International Journal of Phytomedicine 6 (2014) 293-299
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Impact of Turmeric as dietary approach on HER2 status in blood of gastric cancer patients

Akhileshwar Kumar Srivastava [1], Divya Singh[1], Mallika Tewari[2], Hari Shankar Shukla[2], Bijoy Krishna Roy[1*]

***Corresponding author:**

Bijoy Krishna Roy

[1]Department of Botany, BHU,Baranasi India
[2]Department of Surgical Oncology, IMS, BHU, Baranasi India

A b s t r a c t

Curcumin as an active ingredient of turmeric acts as repressor against proteins expressed in cancerous cell. However, due to poor absorption, little is known about the effect of curcumin on HER2 expression in blood. A blood samples were drawn from ten subjects of gastric cancer aged 37 to 80 years using turmeric as random doses in diet. For hypothesising of study, subjects were convinced to use a constant dose (500 mg) of turmeric twice in a day till the five days and then blood was drawn. Out of ten, three subjects had detectable curcumin, whereas in blood of nine subjects including three showed overexpression of HER2 status (>15 ng/ml) at random doses of turmeric. At constant dose (500 mg) of turmeric, six subjects out of ten had increasing curcumin level with decreasing HER2 status in blood, whereas in two subjects, HER2 status was remain unchanged due to no detection of curcumin. In other two subject underwent for chemotherapy had low HER2 status without curcumin detection. An inverse relation of curcumin holds on HER2 status in blood of four subjects with different body mass of same age group after taking constant doses of turmeric powder. The curcumin bioavailability in blood depending on higher doses of turmeric and physical status of subjects may inhibit the HER2 expression.

Keywords: Subjects, Curcumin, HER2, HPLC, Blood, Bioavailability

Introduction

A polyphenolic compound curcumin [1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadien-3, 5-dione] is an active ingredient derived from the rhizomes of turmeric (*Curcuma longa* L.) [1]. Turmeric is widely used as a dietary spice, a coloring, and flavouring agent in various parts of the world, especially in Indian subcontinent [2]. Since centuries, turmeric has been known for treatment of several ailments, including biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis [3, 4]. Turmeric consumption has been associated for beneficial effects on human health; mainly anti-inflammatory and cancer chemopreventive activities [5]. Experimentally, it has been equivocally established that free curcumin induces arresting and or apoptosis in human cancer cell lines derived from solid tumors including colorectal, lung, breast, pancreatic and prostrate carcinoma [6, 7]. Despite of curcumin application in cancer therapy, recently in clinical trial in patients of familial adenomatous polyposis confirmed that curcumin could also make positive changes in organ sites affected with cancer. According to earlier reports epidermal growth in tumor is guided by a growth factor receptor protein (HER2) belongs to a family transmembrane tyrosine kinase growth receptor protein encoded by a proto-oncogene located on a chromosome 17q21 [8, 9]. It has been evidenced that progressive tumor growth linked with positive HER2 status and also resistant to cytotoxicity [9, 10]. It is also helpful in optimising the treatment of some metastatic diseases by the application with different chemopreventive agents [11, 12]. The extracellular domain of HER2 protein has been assayed by ELISA in blood serum after cleavage of ADAM (a disintegrin and metalloproteinase) [13]. The positive HER2 status in serum has been indicated by its overexpression and amplification in tumor tissues (Molina et al. 1996) [14]. Along with several natural or synthetic chemicals as suppressor of the proliferation of tumorogenesis in cancer [15], curcumin also effects on expression of growth factors in cancerous tissues by inhibiting the proteins, tyrosin, and kappaC kinases [16]. During tumorogenesis p21-activated kinases (PAK1) play a vital role in cytoskeletal dynamics along with cyclinD1 [17] and curcumin also acts and leads to HER2 suppression in cancer cells by inhibiting the PAK1 activity. Its efficiency has been explained on the basis of inhibition of transcription factors and oncogene expression [18]. On the other hand increase in level of HER2 in serum is due to overexpression and amplification of HER2 in cancerous tissues [19].

Inspite of showing extra ordinary medicinal properties its commercial formulation is still a challenge because its poor bioavailability solubility and rapid plasma clearance. Due to poor oral bioavailability of curcumin information on the therapeutic action efficiency is limited [20]. Curcumin bioavailability is poorly soluble in water but maximum soluble in aqueous buffer upto low ng/ml (pH 5.0) [21]. The low bioavailability of curcumin in animal after oral intake may be due to less solubility as well as rapid systemic metabolism [22, 23]. However, in gastrointestinal tract curcumin is slightly absorbed and assimilated into its metabolic products by

conjugation and reduction and also accumulation traces in the blood, while maximum amount passes through excretory products of body urine and faeces [24]. In case of rodents curcumin degradation involves a major metabolic pathways that finally reduced to dihydrocurcumin and tetrahydrocurcumin successively and then they conjugate with glucuronic acid [25, 26].

The main purpose of this study was to provide evidence to support the amount of bioavailability and expression of growth factor in blood of gastric cancerous patients through oral delivery. We evaluated the HER2 status in response of curcumin in blood of gastric cancer patients of different age groups and body weight after oral administration of turmeric powder at random and escalated doses.

Material and Method

Clinical trial design for oral administration in human subjects

The study and comprehensive written consent used in this study protocol were approved by ethical committee of Institute of Medical Science, Banaras Hindu University, India. Prior to start this study, male and female subjects of gastric cancer aged between 37 and 80 year, with a body mass 45-64 kg were selected. Selected subjects were seven males and three females (age mean = 52.2 y, and body weight = 55.1 kg) (Table 1). The subject were neither addicted to any drugs nor intoxicated chronically before and during study. The subjects were already taking the random doses of turmeric in their diets. Assuming the low intake and low absorption, subjects were advised to dose constant dose (500 mg).

Table 1. Description of gastric cancer patients

Subjects	Sex	Age (y)	Weight (kg)
I	M	80	51
II	M	60	60
III	F	65	52
IV	M	40	62
V	M	40	49
VI	F	55	57
VII	M	65	63
VIII	M	37	64
IX	F	40	45
X	M	40	48
		Mean=52.2	Mean=55.1

Abbreviation: M, Male and F, Female

Collection of the blood

The blood samples (10 ml) were collected from each subject after 5 h from postprandial periods and distributed into each heparinised and serum tubes. The blood samples were immediately centrifuged at 4000 x g for 15 min to obtain plasma and serum. The samples were stored at -70^{0}C until assayed. The plasma of sheep blood was used for validation of HPLC.

Detection of the serum HER2

The status of HER2 in blood serum were measured by using a kit obtained from the Oncogene Science HER2 Microliter ELISA (Oncogene Science, Cambridge, MA, USA), and procedure was followed in accordance with the instructions of manufacturer.

Extraction of curcumin from plasma

The method of curcumin extraction from plasma was followed according to Heath et al. [27]. An aliquot of each plasma (500 µl) was transferred to 10 ml glass tube and then mixed with water (100 µl) as well as 50 µl of internal standard and vortexed for 30 s. The samples were then mixed with 1,000 U β-glucuronidase (20 µl) in 0.1 mol/L phosphate buffer (pH 6.8) and incubated at 37^{0}C for 1 h to hydrolyze curcumin conjugates. The solution (1 ml) of ethyl acetate/methanol (95:5, v/v) was added to each sample as an extracting reagents and then vortex mixed for 5 min. The samples were centrifuged at 4000 x g for 15 min at 4^{0}C. The upper organic layer was transferred to a 1 ml glass tube, evaporated to dryness using a centrifuge concentrator and then methanol (200 µl) was added to dried extract before HPLC analysis.

HPLC analysis

The HPLC model, HP C-18 column (1250 4.6 mm), and Millipore (0.454 µm) membrane filter were used to assess the amount of curcumin in turmeric and blood plasma in two phases of experiments.

The detection of curcumin in turmeric powder

In the first phase, curcumin was extracted from turmeric powder by solvent extraction method [28]. The turmeric powder was weighed in three different amount 5, 10, and 20 mg, respectively for percentage accuracy to measured amount of curcumin and diluted with methanol. The curcumin was separated by using mobile phase (methanol 24.1%, acetonitrile 38.2%, and deionised water 37.7%) with flow rate of 1 ml/min at 40 ^{0}C, and observed at 425 nm.

Determination of Curcumin level in the blood plasma

In the second phase, mobile phase consisted of 0.1% acetic acid/65% methanol/35% water (v/v/v; A) and 100% methanol (B). Chemicals purchased from Spectrochem laboratories (Mumbai, India). The dried extract was eluted by linear gradient mobile phase starting for separation 100% A at zero time to 100% B in 15 min and then again to 100% A in 2 min at a flow rate of 1 ml/min. The injected volume was 20 µl for all samples and detected at 420 nm. Chromatograms were processed by Empower 2 software.

A standard curve for validation of HPLC analysis was constructed using plasma of sheep blood in which no curcumin was detected. Plasma samples (500 µl) were added to different amount of a standard curcumin solution to make final concentrations of curcumin: 0.1, 0.125, 0.25, 0.5, 1.00, 1.25, 2.5, 5.0, and 10 ng/ml, respectively.

Statistical Analysis

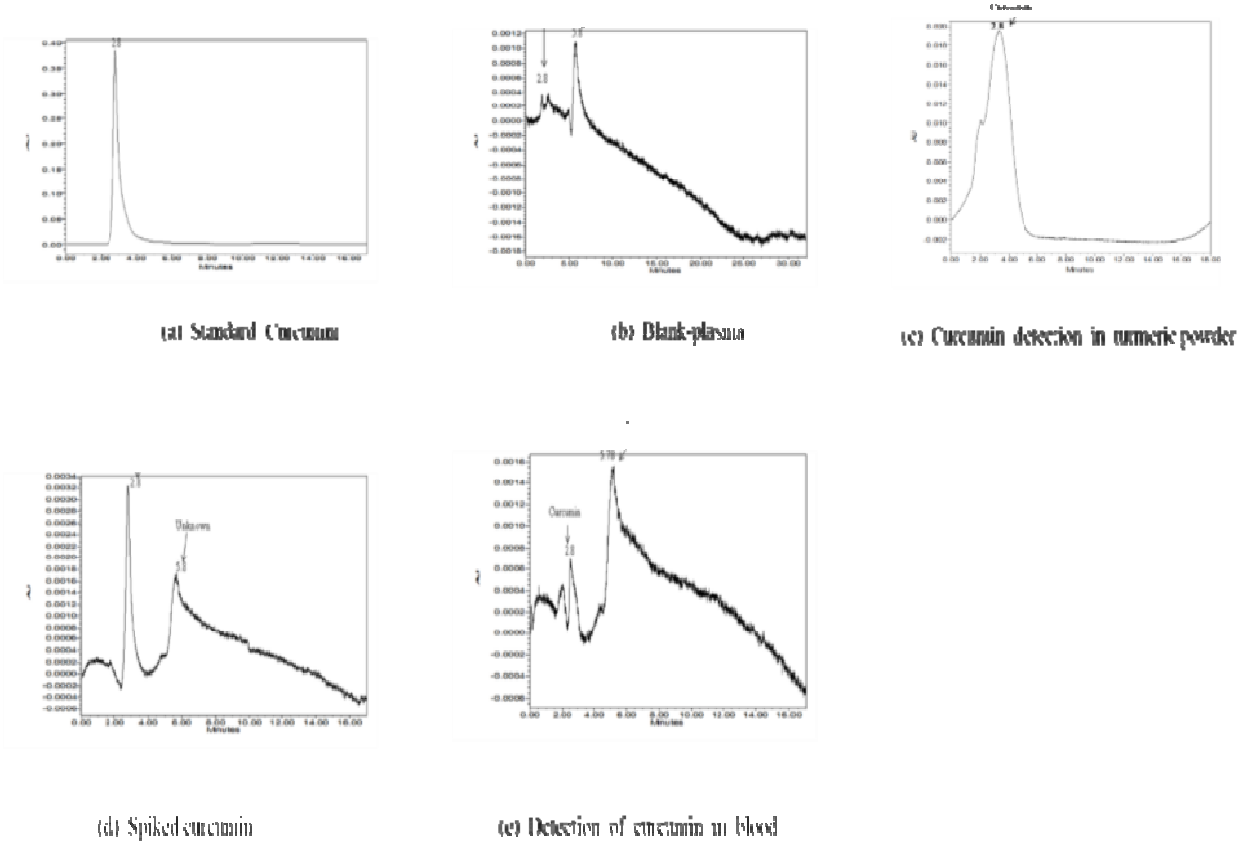
Mann-Whitney U Test was used to test statistically significant/insignificant relation between curcumin and HER2 level in blood of gastric cancer patients. The differences existed regarding variations in curcumin as well as HER2 status in blood serum of subjects using random and constant doses of turmeric was analyzed using pair t-test. All quantitative data were statistically analyzed using SigmaPlot 11.0 statistics software. The data are expressed as mean±standard deviation (S. D.). P values less than 0.05 and 0.01 were considered statistically significant.

Results and Discussion

Recently, the medicinal properties of curcumin is generating an interest in clinical researches. Earlier, it has been reported that curcumin has potential efficacy to cure of several hazardous diseases, hence number of affected patients in Indian continent and other developing countries hope for alternate possibilities for treatment of cancer by using traditional medicine. Ten subjects with gastric cancer were selected for detection of curcumin level and HER2 status in their blood samples using random doses of turmeric powder in daily diet (Table 1). However, due to low solubility and bioavailability of curcumin in body failed to address its medicinal action against severe diseases and main reasons has been explained as rapid metabolic degradation [29]. Therefore, an attempt has been made to enhance curcumin bioavailability in human body, dose of turmeric powder was escalated to 500 mg in diets in twice of a day till five days. After five days no adverse events were reported in the subjects. Then curcumin level as well as HER2 status in blood samples were assessed.

The selectivity of HPLC analysis was studied by using standard curcumin and blank plasma samples, which did not show any interfering components [Figure. 1(a) and (b)]. Curcumin was detected in turmeric powder in chromatogram [Figure. 1(c)]. A typical chromatogram of a curcumin-free plasma samples spiked with standard curcumin is shown in Figure. 1(d). The chromatogram with small peak of curcumin in plasma samples of subjects using turmeric in diets were analyzed [Figure. 1(e)]. The retention time (2.8 min) of peak obtained from processed samples were identified as presence of curcumin in blood similar to standard curcumin.

Fig. 1. Chromatograms of

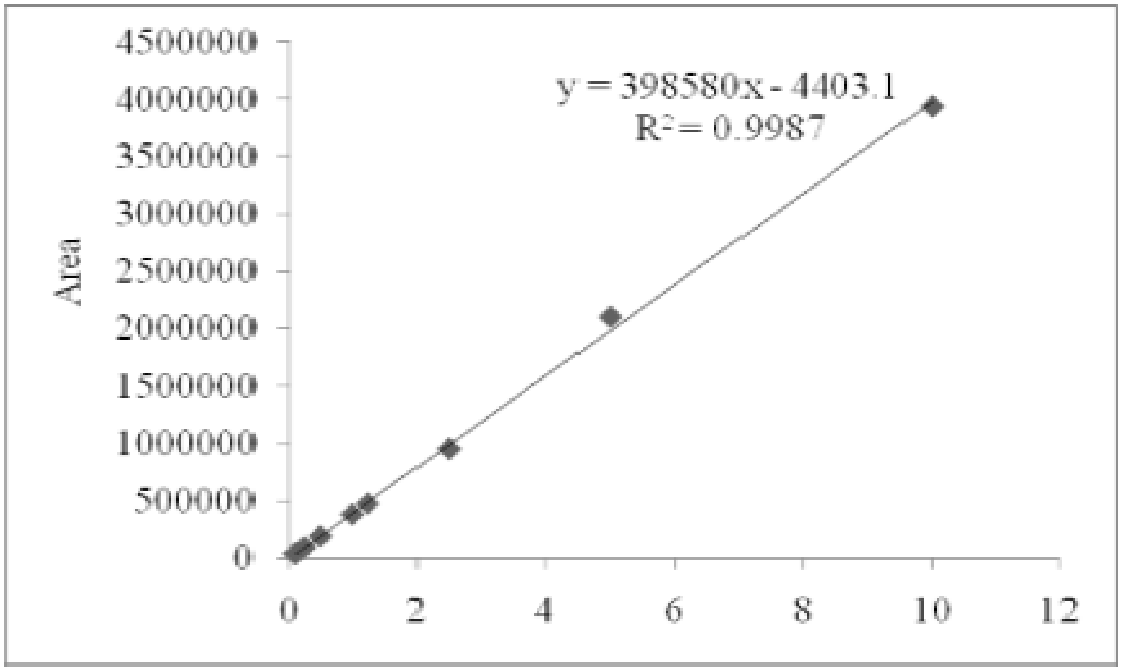


(a) Standard Curcumin (b) Blank-plasma (c) Curcumin detection in turmeric powder

(d) Spiked curcumin (e) Detection of curcumin in blood

The HPLC analysis was validated to construct the calibration by using the value of peak area versus concentration of each analyte. The calibration curve was highly linear (r^2 0.998) within the range of 0.1-10 ng/mL for curcumin spiked in blank plasma (Figure. 2). The values of regression parameters *a* for the slope and *b* for the y-intercept were calculated to be 39858 and -4403.1, respectively. The lower limit of quantitation was 0.16 ng/mL based on this method. The corresponding coefficient of variance was less than 11%.

Figure. 2. A representative standard curve for curcumin in blank plasma extending from 0.1-10 ng/mL.



In the present findings, except some subjects, curcumin was not detected in blood of gastric cancer patients at random doses of turmeric. Results suggested that curcumin could not exist in blood plasma for longer period due to its rapid metabolic process inside the body. Therefore, turmeric dose has been enhanced for clinically study. An evidence showed that no curcumin was detected in blood after 24 h in rat after providing curcumin orally [30]. In our case, out of ten, only three subjects IVth, Vth, and IXth had curcumin level (0.17, 1.00, and 0.29 ng/mL) in blood plasma, whereas HER2 protein were overexpressive (>15 ng/ml) in blood of nine subjects, except only VIth showed normal expression (<15 ng/ml) at random doses of turmeric (Table 2) and supported to findings of Asgeirsson et al. [31]. The result supported to earlier reports that at low level of curcumin in blood after rapid metabolism of turmeric [32] and that could not fully suppress the expression of HER2 Table (2a). However, relation between curcumin level and HER2 status in blood were significant at random doses of turmeric in three subjects (P=0.004).

The amount of curcumin was calculated 100.00 µg (4.00%) in 500 mg turmeric powder that was administered orally in subjects. Curcumin was detected in blood plasma of only six subjects (III, IV, V, VII, IX and Xth) along with reduction in HER2 level after oral administration of constant dose (500 mg) of turmeric (Table 2b). The significant relation between curcumin and reduction in HER level was observed (p=0.001). It has been also proved that high expression of cyclinD1 and abnormal PAK1 activity during tumorogenesis in gastric cancer patients was inhibited or down regulated along with HER2 suppression in cancerous cell by the action of curcumin [19]. The left four subjects had not curcumin in their blood plasma after escalating the turmeric dose upto 500 mg. As it has been earlier explained that even after oral administration of higher doses of curcumin fail to conjugate to plasma [33]. In case of Ist and IInd subjects, no differences in HER2 level (20.13 and 19.07 ng/ml, respectively) as in random doses was found after using constant doses of turmeric, because of no curcumin was detected. No detection of curcumin in blood sample of subjects VIth and VIIIth with lower HER2 level after using constant doses of turmeric were not supporting the results because they were underwent for chemotherapy. The paired t-test of HER2 level in blood serum after using constant doses of turmeric in comparison to random doses in diet was decreasing significantly (p=0.005).

Table 2. Total curcumin and HER2 protein level (ng/ml) were assayed in blood of gastric cancer patients using turmeric in diets

2(a) at random doses.

Subjects	Curcumin	HER2
I	ND	20.13±0.17
II	ND	19.07±0.18
III	ND	19.03±0.35
IV	0.17±0.015	18.47±0.16

2(b) at constant dose (500 mg)

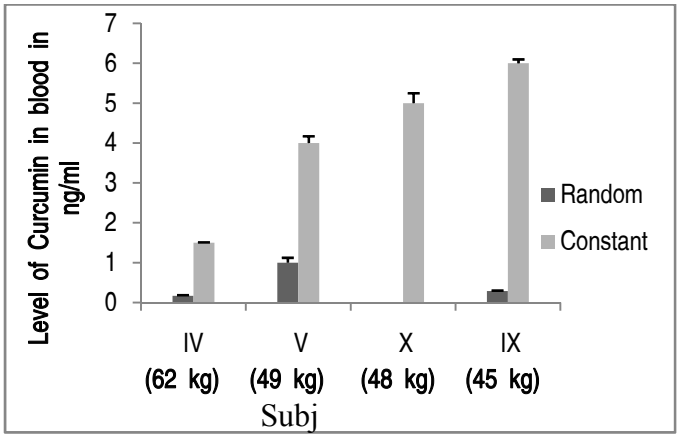
Subjects	Curcumin	HER2
I	ND	20.13±0.17
II	ND	19.07±0.18
III	3.7±0.06	18.90±0.40
IV	1.5±0.01	18.38±0.07

V	1.00±0.122	16.70±0.10	V	4.0±0.17	16.52±0.12
VI	ND	10.13±0.42	VI	ND	10.06±0.49
VII	ND	19.40±0.07	VII	3.0±0.17	19.23±0.04
VIII	ND	20.10±0.20	VIII	ND	19.90±0.40
IX	0.29±0.01	19.83±0.24	IX	6.0±0.10	19.50±0.20
X	ND	18.56±0.16	X	5.0±0.25	18.25±0.03

We also analysed existed difference in curcumin level determined in blood of subjects used random and constant dose of turmeric and found significant (P=0.012). Furthermore, it was also determined the variations existed in HER2 level in blood serum of patients using random and constant doses of turmeric and found significant (P=0.005). This existed differences indicated that escalated dose of turmeric upto certain level may increase its bioavailability and also supress the HER2 expression.

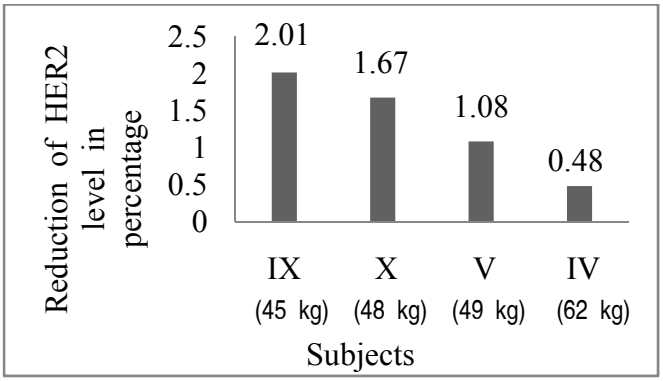
In our study, in blood of subject with different body mass of same age group (40 y), curcumin accumulation were not proportional after using random doses of turmeric, however after escalating constant dose of turmeric, accumulation of curcumin showed relation to body mass. After intake of constant dose (500 mg) of turmeric till five days, it seemed lower curcumin accumulation (1.5 ng/ml) in blood of subject (IV) with higher body mass (62 kg), whereas lower body mass (45 kg) of IXth had accumulated higher curcumin (6.0 ng/ml). This result indicated that curcumin accumulation may depend on body mass, if constant dose of turmeric for same age group subjects is used as shown in plotted histogram (Figure. 3).

Figure. 3 Comparative analysis of curcumin bioavailability in blood of subjects with different body mass at same age (40 y) group after random and escalated dose (500 mg) of turmeric.



After calculating the percentage inhibition of HER2 level in blood at constant dose of turmeric, same pattern was observed as in above subjects of same age group (40 y). Lower inhibition (0.48%) of HER2 was in blood of subject (IV) with higher body mass and higher (2.01%) in low body mass (45) of IXth subject, indicated that its inhibition by curcumin also may depend on body mass (Figure. 4).

Figure. 4 Percentage lowering the HER2 level in blood of subjects with different body mass at same age (40 y) group after providing escalated dose (500 mg) of turmeric for five days.



Conclusion

Curcumin as medicine are far the most commonly used in many diseases, mainly because of the belief that turmeric remedies are safe, cause less side-effect. Hence, study concluded for using of turmeric as medicine to suppress the HER2 expression in blood, curcumin bioavailability played vital role against same. The bioavailability of curcumin in blood depending on two aspects, first physical status of likewise body mass, age, etc. and other increasing dose of turmeric used in diets. Therefore, for curing as well as prevention of mankind from severe diseases by using turmeric, dose should be defined for existing of curcumin in blood plasma on the basis of physical status of body.

Acknowledgments

The authors would like to thank the University Grant Commission for financial support. Furthermore, the assistance of co-workers of the Institute of Medical Science and Department of Botany, Banaras Hindu University, India in performing experiments and analysis are gratefully acknowledged.

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