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ENHANCING THE ABSORPTION OF CURCUMINOIDS FROM FORMULATED TURMERIC EXTRACTS

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ABSTRACT: The Turmeric (*Curcuma Longa.L*) plant, a perennial herb belonging to the ginger family is cultivated extensively in South East Asian countries. The polyphenols Curcumin, De methoxy curcumin and Bisdemethoxy curcumin collectively known as curcuminoids ,which aid in the treatment of degenerative diseases like cancer, alzheimer's, cardio vascular diseases, arthritis, diabetes and immune deficiencies .Curcuminoids are generally present in the rhizome in the ratio Curcumin : DMC : BDMC 55-60% : 19 -21 % :20–24% respectively. When the rhizhome is extracted and the curcuminoids isolated from the extract,there is a composition change from what is naturally found in the rhizhome. The present study gives a comparative data of bio-absorption of two turmeric formulations containing curcuminoids in a varied composition compared to regular turmeric extract. Regular Turmeric extract with 96.5% curcuminoids and two different formulation containing 7.8% curcuminoids and 51% curcuminoids were orally administered to adult albino rats which were divided into 10 groups based on different doses of test item (50 mg/kg, 150mg/kg, 300mg/kg).The absorption of curcuminoids was studied by estimating the percentage of curcuminoids in faeces. The study shows that the absorption of curcuminoids in regular turmeric extract was 68 to 72% in three doses where as the absorption of curcuminoids of Formulation I and Formulation II were 97 to 99% and 99.6% to 99.7% respectively. An increase in absorption rate was evidently seen for turmeric formulations compared to the regular turmeric extract.

INTRODUCTION: India is well-known for its rich history for using plants for medicinal purposes. Turmeric (*Curcuma longa* Linn) is a medicinal plant extensively used in Ayurveda, Unani and Siddha medicine as home remedy for various diseases[1, 2].Curcuma *Longa*., botanically related to ginger (*Zingiberaceae* family), is a perennial plant having a short stem with large oblong leaves and bears ovate, pyriform or oblong rhizomes, which are often branched and brownish yellow in colour.

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Turmeric is used as a dietary spice, coloring agent in foods, textiles and in the treatment of various ailments in Asian countries. It is widely used in traditional medicine to cure bilinary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism, sinusitis [3]. It is also considered as auspicious and is a part of religious rituals. Turmeric paste in slaked lime is a popular home remedy for the treatment of inflammation and wounds. For centuries curcumin has been used at doses up to 100mg/day. Recent phase I clinical trails indicate the human beings can tolerate a dose as high as 8g/day with no side effects [4].

This vibrant yellow spice has a long history of use in traditional medicines of China and India. The rhizome of turmeric has been crushed into a powder and used in Asian cookery, medicine,

cosmetics, and fabric dying for more than 2000 years. Early European explorers to the Asian continent introduced this important spice to the Western world in the 14th century [5]. As part of the ancient Indian medical system, Ayurveda, a poultice of turmeric paste is used to treat common eye infections, to dress wounds, treat bites, burns, acne and various skin diseases.

In Northern India, women are given a tonic of fresh turmeric paste with powder of dried ginger roots and honey in a glass of hot milk twice daily after child birth. Powdered turmeric is taken with boiled milk to cure cough and related respiratory ailments and roasted turmeric is an ingredient used as an anti-dysenteric for children [6.] This ancient remedy is also used to treat dental diseases, digestive disorders such as dyspepsia and acidity, indigestion, flatulence, ulcers, as well to alleviate the hallucinatory effects of hashish and other psychotropic drugs [7].

The potential components of turmeric are 2 to 4% of volatile oil including tumerone, atlantone, zingiberone, 4 to 10% of curcuminoids and 2 to 3% of fixed oil. Other constituents include sugars, proteins and resins. Curcumin was first isolated from turmeric in 1815, and the structure was delineated in 1910 as diferuloylmethane. Commercial curcumin typically contains three major curcuminoids curcumin, demethoxy curcumin, and bis-de-methoxy curcumin. Most commercially available preparation of curcumin contained approximately 72 to 77% curcumin, 14 to 18% of demethoxy curcumin and 3 to 5% bis-demethoxy curcumin.

Curcumin is hydrophobic in nature and soluble in dimethylsulfoxide, acetone, and ethanol. It has absorption maxima around 420nm.When exposed to alkaline conditions, the colour of curcumin turns from yellow to deep red, the form in which it is used for various religious ceremonies. Under acidic conditions, the degradation of curcumin is much slower with less than 20% of total curcumin decomposed in 1 hour [8].

Various studies reported the poor bioavailability for curcuminoids. The reasons for reduced bioavailability of any molecule or ingredient within the body are low intrinsic activity, poor absorption, high rate of metabolism, inactivity of metabolic products and rapid elimination and clearance from the body [9]. However, studies over the past three decades related to absorption, distribution, metabolism and excretion of curcumin have revealed poor absorption and rapid metabolism of curcumin that severely curtails its bioavailability. To improve the bioavailability of curcumin, numerous approaches have been undertaken.

These approaches involve, the use of adjuvants like piperine that interferes with glucuronidation, the use of liposomal curcumin, converting curcumin to nano sized particles, the use of curcumin phospholipid complex and the use of structural analogues of curcumin (e.g., EF-24). Despite the lower bioavailability, therapeutic efficacy of curcumin against various human diseases, including cancer, cardiovascular diseases, diabetes, arthritis, neurological diseases and Crohn's disease, has been documented. Enhanced bioavailability of curcumin in the near future is likely to bring this promising natural product to the forefront of therapeutic agents for treatment of most degenerative diseases [10].

Although curcumin has been isolated in the 19th century; extracts of the rhizomes of Curcuma longa have been in use from the Vedic ages. Some of the medicinal application of turmeric includes to controlling anaemia, atherosclerosis, diabetes, oedema, haemorrhoids, hepatitis, hysteria, indigestion, inflammation, skin disease, urinary disease, wound and bruise healing, psoriasis, anorexia, cough, liver disorders, rheumatism, sinusitis [11].Being a polyphenol, curcumin is an antioxidant, preventing cell and tissue destruction due to free radical activity.

Hence it helps to prevent or retard various cardio-vascular, viral and other chronic diseases like arthritis, cancer and AIDS by neutralizing the existing free radicals [12]. Curcumin on intake depletes substance P, a neurotransmitter of pain impulses in the nerve endings and potentiates adrenal glands which produce anti-inflammatory hormones .When used orally; curcumin has several effects on body like inhibiting leukotriene formulation and platelet aggregation promoting

fibrinolysis (tissue regeneration) and stabilizing the cell membranes [13]. Pharmaco kinetics studies in rats indicate that the absorption of pure curcumin from gastro intestinal tract is about 60 to 65 percent after administration of a single oral dose of 400mg/kg [14].

Curcuma longa rhizome has been traditionally used as antimicrobial agent as well as an insect repellent [15]. Several studies have reported the broad-spectrum antimicrobial activity for curcumin including antibacterial, antiviral, antifungal, and antimalarial activities. Because of the extended antimicrobial activity of curcumin and safety property even at high doses (12g/day) assessed by clinical trials in human, it was used as a structural sample to design the new antimicrobial agents with modified and increased antimicrobial activities through the synthesis of various derivatives related to curcumin [16]**.**This polyphenolic compound due to a variety of biological activities has been gained significant attention of researches all over the world [17, 18]**.**

As many other plant materials, there are differences in the curcumin content for the Curcuma longa from different geographical regions and it could be due to hybridization with other Curcuma species which could be important fact to choose the plant with higher content of curcumin [19]. Mixture of curcumin with other antimicrobial agents is used for the development of antimicrobial skin gels and emulsions with improved skin protection and wound dressing properties [20]**.**

The hexane and methanol extracts of *Curcuma longa* demonstrated antibacterial effect against 13 bacteria, namely *Vibrio harveyi, Vibrio alginolyticus, Vibrio vulnificus, Vibrio parahaemolyticus, Vibrio cholerae, Bacillus subtilis, Bacillus cereus, Aeromonas hydrophila, Streptococcus agalactiae, Staphylococcus aureus , Staphylococcus intermedius, Staphylococcus epidermidis,* and *Edwardsiella tarda*. [21]**.**

The synergistic activity of curcuminoids and ampicillin combination demonstrated pronounced reduction in the MIC of ampicillin against either clinical strain or Staphylococcus aureus ATCC 25923 strain. Bacteriocin subtilosin isolated from Bacillus amyloliquefaciens in combination with encapsulated curcumin revealed partial synergism against wild-type and nisin sensitive strains of Listeria monocytogenes Scott A [22]. Strongly bound metal complexes to antimicrobial agents are introduced as another possible way for synergistic activity of respective antimicrobial agents through elevation of the binding effect of them to the bacterial walls. Complexes of curcumin with cobalt nanoparticles showed increased antibacterial activity against *E.coli* [23].

Additionally, fabrication of silver nano-composite films impregnated with curcumin showed the stronger antibacterial activity against E.coli. It was shown that the bactericidal activity of sodium carboxymethyl cellulose silver nanocomposite films (SCMC SNCFs) as an effective antibacterial material was improved by loading of curcumin with SCMC SNCFs [24].

In another in situ investigation, the synergistic effect of curcumin encapsulated chitosan-[poly (vinyl alcohol)] silver nanocomposite films was shown. The novel antimicrobial films with pronounced antimicrobial exhibition against E. coli proved to be potential antibacterial material for treating infections or wound dressing [25]. It has been demonstrated that curcumin as a plant derivative has a wide range of antiviral activity against different viruses. Inosine monophosphate dehydrogenase (IMPDH) enzyme due to rate-limiting activity in the de novo synthesis of guanine nucleotides is suggested as a therapeutic target for antiviral and anticancer compounds. Among the 15 different polyphenols, curcumin through inhibitory activity against IMPDH effect in either noncompetitive or competitive manner is suggested as a potent antiviral compound via this process [26]. Curcumin was tradionally utilized for its preventive action against tumorigenesis, oxidation, inflammation, apoptosis, hyperlipemia and in the treatment of Alzheimer's disease by multiple site targeted therapy [27, 28]**.**

Due to extensive traditional use of turmeric in food products, various researches have been done in order to study the turmeric and curcumin with the aspect of controlling fungal related spoilage and

fungal pathogens. The study on addition of turmeric powder in plant tissue culture showed that turmeric at the 0.8 and 1.0g/L had appreciable inhibitory activity against fungal contaminations [29]. One of the major complications during therapies against chronic asthma is oropharyngeal candidiasis.

Curcumin as a potential candidate for the treatment of candidosis with anti-inflammatory activity was studied in a murine model of asthma. Oral administrator of curcumin is more effective than dexamethasone in reducing fungal burden in BALB/c mice. It also significantly decreased pathological changes in asthma [30]. The optimum potential of curcumin is limited because of poor oral bioavailability and insufficient solubility in aqueous solvents leading to poor absorption, fast metabolism, and quick systemic elimination [31].

Although curcumin is poorly absorbed after ingestion, multiple studies have suggested that even low levels of physiologically achievable concentrations of curcumin may be sufficient for its chemopreventive and chemotherapeutic activity. Thus, curcumin regulates multiple targets (multi targeted therapy), which is needed for the treatment of most diseases, it is inexpensive and has been found to be safe in human clinical trials.

In spite of its efficacy and safety, curcumin has not yet been approved as a therapeutic agent This gap between the high level of many pre-clinical studies and the limitation of its right clinical use due to poor aqueous solubility, together with low bioavailability, lead to many uncertainties of its therapeutic effect in the Western population [32]. The present study gives a comparative data of the bio-absorption of two different turmeric formulations in comparison with regular turmeric extract.

MATERIALS AND METHODS:

Year of experimentation:

2014 Site of Experimentation: CARe Keralam Ltd, Thrissur, Kerala, India.

The present study protocol has been inspected in the spirit of OECD principles of Good Laboratory Practices {ENV/MC/CHEM (98)17:1997}, reviewed and approved by Institutional Animal Ethics committee (IAEC) of CARe Keralam Ltd.

Chemicals, reagents and samples used:

All solvents and chemicals used were of AR/HPLC grade and obtained from Merck (Mumbai, India).The reference curcuminoids were purchased from Chromadex Company USA.

A. Preparation of Turmeric extracts:

Three different curcuminoids containing turmeric extracts were used as Test items for the present study. They are Regular Turmeric Extract (C 95), Turmeric Extract Formulation I (C 7.5), Turmeric Extract Formulation II (C 50).

1) **Regular Turmeric Extract, TMR (C 95):** Dried turmeric rhizome was extracted with Hexane: Acetone (70:30) and desolventised to form oleoresin turmeric. This oleoresin was crystallized using Iso propyl Alcohol (IPA). The crystals were dried and powdered which contain 96.5% of curcuminoids (Curcumin = 74%, DMC = 17.5%, BDMC = 5%) .

2) **Turmeric Extract Formulation I, TMF I (C 7.5):** Hydro alcoholic extraction of dried turmeric raw material was done .The extracted miscella was desolventised to form the product. The extract contains 7.8% of curcuminoids(Curcumin = 4.27% , DMC =1.56%, BDMC = 1.97%), to retain the composition of curcuminoids almost similar to that in the turmeric root.

3) **Turmeric Extract Formulation II, TMF II (C 50):** Spent turmeric Oleoresin (STO) which was considered as an industrial waste was purified using Diaion HP 20 resin chromatography. Column eluted with 80% methanol and 10 fractions collected. Curcuminoids enriched fractions combined and concentrated. Concentrated fractions dissolved in methanol and precipitated in excess hexane. Precipitated product is filtered and dried to yield curcuminoids of purity 51% (Curcumin = 12%, DMC = 13% , BDMC =26%), with a curcuminoids composition different from that of commercially available regular turmeric extract C 95.

B. Study Design:

The animal species, Wistar albino rats were collected from Veterinary University, Mannuthi, Thrissur, Kerala, India. Female Albino rats which were nulliparous and non-pregnant weighing 150-210g with 8 to 11 weeks age were used in the study. Single animal was housed in a standard polysulphonate cage under standard laboratory conditions which include air conditioned with adequate fresh air supply with IVC system (Air changes 15 per hour),room temperature 21°C to 24°C, relative humidity 57-65% with 12 hours light and 12 hours dark cycle. Water and feed were given *ad-libitum*.

1) **Vehicle:** 5% Tween 80 with distilled water (v/v) was used as vehicle for formulation preparation. The test item forms good suspension with tween 80.Hence tween 80 was used as a suspending agent for test item formulation. Tween 80 is universally accepted and routinely used vehicle in oral toxicity studies.

2) **Dose Formulation** The weighed test item was finely ground in a mortar with the help of the pestle. The ground test item was suspended in 5% Tween 80 to get desired concentration as per the dose (mg/kg body weight).Formulation of the test item was prepared shortly before dosing.

3) **Administration of Test item:** The animals were acclimatized for 5 days and kept on fasting overnight (water provided *ad libitum*) prior dosing. On the sixth day, the test item was administered orally by gavage to each rat as single dose using gavaging needle. The dose volume was 1ml/100g body weight for all animals. The animals were divided into 10 groups based on the dosage of test item, Vehicle control (5% Tween 80 in distilled water) was fed to Group 1 and it was kept as control. Regular turmeric extract and two different turmeric formulation containing curcumoinds 7.8% and 51% were orally administered at different doses (50mg/kg, 150mg/kg and 300mg/kg) to the respective groups. The test groups and the dosage are listed in **Table 1**. The whole faeces were collected separately from each rat up to 24 hour. The faeces of each rat were dried separately for HPLC analysis.

C. Estimation of Curcuminoids by HPLC:

1) **Preparation of Standard**: Accurately weigh 0.0500g standard in to a 100-ml standard flask. Add 60 ml Methanol and swirl well, make upto the volume using methanol. Dilute 2ml to 25ml using methanol. Filter the solution using PVDF 0.45 um HPLC filter and inject 20µl in HPLC.

2) **Preparation of Sample:** Dried faeces were weighed and extracted with acetone for HPLC analysis. The samples were filtered through 0.45 um HPLC filter before injection.

3) **Chromatographic conditions:** Curcuminoids were separated on a Daimonsil C18 column (4.6*100, 5 micron) using acetonitrile-5% acetic acid (75:25 v/v) as mobile phase at a flow rate of 1ml/minute, 20µl of sample was injected, curcuminoids were quantified at a wavelength of 425nm [33] .

TABLE 1: SEGREGATIONS OF RATS FOR THE STUDY

Sl No	GROUPS	DOSE
1	Group 1	Vehicle control (5% Tween 80 in distilled water)
2	Group 2	TMR 50mg/kg
3	Group 3	TMR 150mg/kg
4	Group 4	TMR 300mg/kg
5	Group 5	TMF I 50mg/kg
6	Group 6	TMF I 150mg/kg
7	Group 7	TMF I 300mg/kg
8	Group 8	TMF II 50mg/kg
9	Group 9	TMF II 150mg/kg
10	Group 10	TMF II 300mg/kg

TMR- Regular Turmeric Extract, TMF I - Turmeric Extract Formulation I, TMF II - Turmeric Extract Formulation II.

RESULTS AND DISCUSSION:

Curcuminoids, when given orally or intraperitoneally to rats, is mostly egested in the faeces and only a little in the urine .Only traces of curcuminoids are found in the blood from the heart, liver and kidney. So the absorption of curcuminoids can be evidently studied by estimating the percentage of curcuminoids the faeces of rats. In the present study, rats were fed with regular turmeric extract with 96.5% curcuminiods and two different formulations containing 7.8% and 51% curcuminoids respectively, and the absorption of curcuminoids in the gastro intestinal tract compared.

Groups administered with Turmeric extract formulation I and II had low curcuminoids content in faeces, where as groups administered with Regular turmeric extract had high curcuminoids content when compared with vehicle control. This shows that the absorption of curcuminoids is more in formulations than that of the Regular turmeric extract. The percentage of curcumin content in faeces for each group is shown in **Table 2.**

Based on the percentage of curcuminoids in faeces, the absorption percentage of curcuminoids in gastro intestinal tract is calculated. The bio absorption for regular turmeric extract was found to be lower compared to that of formulation I & II. The absorption percentage of the three formulations is given in **Table 3, Table 4 and Table 5** and graphical representation shown in **Fig.1**.

Curcumin facilitates bile acid secretion which enhances the digestion of lipids and proteins and it eliminates excess cholesterol out of the liver. Curcumin is a pharmacologically safe component - without any side effects with various medicinal properties like anti-carcinogenic ,anti-inflammatory & anti-oxidant activities. The present study revealed the poor absorption of curcuminoids by albino rats from regular turmeric extract was 68 to 72% only in three doses where as the absorption of curcuminoids of two turmeric extract formulations I and II has 97 to 99% and 99.6 to 99.7% respectively.

TABLE 2: CURCUMINOIDS CONTENT IN FAECES

Sl No:	Groups	Curcuminoids Content (%)
1	Group 1 Vehicle control (5% Tween 80 in distilled water)	0.000±0.000
2	Group 2 TMR 50mg/kg	0.0910±0.0420
3	Group 3 TMR 150 mg/kg	0.3025±0.0219
4	Group 4 TMR 300mg/kg	0.4579±0.0759
5	Group 5 TMF I 50mg/kg	0.0006±0.0002
6	Group 6 TMF I 150 mg/kg	0.0006±0.0002
7	Group 7 TMF I 300mg/kg	0.0006±0.0002
8	Group 8 TMF II 50mg/kg	0.0005±0.0002
9	Group 9 TMF II 150 mg/kg	0.00158±0.0004
10	Group 10 TMF II 300mg/kg	0.0030±0.002

Values are expressed as mean ± SEM,
n=5 animals each;
Group II-X was compared with Group I (5% Tween 80 in distilled water).
TMR- Regular Turmeric extract
TMF I – Turmeric extract formulation I
TMF II – Turmeric extract formulation II

TABLE 3: PERCENTAGE OF CURCUMINOIDS ABSORBED IN TEST SPECIES FOR REGULAR TURMERIC EXTRACT (TMR)

SL no:	Group and Dosage	Weight of Animal(g)	Curcuminoid content Fed Orally (mg)	Curcuminoid content in Faeces (mg)	Curcuminoid content Absorbed(mg)	% of Absorption	Average percentage
1		155	7.5	2.5	5	66.66	
2		155	7.5	1.58	5.92	78.93	
3	Group 2	160	7.72	2.2	5.52	71.50	
4	50mg/kg	155	7.48	2.6	4.88	65.24	

							71.19
5		165	7.96	2.1	5.86	73.63	
6		170	24.6	7.38	17.22	70	
7		200	28.95	9.73	19.22	66.39	
8	Group 3	175	25.33	7.21	18.12	71.54	
9	150mg/kg	200	28.95	9.46	19.49	67.32	
10		210	30.40	9.79	20.61	67.79	68.61
11		160	46.32	16.27	30.05	64.87	
12	Group 4	205	59.35	11.56	47.79	80.52	
13	300mg/kg	155	44.87	11.32	33.55	74.77	
14		170	49.22	15.12	34.1	69.28	
15		150	43.43	12.12	31.31	72.09	72.31

TABLE 4: PERCENTAGE OF CURCUMINOIDS ABSORBED IN TEST SPECIES FOR TURMERIC FORMULATION I (TMF I)

SL no:	Group and Dosage	Weight of Animal(g)	Curcuminoid content Fed Orally (mg)	Curcuminoid content in Faeces (mg)	Curcuminoid content Absorbed(mg)	% of Absorption	Average percentage
1		165	0.6435	0.017	0.6265	97.3	
2		155	0.6045	0.0195	0.585	96.77	
3	Group 5 50mg/kg	170	0.663	0.012	0.651	98.19	
4		200	0.78	0.008	0.772	98.97	97.79
5		170	0.663	0.015	0.648	97.73	
6		150	1.755	0.018	1.737	98.97	
7		160	1.872	0.011	1.861	99.41	
8		160	1.872	0.013	1.859	99.30	
9	Group 6 150mg/kg	170	1.989	0.010	1.979	99.49	99.21
10		150	1.755	0.019	1.736	98.91	
11		150	3.51	0.0122	3.4978	99.65	
12		205	4.797	0.0188	4.7782	99.60	
13		170	3.978	0.0133	3.9647	99.67	
14	Group 7	200	4.68	0.0154	4.6646	99.67	
15	300mg/kg	170	3.978	0.0190	3.959	99.52	99.62

TABLE 5: PERCENTAGE OF CURCUMINOIDS ABSORBED IN TEST SPECIES FOR TURMERIC FORMULATION II (TMF II)

SL No:	Group and Dosage	Weight of Animal(g)	Curcuminoid content Fed Orally (mg)	Curcuminoid content in Faeces (mg)	Curcuminoid content Absorbed(mg)	% of Absorption	Average percentage
1		165	4.2075	0.013	4.1945	99.69	
2	Group 8	165	4.2075	0.014	4.1935	99.67	99.65
3	50mg/kg	170	4.335	0.017	4.318	99.61	
4		200	5.1	0.015	5.085	99.71	
5		170	4.335	0.019	4.316	99.56	
6		140	10.71	0.034	10.676	99.68	
7	Group 9	190	14.535	0.035	14.5	99.76	
8	150mg/kg	150	11.475	0.0.9	11.436	99.66	99.70
9		160	12.24	0.041	12.199	99.67	
10		160	12.24	0.033	12.207	99.73	
11		155	23.715	0.107	23.608	99.55	
12	Group 10	175	26.775	0.04	26.735	99.85	
13	300mg/kg	160	24.48	0.102	24.378	99.58	
14		190	29.07	0.091	28.979	99.69	99.66
15		210	32.13	0112	32.018	99.65	

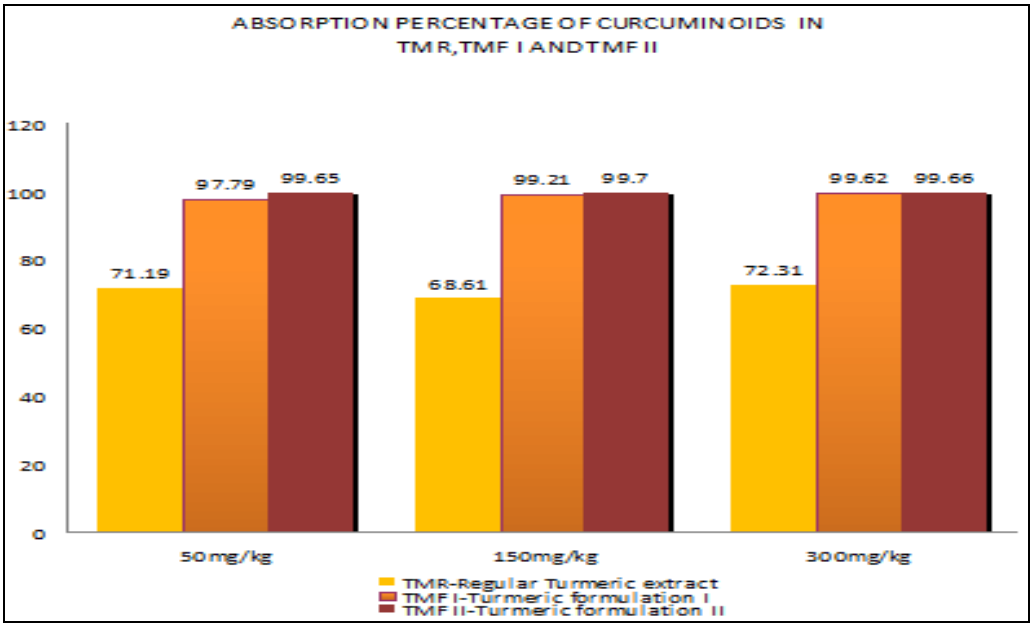

FIG.1: ABSORPTION PERCENTAGE OF CURCUMINOIDS IN TMR, TMF I AND TMF II

CONCLUSIONS: This present study indicates the improved bio-absorption for Turmeric formulation I and Turmeric formulation II compared to regular turmeric extract in animal system. More research is needed to prove its bioavailability and clinical aspects.

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