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INVIO CHARACTERIZATION (BIOAVAILABILITY) OF CURCUMIN NANOPARTICLE

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ABSTRACT Curcumin is used as traditional Indian and Chinese medicine in order to treat various diseases as well as used as a wound healing agent. Curcumin (diferuloylmethane) is one of the potent, nontoxic, and major bioactive components present in turmeric. Curcumin possess remarkable antibacterial, antiviral, and antiprotozoan activity. According to the studies, it shows that curcumin manifests very poor oral bioavailability, and forms numerous curcumin metabolites are formed after metabolism, although the bioavailability is low but the therapeutic activity of the curcumin for the various diseases, and for treatment of the disease enhancement of bioavailability of the curcumin in the future is necessary. The major drawbacks of curcumin are low absorption and poor bioavailability. One of the most important technique/ method to improve the poor biopharmaceutical properties of the curcumin is to enhance aqueous solubility, bioavailability by using nanotechnology and nanoparticles, having a small size in nanometer range. Nanotechnology is one of the most interesting areas of research in modern science. In medicine, nanotechnology-based drug delivery system is an advanced method for treating number of dreadful diseases. In in-vivo study was performed on mice via Oral and Intravenous administration to determine the Bioavailblity of Curcumin.

KEYWORDS : antimicrobial activity; curcumin; curcumin nanoparticles. Curcumin nanoparticles, Bioavailability, Drug delivery, Anticancer, Curcuminoids, bioavailability, nanoparticulate, nanocurcumin, polyphenolic.

INTRODUCTION:-

Curcumin is a polyphenol compound, which is extracted from curcuma long Linn, (Zingiberaceae) rhizome. It is also known as turmeric in Indian culinary art. Curcumin longa also contains three main curcuminiods such as curcumin, desmethoxycurcumin, bismethoxycurcumin which includes 3-5% of the turmeric preparations. Practically the available curcumin contains 75-80% curcumin, 15-20% desmethoxycurcumin and 3-5% bis-desmethoxycurcumin. It is commonly used as a spice in the South and Asian countries, due to its flavour and yellow colour [1]. Curcumin functions as an antioxidant, anti-inflammatory, and anti-atherosclerotic. It inhibits scarring, cataract, gallstone formation, liver injury, and kidney toxicity, and also promotes wound healing and muscle regeneration [2]. It has been used to cure liver problems, digestive disorders, and in treatment of several skin ailments and in wound healing [3]. It has been observed by trials on human and mouse that oral consumption of curcumin demonstrates lesser bioavailability, and it undergoes intestinal metabolism, whereas the absorbed curcumin shows rapid metabolism and excretion in bile [4, 5].

Curcumin possesses better pharmacodynamics but poor pharmacokinetics [6]. b-Diketone moiety in curcumin is the major reason for the instability and weak pharmacokinetic of curcumin. Modifications are performed in the structure of curcumin, and its analogs were synthesized without β-diketone moiety. Because of such structural modifications, the stability and analogs without β-diketone moiety are called monocarbonyl analogs of curcumin (MACs). MACs have shown to have anticancer and anti-inflammatory activities [7]. Recently, it was demonstrated that curcumin can be delivered through inhalation. The pressurized metered dose inhaler could be used as one of the new vehicles for the delivery of curcumin [8]. Nanotechnology-based drug delivery will probably be a suitable method for increasing the biological action of curcumin, which increases its absorption. Curcumin nanoparticles, liposomes, and phospholipid may work as novel approaches to increase the bioavailability of curcumin [5]. Size distribution and charge on nanoparticles can affect the retention property. The smaller the size of the particles is, the more is the retention property of the particle. Zeta potential influences the stability of the particles [9, 10]. During the last decades it was observed in the research that was taking place on antioxidant property of curcumin and the potential property of curcumin was determined. (11),(12). Currently, numerous researches on curcumin proved that this agent can prevent the proliferation of cancer cells and acts as a cell death inducer by inducing many signalling pathways. Therefore, as an inducer of cell death curcumin has become a chemopreventive and anti-cancer agent. This can be proved by performing various in vitro experiments and preclinical studies on the animal models (13). When curcumin is orally administered, after that it metabolise is into curcumin glucuronide and curcuminsulfonate. However, when curcumin is administered systemically or intraperitoneally, it is metabolized into tetrahydrocurcumin, hexhyrdrocurcumin, and hexhydrocurcuminol (14). There are various effect of curcumin on the target cells, Curcumin can modulate numerous different transcription factors, cytokines, growth factors, kinases, and other enzymes (14).

MEDICINAL USE OF CURCUMIN:

Curcumin is also responsible for reducing LDL and increasing HDL, inhibiting platelet aggregation and inflammatory response that reduce the occurrence of myocardial infarction and cardiovascular diseases (16). Curcumin have anti-inflammatory activity by inhibition the molecules, which play an important role in case of inflammation(17). Curcumin has antioxidant, anti-inflammatory, antiviral and antifungal actions (18). Curcumin leads to development of resistance to insulin in patients with diabetes mellitus II (14). The combination of curcumin-encapsulated nanoparticles with electroporation technique in MCF-7 human breast cancer cells depicted better anticancer activity [19].

The nanocurcumin formulation was more reactive against Gram-positive bacteria than the Gram-negative bacteria [20]. In another study, curcumin-encapsulated nanoparticles inhibited the growth of methicillin-resistant S. aureus and P. aeruginosa and enhanced the wound-healing activity in an in vivo murine wound model [21].

In curcumin the pharmacokinetics property is usually studied in animals, then in humans, that are reviewed. According to the studies, it shows that curcumin manifests very poor oral bioavailability, and forms numerous curcumin metabolites are formed after metabolism like- curcumin glucuronide, curcumin sulfate, hexahydro-curcumin, tetrahydrocurcumin, and dihydrocurcumin. In recent years, several derivatives, analogs and drug vehicle combinations of curcumin were developed by various experimental studies, these experimental outcomes helped in improving absorption and enhancing the systemic bioavailability than the parent drug curcumin (22).

PROBLEMS OF CURCUMIN BIOAVAILABILITY:

There are several factors which affect the bioavailability of any agent like high rate of absorption, low intrinsic activity clearance and fast elimination from the body, low serum level, short half- life, apparent rapid metabolism, and limited tissue distribution.

ANIMAL STUDY:

METHOD

Male Swiss mice (20-22 g) were obtained from the University of Panama, Panama. These animals were kept in an environmentally controlled room (24 ± 1°C and 12 h:12 h light-dark cycles) with free access to food and water.

Mice were randomly divided in two groups. One group of mice received saline solution and CLNF (5 mg/kg curcumin) intravenously via the tail vein. Another group was given CLNF orally (25 mg/kg), an oil free curcumin solution (25 mg/kg) and a saline solution. The animals had access to water and food after dosage administration. Blood (1 mL) and tissues samples (brain and heart) were collected in heparinized tubes at 0, 2, 5, 10, 30 min, 1 and 12 h after intravenous administration or at 10, 20, 30, 45 min, 1, 2 and 12 h after oral administration (5 mice were used for each point). The tissue was collected into separate containers and the plasma was separated from the heparinized blood by centrifugation and storage at 20 °C prior to analysis.

SAMPLE PREPARATION

A pre-treatment was required to remove the protein and possible interferences from plasma and tissue sample prior to the HPLC analysis. The organs were weighed and homogenized (1:5 w/v) with a mix of water and acetonitrile (1:1). Then, the organs were centrifuged at 6,000 rpm for 10 min at 4 °C; the supernatant was collected (75 µL) and mixed with 150 µL of acetonitrile. The supernatant-acetonitrile samples were centrifuged (12,000 rpm, 15 min) and a new the supernatant was collected and analyzed by HPLC (24).

HPLC SYSTEM AND ANALYTICAL METHOD VERIFICATION

The analytical method was verified following Li et al. protocol [23]. (Li et al. 2009). The calibration curve was linearity over a concentration range of 0.5 ng/mL to 110 ng/mL of curcumin in mice plasma with a correlation coefficient r = 0.9984. Table 1. shows a summary of intra and inter-day precision and accuracy of curcumin in mice plasma.

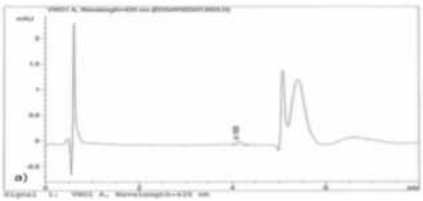
Table 1: Precision and accuracy of HPLC analysis of curcumin.

Nominal Concentration (ng/ml)	Mean Measured Concentration (ng/ml)	Standard Deviation	Precision (%RSD)	Accuracy (%)
Intra-day (n=3)				
5.1	5.3	0.3	5.6	103.9
80.0	80.2	1.5	1.9	100.3
110.0	115.4	5.7	4.9	104.9
Inter-day (n=9)				
5.1	5.2	0.3	5.0	102.4
80.0	81.0	2.3	2.8	101.2
110.0	112.3	3.8	3.4	102.1

RESULTS:

Figure 1: shows the chromatogram of a) standard containing 2.0 ng/mL of curcumin and b) plasma sample collected from mice after 10 min of oral administration. The retention time of curcumin was 4.2 min. There were no interference peaks found at the retention time of analysis in the chromatograms. These results demonstrated that the Poroshell C-18 column (4.6 x 50 mm; particle size 2.7 µm), with mobile phase, composed by acetonitrile (ACN) and a 5% solution of AA, provided a good separation and selectivity for the kinetic analysis of curcumin. The plasma and tissue concentration-time profiles of released curcumin in mice following an oral and intravenous administration of CLNF and a control sample are shown in Figure 2 and 3, respectively. The pharmacokinetic parameters in plasma obtained from the plotted concentration-time data are summarized in Table 4.5. In the curcumin group, the AUCs of curcumin in plasma for oral and intravenous administrations were 6.75 ± 1.13 µg/mL min and 0.77 ± 0.20 µg/mL min respectively, indicating that more free curcumin was available in plasma by oral administration than by the intravenous route. The AUC for the control sample was 0.75 ± 0.3 µg/mL min.

Figure 1: Representative chromatogram of a) standard containing 2.0 ng/mL of curcumin and b) plasma sample collected from mice after 10 min of oral administration.



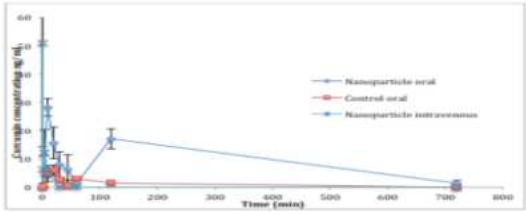


Figure 2: In plasma concentration of curcumin following oral and intravenous administration.

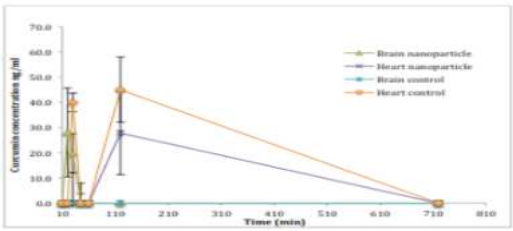


Figure 3: Concentration of curcumin in brain and heart following oral administration.

Table 2: Pharmacokinetic parameters of curcumin and curcumin nanoformulations in mice following oral and intravenous administration.

		Heart		Brain		Plasma	
		CLNF	Control	CLNF	Control	CLNF	Control
AUC (µg/ml min)	Oral	1.35 ± 0.51	1.85 ± 0.43	0.44 ± 0.18	n/d	6.75 ± 1.13	0.75 ± 0.30
	IV	n/d	n/d	n/d	n/d	0.77 ± 0.20	n/d
Cmax	Oral	27.80 ± 16.49	45.01 ± 12.89	28.06 ± 17.56	n/d	28.28 ± 3.02	5.91 ± 2.91
	IV	n/d	n/d	n/d	n/d	50.92 ± 36.78	n/d

DISCUSSION:

Several models of curcumin micro and nanoformulations have been proposed to overcome its limited biomedical applications. Such models were nanoemulsions (26), microparticles (29) or solid dispersions (30). From all of these studies, improvements in bioavailability of curcumin have been demonstrated; however, most of the in vitro effective results remain unachievable on in vivo experiments (28).

In this work, after standardization of CLNF, the pharmacokinetics of CLNF formulation were characterized using HPLC, for intravenous and oral doses of 5 mg/kg and 25 mg/kg, respectively, using Swiss mice (23). Curcumin release profile in plasma after oral and intravenous administration of CLNF and saline are shown in Figure 4.22. As shown Figure 4.22 and Table 4.5, the area under the curve (AUC) of CLNF was 6.75 µg/mL min, while the AUC of free curcumin oily solution was 0.75 µg/mL min after oral administration. This is a 9-

fold higher than no encapsulated curcumin. These results also display a Cmax = 28.28 ng/mL of CLNF, about 5 folds higher than oil free curcumin solution (5.91 ng/mL), suggesting that CLNF can deliver curcumin in vivo and increase its bioavailability after oral administration. In intravenous administration, the AUC was 0.77 µg/mL min, probably due to the partially protonated amino groups of chitosan in physiological pH, that produce a reduction of chitosan solubility and further release of curcumin into plasma (24).

Two factors have been identified that may improve oral bioavailability: 1) chitosan can enhance bioavailability of nanoformulated substances due to its mucoadhesive properties. Mucoadhesion improves time resistance of carrier systems, increasing the bioavailability of poorly absorbed drugs (27) the commonly used surfactant (Tween 80) that has been shown to enhance the solubility of compounds leading to increased absorption of drugs (26).

The ability of CLNF to reach organs such as heart and brain was also evaluated. The HPLC analyses showed the presence of curcumin in brain and heart after oral administration of CLNF (Figure 4.23 and Table 4.5). However, the results present a high standard deviation, probably due to the uneven distribution of the curcumin in the organs (23).

When CLNF were administrated intravenously, not a significant amount of curcumin was found in the samples. Conventional colloidal carriers were rapidly removed from the bloodstream by the reticuloendothelial system, after intravenous administration. A study using different sized chitosan nanoparticles (40, 70 and 100 nm) showed that the liver eliminated larger particles (100 nm) from the blood stream faster compared to the smaller particles (40 nm) and, as a consequence, the efficacy of larger chitosan nanoparticles by intravenous administration decreased (25). These results indicate that CLNF were less effective on intravenous administration than oral administration. CLNF raise the bioavailability of curcumin in oral administration, increasing the retention time of curcumin in plasma.

CONCLUSION

Curcumin is the yellow polyphenolic compound extracted from the rhizome of curcuma longa belong to the ginger family of Zingiberaceae, turmeric is available in yellow colour mainly obtained from polyphenolic pigment and fat-soluble substance called as curcuminoids. There are several factors which effect the bioavailability of curcumin, therefore various approaches have been taken place for the improvement in the bioavailability of curcumin.

There has been a great interest in advancing the production of more stable, efficient and safe nanocarriers for drugs, which guarantees that formulations and manufacturing processes will undoubtedly continue to evolve. The treatment of major diseases of worldwide economic importance has been proven to benefit from these nanosystems, thought there are still few nanodrugs available on the pharmaceutical market.

In the present study, CLNF were successfully prepared, characterized and evaluated as a potential carrier system for oral delivery of curcumin. The in vivo results demonstrate that these nanocapsules increased the oral bioavailability of curcumin compared to curcumin suspensions. In addition, nanoformulated curcumin was found in organs such as heart and brain, while non-formulated curcumin was not.

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