Abstract of the thesis

This thesis is mainly focused on inhibitory role of turmeric against oncogenic factors associated with *Helicobacter pylori* infection. The thesis comprises following studies: systematic reviews to ascertain of current knowledge, qualitative research to ascertain the pharmacological role of curcumin (a constituent of turmeric) against oncogenic factors (cagA and HER2) associated with *H. pylori* and computational approaches to investigate the potentiality of curcumin in comparison to some conventional drugs (clarithromycin, amoxicillin, pantoprazole, and metronidazole) against CagA (cytotoxic associated gene A) oncoprotein. In addition to above, an interactive properties of curcumin biotransformed compounds with CagA oncoprotein were analyzed for revealing the inhibitory potential against CagA+ *H. pylori*. The samples (cancerous tissues and blood) from gastric cancer patients with different body weight and ages were collected from operation theater, Department of Surgical Oncology, IMS, BHU and all the experimental work were performed in laboratory, Department of Botany, BHU and data were compiled. My research works were based on following objective:

1. Determination of curcumin amount in turmeric rhizomes collected from different parts of north-India.
2. Detection of oncogenic factors (cagA and HER2) of *Helicobacter pylori* in gastric cancer tissues.
3. Determination of curcumin level and its relation with HER2 (human epidermal growth factor receptor 2) status in blood of turmeric using patients.
4. *In silico* study of curcumin inhibitory potential in comparison to conventional drugs against CagA oncoprotein of *Helicobacter pylori*.
5. *In silico* investigation of interactive activity of curcumin biotransformed compounds with CagA.
After performing my research based on above objectives, the results and findings has been summarized as below:

1

After physico-chemical characterisation of soil collected from the different locations (Varanasi, Darbhanga, Mirzapur and Allahabad) and concluded that the curcumin production depends on the distribution of fertilizers N, K, or in combination of NPK along with other nutrients present in soil. The correlation of variable data of N (R = 0.76; P < 0.05) and K (R = 0.89; P < 0.01) in soil showed a significant co-relation with curcumin production among different locations of North-India.

The variable data of combined NPK (R = 0.83; P < 0.05) level along with other nutrients in soils at suitable doses showed positive role in the enhancement of curcumin production.

The highest curcumin (9.03%) content in turmeric rhizomes of Varanasi and lowest (4.43%) in Allahabad indicated the variations in curcumin contents depending on nutrients level in soils and geographical distribution. These findings could be added to the agronomic practices for future line of action for cultivation of turmeric subject to the enhancement of curcumin production. This could be an important information for users.

2

Before testing the efficacy of turmeric, the study has been undertaken to test the presence of oncogenic factors (cagA and HER2) associated with H. pylori in biopsies of subjects using turmeric at random and constant doses.

PCR amplification of H. pylori-specific 16S rDNA in isolates of infected tissues confirmed the results of RUT (rapid urease test) and among 85 biopsies, 54 biopsies were HP-positive and 31 as HP-negative. Amplified DNA templates
confirmed the cagA+ and cagA- strains of *H. pylori* with positive amplification in 34 template for cagA+ and the rest for cagA- strains.

Subsequently, out of 85, 21 biopsies showed HER2+ by IHC (immunohistochemistry) study, among them 15 biopsies showed cagA+ HP for HER2 (71.43%) expression and two were cagA- strains for negative expression. During PCR amplification of 21 biopsies, HER2 (human epidermal growth factor receptor 2) positive gene were amplified in 17 biopsies by expressing with a value of 81.50% and rest could not amplified indicated cagA- strains.

HER2 status was supported by AUC (area under curve) values of 0.750 and 0.875 for IHC expression and HER2 gene amplification respectively. Result showed a positive correlation between cagA+ *H. pylori* and HER2 status in cancerous tissues.

Our study also indicated that the cagA+ *H. pylori* strain showed positive relation with HER2 gene amplification and expression in gastric cancer, therefore cagA gene could also be used as a potent biomarker for gastric cancer in prognostic diagnosis to apply the therapeutic action for cagA+ *H. pylori* patients.

Further it has been added that male were more prone to *H. pylori* infection possessing cagA and HER2 oncogenic factors.

Our results could be helpful to researchers for improving the quality of diagnosis as well as therapy for patients infected with *H. pylori*.

### 3

Determination of curcumin level in blood of gastric cancer patients using turmeric in their usual diets by HPLC showed that the accumulation of curcumin in blood plasma at random doses had no linear relationship with physical status of patients.

However, at constant dose (500 mg) of turmeric, higher (6.0 ng/ml) curcumin amount in blood plasma was found to be accumulated in lower body mass (45
kg) and lower amount (1.5 ng/ml) in higher body mass (62 kg) of same age group (40 y).

This outcome of curcumin accumulation in blood plasma indicates that the bioavailability of curcumin depends on (a) physical status of subjects such as body mass and age (b) doses of turmeric in diets.

HER2 level in blood sample was determined by using a kit obtained from the Oncogene Science HER2 Microliter ELISA and inhibitory relation with curcumin level was not apparent at random doses turmeric intake but after intake of a constant dose (500 mg) of turmeric showed reduction in HER2 level in blood serum and indicated an inhibitory role for HER2 expression.

In similar way, inhibition of HER2 (2.01%) level in blood of gastric cancer patients was higher in lower body mass (45 kg) and lower (0.48%) in higher body mass (62 kg) of same age group (40 y). Results indicated that HER2 inhibition depends on curcumin bioavailability, physical status of patients, and intake doses of turmeric.

Hence, this study concluded that the turmeric as a drug is effective to control the oncogenic factor. The presence of curcumin in blood depends on its bioavailability in body and played an essential role in lowering the HER2 level in blood of gastric cancer patients infected with *H. pylori*.

4

In silico studies showed the physicochemical pharmacokinetic properties of curcumin as drugs similar to conventional drugs according to Lipinski’s rule of five except clarithromycin.

Further examination of druglikeness properties, curcumin exhibited positive druglikeness scoring (>0.50) and showed overall superiority than clarithromycin, amoxicillin, pantoprazole, and metronidazole.
Molecular docking analysis of curcumin and conventional drugs targeting with cagA, curcumin showed positive interaction with conserved residues (LYS644 and ALA653) and disrupted the integrity of protein of cagA.

The docking analysis also revealed the global binding energy (-36.37 kcal/mol) of curcumin, which has comparatively higher than other conventional drugs except clarithromycine (51.06 kcal/mol). Result indicated the strong and stable interaction of curcumin with CagA oncoprotein.

Our computational approach considering Lipinski’s rule, druglikeness score as well as molecular docking for lead compounds also supports the previous reports, and evidenced that the curcumin could be one of the potential therapeutic agent, and can replace conventional drugs failure against H. pylori infected patients.

During the in silico investigation of inhibitory potential of curcumin biotransformed (conjugated/reduced) compounds, all compounds except conjugated curcumin glucuronide have shown good ADME properties, whereas druglikness properties have been followed by all lead compounds.

It has been evidenced that all the biotransformed compounds are capable to interact with conserved residues of CagA similar to curcumin.

The docking studies of lead compounds with CagA oncoprotein have been effectively quantified by the molecular contribution as well as different energetic attribution: global energy, attractive vdw, and ACE. The global energy for binding of compounds with CagA oncoprotein demonstrated the inhibitory potential of curcumin and its biotransformed compounds against CagA+ H. pylori.

The higher binding energy of curcumin in conjugated form (CUR-GLR and CUR-SUL) revealed interaction with CagA and showed more inhibitory potential against CagA+ H. pylori than the reduced form. The global energy displays the inhibition potential in the order: CUR-GLR > CUR-SUL > CUR > DHC > THC > HHC.
On the basis of in silico approaches, it can be suggested that the oral administration of turmeric should be more preferred than intravenous administration in case of CagA+ *H. pylori* infection.

In view of above, our studies conclude that turmeric as natural drug may play similar pharmacological role to conventional drugs against oncogenic activities of *H. pylori* in infected patients. Hence curcumin (a product of turmeric) proved to be alternative of that drugs in prevention of oncogenic activities of CagA+ *H. pylori*.

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