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Toxicity prediction of compounds from turmeric (*Curcuma longa* L)

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ABSTRACT

Turmeric belongs to the ginger family *Zingiberaceae*. Currently, cheminformatics approaches are not employed in any of the spices to study the medicinal properties traditionally attributed to them. The aim of this study is to find the most efficacious molecule which does not have any toxic effects. In the present study, toxicity of 200 chemical compounds from turmeric were predicted (includes bacterial mutagenicity, rodent carcinogenicity and human hepatotoxicity). The study shows out of 200 compounds, 184 compounds were predicted as toxigenic, 136 compounds are mutagenic, 153 compounds are carcinogenic and 64 compounds are hepatotoxic. To cross validate our results, we have chosen the popular curcumin and found that curcumin and its derivatives may cause dose dependent hepatotoxicity. The results of these studies indicate that, in contrast to curcumin, few other compounds in turmeric which are non-mutagenic, non-carcinogenic, non-hepatotoxic, and do not have any side-effects. Hence, the cost-effective approach presented in this paper could be used to filter toxic compounds from the drug discovery lifecycle.

1. Introduction

Natural products from plants find application in several therapeutic formulations. These compounds belong to different chemical classes (alkaloids, phenolics, terpenoids, etc.) and have chemically diverse and complex structures. Because of these complex structures, many of the natural compounds are hard to synthesize (Mann, 1994) but the plant cells do with ease. Natural products have a wealth of applications. Some of them are used as drugs, while others possess important biological properties or are used as dietary supplements, as dyes, flavoring agents, or ingredients in the cosmetics industry (Cordell, 2002). However, finding specific chemical compounds in tens of thousands of plant species can be compared to finding a needle in a haystack (Hristozov et al., 2008). Currently, both academia and industry are interested in finding different plant sources of such compounds (Abel et al., 2002). Traditional medicines offer a rich and largely unexplored source of therapeutic leads for the pharmaceutical industry (Corson and Crews, 2007).

Turmeric (*Curcuma longa* Linn. Syn *C. domestica* Valeton) is extensively used as a spice, food preservative and colouring material commonly used in the Indian subcontinent (Aggarwal et al., 2007; Chattopadhyay et al., 2004). Traditionally many medicinal properties are attributed to this spice. Since the time of Ayurveda (1900 Bc) numerous therapeutic activities have been assigned to turmeric for a wide variety of diseases and conditions, including those of the skin, pulmonary, and gastrointestinal systems, aches, pains, wounds, sprains, and liver disorders (Aggarwal et al., 2007). Turmeric contains a wide variety of phytochemicals, including but not limited to curcumin, demethoxycurcumin, bisdemethoxycurcumin, zingiberene, curcumenol, curcumol, eugenol, tetrahydrocurcumin, triethylcurcumin, turmerin, turmerones, and turmeronols (Chattopadhyay et al., 2004). Extensive research within the last half century has proven that most of these activities, once associated with turmeric, are due to curcumin. Turmeric contains three different analogues of curcumin (i.e., diferuloylmethane, demethoxycurcumin, and bisdemothycurcumin). It is not clear whether all the three analogues exhibit equal activity. Although in most systems curcumin was found to be most potent (Sreejayan and Rao, 1996; Ahsan et al., 1999) in some systems bisdemethoxycurcumin was found to exhibit higher activity (Syu et al., 1998; Thapliyal and Maru, 2001). There are also suggestions that the mixture of all three is more potent than either one alone (Huang et al., 1998; Sreejayan and Rao, 1997). Tetrahydrocurcumin (a metabolite of curcumin) has been shown to be active in some systems (Sugiyama et al., 1996; Khopde et al., 2000; Okada et al., 2001; Pari and Murugan, 2004; Pari and Amali, 2005) and not in others (Nakamura et al., 1998; Ireson et al., 2001). Whether other metabolites of curcumin exhibit biological activity is not known. However, there are still several unanswered questions. The optimum dosage of curcumin for the treatment of a given disease is not clear. Serum levels of curcumin tend to be low (Lao et al., 2006). Second, the tissue concentration of curcumin and in cell cul-

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ture conditions is not known. Third, whether any components of turmeric other than curcumin that has beneficial effects either alone or in combination with curcumin need to be determined. For instance, numerous activities have been assigned to turmeric oil (Apisariyakul et al., 1995; Negi et al., 1999; Wuthi-udomlert et al., 2000; Skrzypczak-Jankun et al., 2000, 2003; Jankun et al., 2006). Fourth, structural analogues of curcumin that are more bioavailable and efficacious are needed. Last but not least, toxic effects of turmeric compounds were not studied extensively. However, there are some evidences to suggest that turmeric extracts can be toxic (Deshpande et al., 1998; Kandarkar et al., 1998). Hence in the present study we have adopted *in silico* methods to predict the toxicity of 200 compounds from turmeric.

2. Materials and methods

Two hundred chemical structures from turmeric were collected from (1) experimental identification (GC–MS profile obtained from the extracted essential oil samples of Turmeric), (2) reported compounds from literature and (3) from the NCBI PubChem database. The collected structures (Table 1) were drawn by using ACD/Chemsketch and were used to predict toxicity from chemical structures through descriptor predictions using DRAGON Professional Ver 5.4-2006 (TALETE Srl, Milano, Italy) and PreADMET Ver 1.0 (http://preadmet.bmdrc.org/preadmet), respectively. Because removing non-drug-like compounds from the drug discovery lifecycle in the early stages can lead to tremendous savings of resources (Cheng and Merz, 2003).

2.1. Toxicity prediction

2.1.1. Ames mutagenicity assay

Mortelmans and Zeiger (2000) described a short-term bacterial mutation assay caused by chemical substances. According to the data set of National Toxicology Program (NTP), the built biological model for toxicity prediction includes three strains: TA98, TA100, and TA1535.

2.1.2. Rodent carcinogenicity

The Predictive Toxicology Challenge (PTC) was initiated for the development of advanced technology for predictive toxicology models. We have used computational models for carcinogenicity prediction created by Helma and Kramer (2003) with the data set of both National Toxicology Program (NTP) (Benigni, 1997) and Food and Drug Administration (FDA).

2.1.3. Hepatotoxicity

The hepatotoxicity model (Dixon and Villar, 1999; Cheng and Dixon, 2003) predicts potential organ toxicity for a wide range of structurally diverse compounds. The model was developed from literature data of 382 compounds known to exhibit liver toxicity (positive dose-dependent hepatocellular, cholestatic, neoplastic, etc.) or trigger dose-related elevated aminotransferase levels in more than 10% of humans. An ensemble of recursive partition trees were trained against AlogP98 and 1D similarity data. Accuracy against 54 compounds reserved for testing was 80%. The model classifies compounds as either 0 or 1, meaning either "non-toxic" or "toxic", and provides an average-class-value estimate of confidence.

3. Results

The collected compounds from turmeric were screened for toxicity by using *in silico* models. The results are tabulated for mutagenicity, rodent carcinogenicity and also for hepatotoxicity (refer Table 1) in consistent with the US Food and Drug Administration (FDA) Critical Path Initiative for regulatory risk assessment and scientific decision support for highly sensitive endpoints such as carcinogenicity, mutagenicity etc. (Valerio et al., 2007).

3.1. Mutagenicity prediction (Ames test)

The Ames test (Mortelmans and Zeiger, 2000) is used worldwide as an initial screen to determine the mutagenic potential of new chemicals and drugs. The test is also used for submission of data to regulatory agencies for registration or acceptance of many chemicals, including drugs and biocides. International guidelines have been developed for use by corporations and testing laboratories to ensure uniformity of testing procedures. Hence the mutagenicity was predicted for the analysis data set. The compounds which were predicted as mutagens as well as non-mutagens are listed in Table 1.

Fifteen compounds belong to *Bisabolane type Sesquiterpinoids*, (refer Table 1, Sl. Nos. 4, 5, 7, 8, 9, 16, 18, 20, 22, 23, 24, 25, 26, 27 and 28) were predicted as non-mutagens. Similarly, eleven compounds (Nos. 54, 55, 56, 57, 61, 62, 63, 64, 65, 66 and 68) of the class *Diphenylheptanoids*, six compounds (Nos. 184, 185, 187, 189, 193 and 194) of the class *Sesquiterpinoids* (*ST*), a couple of compounds (Nos. 41 and 42) of the class *Carabrane type ST*, a couple of compounds (Nos. 46 and 48) of the class *Curcumane type ST*, three compounds (Nos. 92, 96 and 102) of the class *Germacrane type ST*, 17 compounds (Nos. 111, 113, 115, 116, 117, 118, 119, 120, 122, 126, 127, 128, 135, 136, 138, 139 and 140) of the class *Guaiane type ST* and four compounds (Nos. 179, 180, 181 and 182) belong to *Sesquiterpene dimers* were also predicted as non-mutagens.

All the analysed *Elemane type ST*, were predicted as mutagens except elemol (No. 79), which is a non-mutagen. Similarly, mutagenicity was observed among the analysed *Monomeric phenylpropene derivatives* except calebin A (No. 143). Only citronellol (No. 164) was predicted as non-mutagen among the *Monoterpenoids*, the remaining compounds were predicted as mutagenic. All the analysed compounds from *Cadalene type ST*, *Cadinane and furanocadinane type ST*, *Dimeric phenylpropene derivatives*, *Diphenylpentanoids*, *Eudesmane and furanoeudesmane type ST*, *Labdane type diterpenoid* and other *miscellaneous* compounds were predicted as mutagens.

3.2. Rodent carcinogenicity prediction

Valerio et al. (2007) studied rodent carcinogenicity to estimate the carcinogenic potential of small, organic, naturally occurring chemicals found in the human diet and found that the *in silico* analysis was capable of identifying the rodent carcinogenic potential of naturally occurring organic molecules found in the human diet with a high degree of sensitivity. Similarly, the prediction approach found that the following compounds were non-carcinogens.

Nine compounds (Nos. 3, 7, 15, 23, 24, 25, 26, 27 and 32) belong to *Bisabolane type ST*, only one compound (8-hydroxycadalene, No. 35) belong to *Cadalene type ST*, a couple of compounds (Nos. 38 and 39) belong to *Cadinane and furanocadinane type ST* and 16 compounds (Nos. 111, 112, 114, 124, 125, 126, 127, 128, 129, 130, 132, 133, 135, 136, 139 and 140) belong to *Guaiane type ST* were predicted as non-carcinogens. 4S-dihydrocurcumenone (No. 41) belongs to *Carabrane type ST* is the only non-carcinogen among the analysed compounds of this group. Germacrone 4,5-epoxide (No. 105) belongs to *Germacrane type ST* is the only non-carcinogen, all others belong to this group are predicted as carcinogens.

The analysis of diphenylheptanoids showed that five compounds (Nos. 59, 60, 61, 62 and 70) were non-carcinogens. Three of the *Monomeric phenylpropene derivatives* (Nos. 143, 144 and 145) are non-carcinogens. Only three of the *Monoterpenoids*, α-terpineol (No. 155), carvacrol (No. 163) and p-cymene-8-ol (No. 173) are non-carcinogenic, the rest are carcinogenic. Four of the parviflorene (Nos. 179, 180, 181 and 183) of the class *Sesquiterpene dimers* were predicted as non-carcinogens. A couple of compounds, 1-4-methylphenylethanol (No. 195) and 3,7-dimethyl-5-indanecarboxylic acid (No. 196) were also predicted as non-carcinogens among other *miscellaneous compounds*.

All the analysed compounds belongs to ST, Labdane type diterpenoid, Eudesmane and furanoeudesmane type ST, Elemane type ST, Diphenylpentanoids, Dimeric phenylpropene derivatives, Curcumane type ST were predicted as carcinogens.

Table 1
Toxicity prediction profile of the discovery leads from turmeric.

Sl. No.		Ames mutagenicity test						Rodent carcinogenicity				Hepato-toxicity
			−S9			+S9						
Bisabolane type sesquiterpinoids		**TA98**	**TA100**	**TA1535**	**TA98**	**TA100**	**TA1535**	**Total Result**	**Mouse**	**Rat**	**TotalResult**	**Result**
1	1,3,5,10-Bisabolapentaen-9-ol	–	–	–	+	–	–	+	+	–	+	0
2	1,3,5,10-Bisabolatetraene	+	–	–	+	–	–	+	+	–	+	0
3	1,3,5,11-Bisabolatetraene	+	–	–	+	–	–	+	–	–	–	0
4	1,10-Bisaboladiene-3,4-diol	–	–	–	–	–	–	–	+	–	+	0
5	2,5-Dihydroxybisabola-3,10-diene	–	–	–	–	–	–	–	+	–	+	1
6	2,10-Bisaboladiene-1,4-diol	–	–	–	–	–	–	–	+	–	+	1
7	3-Hydroxy-1,10-bisaboladien-9-one	–	–	–	–	–	–	–	–	–	–	0
8	4,5-Dihydroxybisabola-2,10-diene	–	–	–	–	–	–	–	+	–	+	0
9	4-Hydroxy-3-methoxy-2,10-bisaboladien-9-one	–	–	–	–	–	–	–	–	+	+	0
10	4-Hydroxybisabola -2,10-dien-9-one	–	–	–	–	–	–	+	+	–	+	0
11	4-Methoxy-5-hydroxy-bisabola-2,10-diene-9-one	–	–	–	+	–	–	+	+	+	+	0
12	a_Atlantone	+	–	–	–	–	–	+	+	+	+	0
13	a_Turmerone	–	–	–	+	–	–	+	+	+	+	0
14	a-Curcumene	+	–	–	+	–	–	+	+	–	+	0
15	ar-Turmerone	–	–	–	+	–	–	+	–	–	–	0
16	b_Atlantone	–	–	–	–	–	–	–	–	+	+	0
17	b_Bisabolene	+	–	–	–	–	–	+	–	+	+	0
18	b_Turmerone	–	–	–	–	–	–	–	+	+	+	0
19	b-Curcumene	+	–	–	–	–	–	+	+	+	+	0
20	Bisabola-3,10-diene-2-one	–	–	–	–	–	–	–	+	+	+	0
21	Bisacumol	–	–	–	+	–	–	+	+	–	+	0
22	Bisacurol	–	–	–	–	–	–	–	+	–	+	0
23	Bisacurone	–	–	–	–	–	–	–	–	–	–	0
24	Bisacurone A	–	–	–	–	–	–	–	–	–	–	0
25	Bisacurone B	–	–	–	–	–	–	–	–	–	–	0
26	Bisacurone C	–	–	–	–	–	–	–	–	–	–	0
27	Bisacurone epoxide	–	–	–	–	–	–	–	–	–	–	0
28	Curlone	–	–	–	–	–	–	–	+	+	+	0
29	g_Curcumene	–	–	–	+	–	–	+	+	+	+	0
30	Turmerone	–	–	–	+	–	–	+	+	+	+	0
31	Turmeronol A	–	–	–	+	–	–	+	+	–	+	0
32	Turmeronol B	–	–	–	+	–	–	+	–	–	–	0
33	Xanthorrhizol	+	–	–	–	–	–	+	+	–	+	0
34	Zingiberene	+	–	–	+	–	–	+	+	+	+	0
Cadalene type Sesquiterpinoids		**TA98**	**TA100**	**TA1535**	**TA98**	**TA100**	**TA1535**	**Result**	**Mouse**	**Rat**	**Result**	**Hepato-toxicity**
35	8-Hydroxycadalene	+	–	–	+	–	+	+	–	–	–	0
36	a-calacorene	+	–	–	+	–	–	+	+	–	+	0
37	Cadalenequinone	+	+	–	+	–		+	+	–	+	0
Cadinane and furanocadinane type ST												
38	a-Cadinol	–	–	–	–	–	+	+	–	–	–	1
39	Curzeone	+	+	–	+	–	+	+	–	–	–	1
40	Pyrocurzerenone	+	+	–	+	–	+	+	+	+	+	0
Carabrane type ST												
41	4S-Dihydrocurcumenone	–	–	–	–	–	–	–	–	–	–	0
42	Curcumenone	–	–	–	–	–	–	–	–	+	+	0
43	Curcurabranol_A	–	–	+	–	–	–	+	–	+	+	0
44	Curcurabranol_B	–	–	+	–	–	–	+	–	+	+	0
Curcumane type ST												
45	Curcumadione	–	–	–	+	–	–	+	–	+	+	0

(*continued on next page*)

Table 1 (*continued*)

Sl. No.		Ames mutagenicity test						Rodent carcinogenicity				Hepato-toxicity
46	Curcumenone	–	–	–	–	–	–	–	–	+	+	0
47	Curmadione	–	–	–	+	–	–	+	–	+	+	0
48	Isocurcumadione	–	–	–	–	–	–	–	–	+	+	0
Dimeric phenylpropene derivative												
49	1-Feruloyloxy-2-methoxy-cinnamic acid	–	–	–	+	–	–	+	+	+	+	1
50	1-Feruloyloxy-cinnamic acid	–	–	–	+	–	–	+	+	+	+	1
51	1-p-Coumaroyloxy-cinnamic acid	–	–	–	+	+	–	+	+	+	+	1
diphenylheptanoids		**TA98**	**TA100**	**TA1535**	**TA98**	**TA100**	**TA1535**	**Result**	**Mouse**	**Rat**	**Result**	**Hepato-toxicity**
52	(1E,3E)-1,7-Diphenyl-1,3-heptadien-5-ol	–	–	–	+	+	–	+	+	–	+	1
53	(1E, 3E)-1,7-Diphenyl-1,3-heptadien-5-one	–	–	–	+	+	–	+	+	+	+	0
54	(1E, 4E, 6E)-1,7-bis-(4-Hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one	–	–	–	–	–	–	–	+	+	+	1
55	(1E, 4E, 6E)-1,7-bis-(4-Hydroxyphenyl)-1,4,6-heptatrien-3-one	–	–	–	–	–	–	–	+	+	+	1
56	(1E, 6E)-1-(4-Hydroxy-3-methoxyphenyl)-7-(3,4-dihydroxy phenyl) -1,6-heptadiene-3,5-dione	–	–	–	–	–	–	–	–	+	+	1
57	(E)-1,7-bis-(4-hydroxy- 3-methoxyphenyl)- 1-heptene-3,5-dione	–	–	–	–	–	–	–	–	+	+	1
58	(E)-1,7-bis-(4-hydroxyphenyl)-1-hepten-3,5-dione	+	–	–	–	–	–	+	–	+	+	1
59	(E)-1,7-Diphenyl-1-hepten-5-one	–	–	–	+	+	–	+	–	–	–	0
60	(E)-1,7-Diphenyl-3-hydroxy-1-hepten-5-one	–	–	–	+	+	–	+	–	–	–	0
61	(E)-5-Hydroxy-7-(4-hydroxyphenyl)-1-phenyl-1-heptene	–	–	–	–	–	–	–	–	–	–	1
62	(E)-7-(3,4-dihydroxyphenyl)-5-hydroxy-1-phenyl-1-heptene	–	–	–	–	–	–	–	–	–	–	1
63	(E)-7-Hydroxy-1,7-bis-(4-hydroxy-3-methoxyphenyl)-1-heptene-3,5-dione	–	–	–	–	–	–	–	+	+	+	1
64	5'-Methoxycurcumin	–	–	–	–	–	–	–	+	+	+	1
65	bis-Demethoxycurcumin	–	–	–	–	–	–	–	+	+	+	1
66	Curcumin	–	–	–	–	–	–	–	–	+	+	1
67	Cyclocurcumin	+	–	–	+	–	+	+	+	+	+	1
68	Demethoxycurcumin	–	–	–	–	–	–	–	–	+	+	1
69	Dihydrocurcumin	+	–	–	–	–	–	+	–	+	+	1
70	Tetrahydro-bis-demethoxycurcumin	+	–	–	–	–	–	+	–	–	–	1
71	Tetrahydrodemethoxycurcumin	+	–	–	–	–	–	+	–	+	+	1
Diphenylpentanoids		**TA98**	**TA100**	**TA1535**	**TA98**	**TA100**	**TA1535**	**Result**	**Mouse**	**Rat**	**Result**	**Hepato-toxicity**
72	(1E, 4E)-1-(4-Hydroxy-3-methoxyphenyl)-5-(4-hydroxyphenyl)-1,4-pentadien-3-one	+	–	–	–	–	–	+	+	+	+	1
73	(1E, 4E)-1,5-bis-(4-Hihydroxy-3-methoxyphenyl)-1,4-pentadien-3-one	+	–	–	–	–	–	+	–	+	+	1
Elemane type ST												
74	5-epi-Curzerenone	–	–	–	–	–	+	+	+	+	+	1

75	b-Elemene	+	–	–	–	–	–	+	–	+	+	0
76	b-Elemenone	–	–	–	–	–	+	+	–	+	+	0
77	Curzerene	–	–	–	–	–	+	+	+	+	+	0
78	Curzerenone	–	–	–	–	–	+	+	+	+	+	1
79	Elemol	–	–	–	–	–	–	–	–	+	+	0
80	g-Elemene	+	–	–	–	–	–	+	–	+	+	0
Eudesmane and furanoeudesmane type ST												
81	1,4-Dihydroxyfuranoeremophilan-6-one	+	–	–	–	–	–	+	–	+	+	0
82	a-Silenene (a-eudesmene)	+	–	–	–	–	–	+	–	+	+	0
83	b-Dictyopetrol	+	–	–	–	–	+	+	–	+	+	1
84	b-Eudesmol	+	–	–	–	–	–	+	–	+	+	1
85	b-Silenene	+	–	–	–	–	–	+	–	+	+	1
86	Curcolone	+	+	–	–	–	+	+	–	+	+	0
87	Curcolonol	+	–	–	–	–	–	+	–	+	+	0
Germacrane type ST		**TA98**	**TA100**	**TA1535**	**TA98**	**TA100**	**TA1535**	**Result**	**Mouse**	**Rat**	**Result**	**Hepato-toxicity**
88	(1a,4b,5a,10b) 1,10 4,5-Diepoxy-7(11)-germacren-8-one	–	+	–	–	–	–	+	–	–	+	0
89	(1R,10R)-Epoxy-(-)-1,10-dihydrocurdione	–	–	–	–	–	+	+	–	+	+	0
90	(1S,10S),(4S,5S)-Germacrone-1(10),4-diepoxide	–	+	–	–	+	+	+	+	–	+	0
91	3,4-Epoxy-6,9-germacranedione	–	–	–	–	–	+	+	–	+	+	0
92	4,5-Epoxy-12-acetoxy-7a,11a-dihydrogermacradien-8-one	–	–	–	–	–	–	–	+	+	+	0
93	4,5-Epoxy-12-hydroxy-1(10),7(11)-germacradien-8-one	–	+	–	–	–	+	+	+	–	+	0
94	13-Hydroxydehydrocurdione	–	–	–	+	–	+	+	+	–	+	0
95	13-Hydroxygermacrone	–	+	–	–	–	+	+	+	–	+	0
96	Acetoxyneocurdione	–	–	–	–	–	–	–	+	+	+	0
97	Curdione	–	–	–	–	–	+	+	+	+	+	0
98	Dehydrocurdione	–	–	–	–	–	+	+	+	+	+	0
99	Furanodiene	–	+	–	–	–	+	+	+	+	+	0
100	Furanodienone	–	+	–	–	–	+	+	+	+	+	0
101	Furanogermenone	–	+	–	–	–	+	+	+	+	+	0
102	Germacrene_B	–	–	–	–	–	–	–	–	+	+	0
103	Germacrone	–	+	–	–	–	+	+	+	+	+	0
104	Germacrone-13-al	–	+	–	+	–	–	+	+	+	+	0
105	Germacrone 4,5-epoxide	–	+	–	–	–	+	+	–	–	–	0
106	Glechomanolide	–	+	–	–	–	+	+	+	+	+	1
107	Isofuranodienone	–	+	–	–	–	+	+	+	+	+	0
108	Neocurdione	–	–	–	–	–	+	+	+	+	+	0
109	Wenjine	–	+	–	–	–	–	+	+	–	+	1
110	Zederone	+	+	–	–	–	+	+	+	+	+	0
Guaiane type ST		**TA98**	**TA100**	**TA1535**	**TA98**	**TA100**	**TA1535**	**Result**	**Mouse**	**Rat**	**Result**	**Hepato-toxicity**
111	1b,4b,5b,10b-zedoaronediol	–	–	–	–	–	–	–	–	–	–	0
112	1-Epi-procurcumenol	–	–	–	–	–	+	+	–	–	–	0
113	4-Epi-curcumenol	–	–	–	–	–	–	–	+	–	+	1
114	4-Hydroxy-7(11),10(14)-guaiadien-8-one	+	–	–	–	–	+	+	–	–	–	1
115	7a,11a,-Epoxy-5b-hydroxy-9-guaiaen-8-one	–	–	–	–	–	–	–	+	+	+	0
116	9-oxo-Neoprocurcumenol	–	–	–	–	–	–	–	–	+	+	0
117	Aerugidiol	–	–	–	–	–	–	–	–	+	+	0
118	Alismoxide	–	–	–	–	–	–	–	+	–	+	0
119	Curcumadiol	–	–	–	–	–	–	–	+	–	+	0
120	Curcumenol	–	–	–	–	–	–	–	+	–	+	1

(*continued on next page*)

Table 1 (*continued*)

Sl. No.		Ames mutagenicity test						Rodent carcinogenicity				Hepato-toxicity
121	Curcumol	+	–	–	–	–	–	+	–	+	+	1
122	Epi-curcumenol	–	–	–	–	–	–	–	+	–	+	1
123	Gweicurculactone	–	+	–	+	+	+	+	–	+	+	0
124	Isocurcumenol	+	–	–	–	–	–	+	–	–	–	1
125	Isoprocurcumenol	+	–	–	–	–	+	+	–	–	–	1
126	Isospathulenol	–	–	–	–	–	–	–	–	–	–	0
127	Isozedoarondiol	–	–	–	–	–	–	–	–	–	–	0
128	Methylzedoarondiol	–	–	–	–	–	–	–	–	–	–	1
129	Neocurcumenol	–	–	–	+	–	–	+	–	–	–	1
130	Neoprocurcumenol	–	–	–	–	–	+	+	–	–	–	0
131	Oxycurcumenol	–	–	–	–	–	+	+	+	–	+	1
132	Procurcumadiol	–	–	–	–	–	+	+	–	–	–	0
133	Procurcumenol	–	–	–	–	–	+	+	–	–	–	0
134	Spathulenol	+	–	–	–	–	–	+	+	+	+	1
135	Zedoalactone_A	–	–	–	–	–	–	–	–	–	–	1
136	Zedoalactone_B	–	–	–	–	–	–	–	–	–	–	1
137	Zedoarol	–	–	–	–	–	+	+	–	+	+	0
138	Zedoarolide_A	–	–	–	–	–	–	–	–	+	+	1
139	Zedoarolide_B	–	–	–	–	–	–	–	–	–	–	1
140	Zedoarondiol	–	–	–	–	–	–	–	–	–	–	0
Labdane type diterpenoid 41 (17),12-Labdadiene-15,16-dial		**TA98**	**TA100**	**TA1535**	**TA98**	**TA100**	**TA1535**	**Result**	**Mouse**	**Rat**	**Result**	**Hepato-toxicity**
		+	–	–	–	–	–	+	–	+	+	0
Monomeric phenylpropene derivatives												
142	Caffeic acid	–	+	–	+	–	–	+	–	+	+	1
143	Calebin A	–	–	–	–	–	–	–	–	–	–	1
144	Cinnamaldehyde	–	–	+	+	+	–	+	–	–	–	0
145	Cinnamic acid	–	–	–	+	+	–	+	–	–	–	0
146	ethyl-*p*-Methoxycinnamate	–	–	+	+	+	–	+	+	–	+	0
147	*p*-Coumaric acid	–	–	–	+	+	–	+	+	–	+	1
148	*p*-Methoxycinnamic acid	–	–	–	+	+	–	+	+	–	+	0
Monoterpenoids		**TA98**	**TA100**	**TA1535**	**TA98**	**TA100**	**TA1535**	**Result**	**Mouse**	**Rat**	**Result**	**Hepato-toxicity**
149	(E)-b-Ocimene	+	–	–	+	–	–	+	+	+	+	0
150	(Z)-b-Ocimene	+	–	–	+	–	–	+	+	+	+	0
151	1,8-Cineole	–	+	–	–	+	+	+	+	+	+	0
152	a_Phellandrene	+	–	–	+	+	–	+	+	+	+	0
153	a_Pinene	–	–	–	–	+	–	+	–	+	+	0
154	a_Terpinene	+	–	–	+	–	–	+	+	+	+	0
155	a_Terpineol	–	–	–	–	–	–	+	–	–	–	0
156	b_3_Carene	+	–	–	–	+	–	+	–	+	+	1
157	b_Phellandrene	+	–	–	+	+	–	+	–	+	+	0
158	b_Pinene	+	–	–	–	+	–	+	–	+	+	0
159	Borneol	–	–	–	–	–	+	+	–	+	+	0
160	Bornyl acetate	–	–	+	–	–	+	+	–	+	+	0
161	Camphene	+	–	–	–	+	–	+	–	+	+	1
162	Camphor	–	–	–	–	–	+	+	–	+	+	0
163	Carvacrol	+	–	+	+	+	+	+	–	–	–	0
164	Citronellol	–	–	–	–	–	–	––	+	–	+	0
165	D-sabinene	+	–	+	+	+	–	+	+	+	+	0
166	g_Terpinene	–	–	–	+	–	–	+	+	+	+	0
167	Isoborneol	–	–	–	–	–	+	+	–	+	+	0
168	Limonene	+	–	–	+	–	–	+	–	+	+	0
169	Linalool	–	–	+	–	+	+	+	–	–	+	0
170	Myrcene	–	–	+	+	–	–	+	–	+	+	0
171	Nerol	–	+	–	+	–	–	+	+	–	+	0
172	p_Cymene	+	–	–	+	+	–	+	+	–	+	0

173	p-Cymene-8-ol	–	–	+	–	–	+	+	– –	–	–	0
174	Perillene	–	+	+	+	–	–	+	+	+	+	0
175	Terpinen-4-ol	+	–	–	–	–	+	+	+	–	+	0
176	Terpinolene	–	–	–	+	–	–	+	+	+	+	0
Sesquiterpene dimers												
177	Difurocumenone	+	–	–	–	–	–	+	–	+	+	1
178	Parviflorene_A	+	–	–	–	–	–	+	+	–	+	1
179	Parviflorene_B	–	–	–	–	–	–	–	–	–	–	1
180	Parviflorene_C	–	–	–	–	–	–	–	–	–	–	1
181	Parviflorene_D	–	–	–	–	–	–	–	–	–	–	1
182	Parviflorene_E	–	–	–	–	–	–	–	+	–	+	1
183	Parviflorene_F	+	–	–	–	–	–	+	–	–	–	1
Sesquiterpinoids		**TA98**	**TA100**	**TA1535**	**TA98**	**TA100**	**TA1535**	**Result**	**Mouse**	**Rat**	**Result**	**Hepato-toxicity**
184	a_Caryophyllene or a_humulene	–	–	–	–	–	–	–	–	+	+	0
185	a_Copaene	–	–	–	–	–	–	–	–	+	+	1
186	b_Caryophyllene or b_humulene	+	–	–	–	+	–	+	–	+	+	0
187	b_Farnesene	–	–	–	–	–	–	–	+	+	+	0
188	b_Himachalene	–	–	–	–	+	–	+	+	+	+	0
189	Calarene	–	–	–	–	–	–	–	+	+	+	1
190	Curcumalactone	+	–	–	–	–	+	+	+	+	+	0
191	Curcumanolide_A	+	–	–	–	–	+	+	+	+	+	0
192	Curcumanolide_B	+	–	–	–	–	+	+	+	+	+	0
193	Curcumenolactone_C	–	–	–	–	–	–	–	–	+	+	0
194	Farnesol	–	–	–	–	–	–	–	+	–	+	0
Miscellaneous												
195	1–4-Methylphenylethanol	–	–	+	–	–	+	+	–	–	–	0
196	3,7-Dimethyl-5-indanecarboxylic acid	+	–	–	+	+	+	+	–	–	–	0
197	Eugenol	–	+	+	+	+	+	+	+	–	+	0
198	Phloracetophenone	–	+	+	+	–	+	+	–	+	+	1
199	Syringic_acid	–	–	+	+	–	+	+	–	+	+	0
200	Vanillic acid	–	+	+	+	+	+	+	–	+	+	1

3.3. Human hepatotoxicity prediction

Human hepatotoxicity prediction showed that only two compounds belong to *Bisabolane type ST*, i.e., 2,5-dihydroxybisabola-3,10-diene (No. 5) and 2,10-bisaboladiene-1,4-diol (No. 6) were predicted as hepatotoxic. All the *Dimeric phenylpropene derivatives* of the analysed dataset showed hepatotoxicity. A couple of compounds, α-cadinol (No. 38) and curzeone (No. 39) both belong to *Cadinane and furanocadinane type ST* was predicted as hepatotoxic. Both the compounds (Nos. 72 and 73) belong to *Diphenylpentanoids* were also predicted as hepatotoxic.

A couple of compounds, 5-epi-curzerenone (No. 74) and curzerenone (No. 78) belong to *Elemane type ST*, three of the *Eudesmane and furanoeudesmane type ST* (Nos. 83, 84 and 85), two *Germacrane type ST* (glechomanolide, No. 106 and wenjine, No. 109), 15 of the *Guaiane type ST* (Nos. 113, 114, 120, 121, 122, 124, 125, 128, 129, 131, 134, 135, 136, 138 and 139), three of the *Monomeric phenylpropene derivatives* (Nos. 142, 143 and 147), a couple of *Sesquiterpinoids*, α-copaene (No. 185) and calarene (No. 189) and two other compounds (Nos. 198 and 200) grouped under *miscellaneous* were all predicted as hepatotoxic. All of the analysed *Sesquiterpene dimers* showed hepatotoxicity, whereas, only two *Monoterpenoids* (Nos. 156 and 161) were predicted as hepatotoxic among the analysis dataset.

All the compounds belong to *Cadalene type ST* were predicted as hepato non-toxic. Similarly, all compounds belonging to *Curcumane and Carabrane type Sesquiterpinoids* were predicted as hepato non-toxic. Among *Diphenylheptanoids*, three compounds (Nos. 53, 59 and 60) were found to be hepato non-toxic, the rest of the compounds (including curcumin and its derivatives) were likely to cause dose-dependent liver injuries.

4. Discussion

The study shows, out of 200 compounds, 184 compounds were predicted as toxigenic, 136 compounds are mutagenic, 153 compounds are carcinogenic and 64 compounds are hepatotoxic. To cross validate our results, we have chosen the popular curcumin (No. 66), because of extensive research on curcumin within the last half century.

Our predictions are in mutual agreement with Shankar et al. (1980) on mutagenicity of turmeric oleoresin (major component 79% curcumin) on *Salmonella*, which was found to be negative. *In vitro* cytogenetics studies were also identified that chromosome aberration was weakly positive and sister chromatid exchange was also weakly positive. In the studies of Kawachi et al. (1980) on short-term assays of genetic toxicity, positive results were reported in the rec assay (*B. subtilis*) and for chromosomal aberrations in hamster lung fibroblasts. All the analysed compounds belong to *Sesquiterpinoids* were predicted as carcinogens. This is in concordance with Shankar et al. (1980) on toxicology and carcinogenesis of turmeric oleoresin (major component 79% curcumin) identified by feed studies in rats and mice.

Among *Diphenylheptanoids*, curcumin and its derivatives were likely to cause dose-dependent liver injuries. This can be correlated with the laboratory findings that turmeric extracts can be toxic to the liver when taken in high doses or for a prolonged period of time (Babu and Srinivasan, 1997; Kandarkar et al., 1998). For this reason, turmeric products should probably be avoided by individuals with liver diseases, heavy drinkers, and those who take prescription medications that are hard on the liver. It may cause skin problems and also cause stomach ulcers if used for a long time (Fetrow and Avila, 1999). In addition, due to curcumin's stimulating effects on the gallbladder, individuals with gallbladder disease should use curcumin only on the advice of a physician. Administration of high dose of tumeric or ethanolic tumeric extract for variable periods was found to induce hepatotoxic effects in mice and rats (Deshpande et al., 1998; Kandarkar et al., 1998). This was mostly in the form of focal coagulation necrosis associated in some cases with focal necrotic changes in the spleen and kidney (Deshpande et al., 1998). On the other hand, toxic effects of turmeric or curcumin were not observed by some authors in rats, guinea pigs, monkeys and pigs (Wahlstrom and Blennow, 1978; Bhavanishankar et al., 1980; Bille et al., 1985). The toxic effect of turmeric seems to depend on animal species, dose and duration of treatment (Al-Sultan and Gameel, 2004) for example, Kaur et al. (2006) investigated the effect of curcumin in salvaging endotoxin-induced hepatic dysfunction and oxidative stress in the liver of rodents.

At this point, it is worthy to discuss the reproductive toxicity of curcumin in Wistar rats, studied by Ganiger et al. (2007) in order to generate additional relevant toxicity information for the use of curcumin in humans by oral administration. The curcumin, mixed in the experimental diet was fed to three groups of rats, i.e., low, mid and high dose groups, and studied for two successive generations. This study was the final toxicology study on curcumin reviewed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at the 61st Meeting, 2003. The JECFA group considered that the small body weight reduction in the F2 pups of the highest dose group and allocated an Acceptable Daily Intake (ADI) for curcumin of 0–3 mg/kg bw.

The predicted toxicity of the analysis data set from turmeric is in clear concordance with wet lab studies of several researchers. The important ones are listed here with their findings. Al-Sultan and Gameel (2004) have studied hyperaemia and mononuclear cell infiltration in the liver sections of broiler chicken supplemented with turmeric. On the other hand, dilation of bile ducts, hyperplasia of biliary epithelium and periportal hepatocyte degeneration were also observed. Earlier studies in rats (Jentzsch et al., 1959) and dogs (Ramaprasad and Sirsi, 1956) indicated that curcumin had both choleretic and cholagogic actions. The periportal hepatocyte degeneration may possibly be due to leakage of bile from the dilated bile ducts (Al-Sultan and Gameel, 2004). Haemorrhage and cholangeolar cell hyperplasia were previously observed in mice fed low doses of turmeric (0.1%) for 2 weeks but not in mice receiving higher dose (0.5%) this has been related to the anti-inflammatory and anti-proliferative effects of curcuma (Deshpande et al., 1998). The study of Al-Sultan and Gameel (2004) suggests that feeding turmeric to chicken through diet can induce hepatic changes and that these changes are not dose or time dependent.

5. Conclusion

Screening of 200 compounds from turmeric (Table 1) based on their toxicity, which includes bacterial mutagenicity, rodent carcinogenicity and human hepatotoxicity yielded 16 candidates out of 200 compounds. They are as follows; nine from *Bisabolane type ST*, *viz*., 1,3,5,11-bisabolatetraene, 3-hydroxy-1, 10-bisaboladien-9-one, ar-turmerone, bisacurone, bisacurone (A, B and C), bisacurone epoxide and turmeronol B, only one (4S-dihydrocurcumenone) from *Carabrane type Sesquiterpinoid*, two from *Diphenylheptanoids*, (E)-1,7-diphenyl-1-hepten-5-one, and (E)-1,7-diphenyl-3-hydroxy-1-hepten-5-one, respectively, and four from *Guaiane-type Sesquiterpinoids* viz., 1b,4b,5b,10b-zedoaronediol, Isospathulenol, Isozedoarondiol and Zedoarondiol. All these compounds were non-mutagenic, non-carcinogenic, non-hepatotoxic and may not have any side-effects. Of the 200 compounds, 184 compounds were predicted to be toxic in some capacity; 136 compounds were mutagenic, 153 were carcinogenic and 64 were hepatotoxic. Our results will guide the preclinical development studies to be designed and conducted in a timely, cost-efficient manner and thus most likely allow the candidate to have an earlier entry into the clinic.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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