462.
2. Gwinn MR, Vallyathan V. Nanoparticles: health effects--pros and cons. *Environ Health Perspect* 2006; 114(12): 1818-25.
3. de la Monte SM, Wands JR. Alzheimer's disease is type 3 diabetes-evidence reviewed. *J Diabetes Sci Technol* 2008; 2(6): 1101-13.
4. Hollman PC, Katan MB. Dietary flavonoids: intake, health effects and bioavailability. *Food Chem Toxicol* 1999; 37(9-10): 937-42.
5. Subramanian SSAN. Flavonoids of the seeds of Crotalaria .retusa and C. striata. *Current Scienc*e 1969; 38: 65-68.
6. Yadav S, Sheorain AK, Madan N et al. Esthetics with prosthetics in case of maxillary canine transposition: a clinical report. *Nigerian Journal of Clinical Practice* 2012; 15(1): 108- 11.
7. Usman H, Abdulrahman F, Usman A. Qualitative phytochemical screening and in vitro antimicrobial effects of methanol stem bark extract of Ficus thonningii (Moraceae). *Afr J Tradit Complement Altern Med* 2009; 6(3): 289-95.
8. Bello IA, Ndukwe GI, Audu OT et al. A bioactive flavonoid from Pavetta crassipes K. Schum. *Org Med Chem Lett* 2011; 1(1): 14.
9. Thirumalairaj MPVVK, Durairaj G, Shanmu- gaasokan Let al. Potential antibacterial activity of crude extracts and silver nanoparticles synthesized from Sargassum wightii. *International Current Pharmaceutical Journal* 2014; 3(10): 322-25.
10. Seethalakshmi ASAS. Phytochemical Synthesis and Preliminary Characterization of Silver Nanoparticles Using Hesperidin. *Journal of Nanoscience* 2013.
11. Whittaker VP. Thirty years of synaptosome research. *J Neurocytol* 1993; 22(9): 735-42.
12. Breukel AI, Besselsen E Ghijsen WE. A model system to study release of multiple classes of neurotransmitters. *Methods Mol Biol* 1997; 72: 33-47.
13. Trojan J, Cloix JF, Ardourel MY et al. Insulin- like growth factor type I biology and targeting in malignant gliomas. *Neuroscience* 2007; 145(3): 795-811.
14. Scheff SW, Price DA. Alzheimer's disease- related synapse loss in the cingulate cortex. *J Alzheimers Dis* 2001; 3(5): 495-505.
15. Scheff SW, Price DA, Sparks DL. Quantitative assessment of possible age-related change in synaptic numbers in the human frontal cortex. *Neurobiol Aging* 2001; 22(3): 355-65.
16. Yin S, Girnita A, Stromberg T et al. Targeting the insulin-like growth factor-1 receptor by picropodophyllin as a treatment option for glioblastoma. *Neuro Oncol* 2010; 12(1): 19-27.

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