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IN-SILICO STUDY OF TOXICITY MECHANISMS FOR METABOLITES OF PHYTO-COMPOUNDS FROM MUSA SP. COMPARED TO SYNTHETIC MEDICINE RANITIDINE

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ABSTRACT: An *in-silico* attempt to predict rat oral acute toxicity, hepatotoxicity, immunotoxicity, genetic toxicity endpoints, nuclear receptor (NR) signaling, and stress response (SR) pathways of metabolites of synthetic medicine Ranitidine and flavonoids of *Musa* sp. The metabolites of common flavonoids and synthetic medicine were taken from literature, and the prediction was done by using ProTox-II webserver. The predictive results for the toxicity of these metabolites, N-nitrosodimethylamine obtained the lower LD_{50} value (26 mg/kg) as highest toxicity of class II, *i.e.*, prescribed as fatal after swallowing ranged between >5 and ≤50, and rest compounds were class IV and V *i.e.*, harmful or may be harmful if swallowing ranged between >300 and ≤2000 and >2000 and ≤5000. None of these were showed hepatotoxic as well as not cytotoxic and mutagenic active, but few were immunotoxic, and all metabolites of synthetic origin and two phytometabolites *viz*. quercetin-3-glucuronide and 5-O-methylmyricetin were obtained carcinogenic active. In the case of NR signaling pathways and SR pathways, three compounds were active on different parameters. In conclusion, this *in-silico* study indicated that the metabolite (N-nitrosodimethylamine) of synthetic medicine, namely ranitidine showed highly toxic as well as carcinogenic while metabolites as Quercetin-3-glucuronide and 5-O-Methylmyricetin also showed carcinogenic, which may cause at a higher dose and chronic exposure. The present results are suitable for further experimental research on toxicity mechanisms with these metabolites with a narrow range. This predictive study is suggested for future experimental assays to validate the present results of these metabolites.

INTRODUCTION: The medicine of synthetic origin, namely ranitidine and is well-established as an H_2-receptor antagonist. It has been used to treat common diseases such as gastroesophageal reflux disease and peptic ulcers [1-3].

The study on patients revealed that the metabolite as N-nitrosodimethylamine (NDMA) was increased in the urine of patients who used Ranitidine [3]. On the other hand, updated research by FDA [4] notified the patients and healthcare professionals restricted use of ranitidine due to the presence of unacceptable levels of NDMA.

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The fruit is commonly known as banana (*Musa* sp.) belonging to the family Musaceae and has high medicinal and nutritive value due to the presence of several phytocompounds [5-6]. In an earlier study, it was identified that three phytocompounds *viz*. Quercetin, myricetin, and kaempferol showed favorable binding energy and binding interaction compared to a synthetic medicine namely ranitidine on matrix metalloproteinases-9 or MMP-9 [7] but the prediction of ADME and NR signaling pathways, as well as SR pathways, is lacking for the effectiveness of a new drug to prevent gastric-ulcer.

Moreover, the predictive acute toxicity study as well as liver toxicity, genotoxicity, especially cytotoxicity, mutagenicity, carcinogenicity, along with NR as well as SR pathways of each organic chemical, is suitable to know the toxicity mechanisms easily. This *in-silico* study is an alternative to long-duration, cost involvement and animal testing experiments [8], and it was observed that studied parameters have different experimental designs.

In this context, researchers have been developed the predictive toxicity study through computational simulation. Interestingly, several compounds can be screened within an hour to obtain the output of toxicity mechanisms [9-13].

Present *in-silico* study was attempted to detect rat oral acute toxicity, hepatotoxicity, immunotoxicity, genetic toxicity endpoints, nuclear receptor signalling, and stress response pathways of metabolites of synthetic medicine and flavonoids phyto-compounds of *Musa* sp. by using ProTox-II webserver.

MATERIALS AND METHODS:

Selection of phytochemicals and its metabolites: According to the virtual screening [7] three phytochemicals *viz*. quercetin, myricetin, and kaempferol were studied earlier. From earlier studied compounds, the metabolites such as quercetin-3-glucuronide and isorhamnetin of quercetin, 3,5-dihydroxyphenylacetic acid, 3,4,5-trihydroxyphenylacetic acid, and 5-o-methyl-myricetin of myricetin and kaempferol-3-O-diglucoside and kaempferol-3-O-glucoside of kaempferol were taken from literature for the present predictive study [14-16].

Selection of Synthetic Medicine and its Metabolites: The synthetic medicine namely ranitidine and its metabolites such as ranitidine n-oxide, ranitidine s-oxide, desmethyl ranitidine and n-nitrosodimethylamine were selected as per literature for the present predictive study [3-4].

Evaluation of Toxicity Mechanisms of Metabolites of Synthetic and Phyto-compounds: *In-silico* study was done by using ProTox-II webserver developed by Drwal *et al*., [9] and the parameters such as rat oral median lethal dose (LD_{50}), hepatotoxicity, immunotoxicity, cytotoxicity, mutagenicity, carcinogenicity, nuclear receptor signaling (AhR, AR, AR-LBD, ER, ER-LBD, and PPARGamma), and stress response pathways (nrf2/ARE, HSE, MMP, p53, and ATAD5) were predicted for above-mentioned metabolites of natural products and synthetic medicine as per protocol followed of Banerjee *et al*., [10] Ghosh *et al*., [11] Biswas and Talapatra [12] and Roy Goswami [13].

RESULTS AND DISCUSSION: The prediction was carried out on seven metabolites of natural products such as quercetin, myricetin, and kaempferol and four metabolites of synthetic medicine, namely Ranitidine.

In **Table 1**, it was predicted the rat oral acute toxicity (LD_{50}) as mg/kg through different toxicity classes (I–VI) and prediction accuracy in percentage (%) for different studied metabolites. Among 4 metabolites of Ranitidine, N-nitroso-dimethylamine obtained the lower LD_{50} value (26 mg/Kg) as the highest toxicity of class II, *i.e.*, prescribed as fatal after swallowing, ranged between >5 and ≤50 with 100% prediction accuracy. Rest three metabolites such as ranitidine N-oxide, ranitidine s-oxide, and desmethyl ranitidine obtained the LD_{50} values 1100, 1100, and 884 as class IV *i.e.*, harmful if swallowing ranged between >300 and ≤2000 with prediction accuracy 72.9%, 70.97%, and 100% respectively.

On the other hand, the natural products of five metabolites such as quercetin-3-glucuronide, Isorhamnetin, 5-O-methylmyricetin, kaempferol-3-O-diglucoside, and kaempferol-3-O-glucoside were found same LD_{50} value of 5000 mg/Kg as class V *i.e.*, may be harmful if swallowing ranged between >2000 and ≤5000 with prediction accuracy of

70.97% for former compounds and 72.90% for other compounds while two metabolites *viz*. 3,5-dihydroxyphenylacetic acid and 3,4,5-trihydroxy-phenylacetic acid were obtained LD_{50} value of 800 and 1400 mg/kg as class IV with a prediction accuracy of 69.26%.

TABLE 1: PREDICTION OF ORAL ACUTE TOXICITY, CLASS AND ACCURACY VALUE OF STUDIED METABOLITES

S. no.	Compounds name	Rat oral LD_{50} value (mg/Kg)	Predicted toxicity class	Prediction accuracy (%)
1	Quercetin-3-glucuronide	5000	V	70.97
2	Isorhamnetin	5000	V	70.97
3	3,5-Dihydroxyphenylacetic acid	800	IV	69.26
4	3,4,5-Trihydroxyphenylacetic acid	1400	IV	69.26
5	5-O-Methylmyricetin	5000	V	70.97
6	Kaempferol-3-O-diglucoside	5000	V	72.90
7	Kaempferol-3-O-glucoside	5000	V	72.90
8	Ranitidine N-oxide	1100	IV	72.9
9	Ranitidine S-oxide	1100	IV	70.97
10	Desmethyl ranitidine	884	IV	100.00
11	N-nitrosodimethylamine	26	II	100.00

Class I: fatal if swallowed ($LD_{50} \leq 5$); Class II: fatal if swallowed ($5 < LD_{50} \leq 50$); Class III: toxic if swallowed ($50 < LD_{50} \leq 300$); Class IV: harmful if swallowed ($300 < LD_{50} \leq 2000$); Class V: may be harmful if swallowed ($2000 < LD_{50} \leq 5000$) and Class VI: non-toxic (LD_{50} >5000)

In the present predictive rat oral acute toxicity study indicated that the metabolite of Ranitidine as N-nitrosodimethylamine obtained the lower LD_{50} value (26 mg/Kg) as highest toxicity of class II, *i.e.,* prescribed as fatal after swallowing ranged between >5 and ≤50 while the established phytocompounds from *Musa* sp. and its metabolites such as quercetin-3-glucuronide, isorhamnetin, 5-O-methylmyricetin, kaempferol-3-O-diglucoside and kaempferol-3-O-glucoside were found same LD_{50} value of 5000 mg/Kg as class V *i.e.*, may be harmful if swallowing ranged between >2000 and ≤5000 **Table 1**, which are supported by other researchers that polyphenols are least toxic and some flavonoids have low acute toxicity effect on mice [17-18]. Moreover, flavonoid containing extract of *Musa* sp. was prevent gastric ulcer in mice reported by Rao *et al*., [6] and flavonoids as natural products are also suitable for gastroprotective effect reviewed by de Lira Mota *et al*. [19] On the other hand, an *in-silico* approach through molecular docking and interaction revealed that quercetin and Myricetin followed by kaempferol of *Musa* sp. suitable for gastroprotection and these three phyto-compounds were found to inhibit MMP-9 [7].

Updated research by FDA[4] notified the patients and healthcare professionals restricted use of ranitidine due to the presence of unacceptable levels of NDMA. In recent research ranitidine metabolite *viz*. n-nitrosodimethylamine was declared as carcinogen [20,] but in the present study, all metabolites of ranitidine showed carcinogenic activity. However, in the present predictive results, Quercetin-3-glucuronide and 5-O-methylmyricetin were obtained carcinogenic active, which is supported by earlier experiment on rats, and it was observed very higher dose, *i.e.*, beyond 40,000ppm [21]. In the case of 5-O-Methylmyricetin, which obtained carcinogenic activity may be due to methyl group.

In **Table 2**, the prediction of organ toxicity, especially liver toxicity or hepatotoxicity and immunotoxicity, was done. For hepatotoxicity, all metabolites of synthetic medicine and phyto-compounds showed hepatotoxic inactive with probability score of 75%, 72%, 65%, 67%, 72%, 83%, 82%, 54%, 52%, 54% and 65% for quercetin-3-glucuronide; isorhamnetin; quercetin-3′-sulfate; 3,5-dihydroxyphenylacetic acid; 3,4,5-trihydroxy-phenylacetic acid; 5- O- methylmyricetin; kaempferol- 3- O- diglucoside; kaempferol-3-O-glucoside; ranitidine N-oxide, ranitidine S-oxide, desmethyl ranitidine and N-nitrosodimethylamine respectively.

Among seven metabolites of phytocompounds, four metabolites such as quercetin-3-glucuronide; Iso-rhamnetin; 5-O-methylmyricetin and kaempferol-3-O-diglucoside were obtained immunotoxic active with probability score of 58% for former two compounds and 69% and 82% for other two compounds while rest three metabolites such as

3,5-dihydroxyphenylacetic acid, 3,4,5-trihydroxy-phenylacetic acid, and kaempferol-3-O-glucoside were obtained non-immunotoxic or immnotoxic inactive with probability score 65%, 67% and 82% respectively **Table 2**.

TABLE 2: PREDICTION OF ORGAN TOXICITY AND IMMUNOTOXICITY ENDPOINTS OF STUDIED METABOLITES

S. no.	Compounds name	HT	P	IT	P
1	Quercetin-3-glucuronide	I	0.75	A	0.58
2.	Isorhamnetin	I	0.72	A	0.58
3	3,5-Dihydroxyphenylacetic acid	I	0.65	I	099
4	3,4,5-Trihydroxyphenyl-acetic acid	I	0.67	I	0.99
5	5-O-Methylmyricetin	I	0.72	A	0.69
6	Kaempferol-3-O-diglucoside	I	0.83	A	0.82
7	Kaempferol-3-O-glucoside	I	0.82	I	0.64
8	Ranitidine N-oxide	I	0.54	A	0.51
9	Ranitidine S-oxide	I	0.52	A	0.98
10	Desmethyl ranitidine	I	0.54	I	0.84
11	N-nitrosodimethylamine	I	0.65	I	0.99

HT = Hepatotoxicity; IT = Immunotoxicity; I = Inactive; A = Active and PS = Probability score

Table 3 describes the prediction results of the genotoxicity, especially cytotoxicity, mutagenicity, and carcinogenicity for all studied metabolites. For cytotoxicity test, all metabolites of synthetic medicine and phytocompounds showed hepatotoxic inactive with probability score of 91%, 95%, 80%, 88%, 95%, 67%, 69%, 69%, 57%, 66% and 70% for quercetin-3-glucuronide; isorhamnetin; 3,5-dihydroxyphenylacetic acid; 3,4,5-trihydroxy-phenylacetic acid; 5-O-methylmyricetin; kaem-pferol-3-O-diglucoside; kaempferol-3-O-glucoside; Ranitidine N-oxide, ranitidine S-oxide, desmethyl ranitidine and N-nitrosodimethylamine respectively.

For mutagenicity test, all metabolites of phytocompounds showed mutagenic inactive or non-mutagenic with probability score of 91%, 95%, 80%, 88%, 95%, 67% and 69% for quercetin-3-glucuronide; isorhamnetin; 3,5-dihydroxyphenyl-acetic acid; 3,4,5-trihydroxyphenylacetic acid; 5-O-methylmyricetin; kaempferol-3-O-diglucoside; kaempferol-3-O-glucoside respectively while all four metabolites of synthetic medicine such as ranitidine N-oxide, ranitidine S-oxide, desmethyl ranitidine, and N-nitrosodimethylamine were obtained mutagenic active with probability score 69%, 57%, 66% and 70% respectively **Table 3**.

For carcinogenicity test, five metabolites of phytocompounds such as Isorhamnetin; 3,5-dihydroxyphenylacetic acid; 3,4,5-trihydroxy-phenylacetic acid; kaempferol-3-O-diglucoside; kaempferol-3-O-glucoside showed carcinogenic inactive or non-carcinogenic with a probability score of 68%, 77%, 66%, 85%, and 85% except two metabolites *viz*. quercetin-3-glucuronide and 5-O-methylmyricetin were active with probability score 50% and 55% respectively while all four metabolites of synthetic medicine such as ranitidine N-oxide, ranitidine S-oxide, desmethyl ranitidine, and N-nitrosodimethylamine were obtained carcinogenic active with probability score 62%, 60%, 68% and 98% respectively **Table 3**.

TABLE 3: PREDICTION OF CYTO-GENOTOXICITY END POINTS OF END POINTS OF STUDIED METABOLITES

S. no.	Compounds name	CT	PS	MG	PS	CG	PS
1	Quercetin-3-glucuronide	I	0.91	I	0.68	A	0.50
2	Isorhamnetin	I	0.95	I	0.94	I	0.68
3	3,5-Dihydroxyphenylacetic acid	I	0.80	I	0.90	I	0.77
4	3,4,5-Trihydroxyphenylacetic acid	I	0.88	I	0.84	I	0.66
5	5-O-Methylmyricetin	I	0.95	I	0.61	A	0.55
6	Kaempferol-3-O-diglucoside	I	0.67	I	0.74	I	0.85
7	Kaempferol-3-O-glucoside	I	0.69	I	0.76	I	0.85
8	Ranitidine N-oxide	I	0.69	A	0.62	A	0.62
9	Ranitidine S-oxide	I	0.57	A	0.65	A	0.60
10	Desmethyl ranitidine	I	0.66	A	0.67	A	0.68
11	N-nitrosodimethylamine	I	0.70	A	0.97	A	0.98

CT = Cytotoxicity; MG = Mutagenicity; CG = Carcinogenicity; I = Inactive; A = Active and PS = Probability score

Table 4 describes the predicted results of Tox21-nuclear receptor signalling pathways and seven parameters such as AhR, AR, AR-LBD, Aro, ER, ER-LBD and PPAR-Gamma were predicted for all metabolites of synthetic medicine and phyto-compounds. All the studied nine compounds such as quercetin-3-glucuronide; 3,5-dihydroxyphenyl-acetic acid; 3,4,5-Trihydroxyphenylacetic acid; Kaempferol- 3- O- diglucoside; Kaempferol-3-O-glucoside; ranitidine N-oxide, Ranitidine S-oxide, desmethyl ranitidine and N-nitrosodimethylamine were observed Ahr inactive with probability scores 60%, 83%, 81%, 95%, 92%, 96%, 95%, 96% and 99% while two compounds *viz*. isorhamnetin and 5-O-methylmyricetin were Ahr active with a probability score of 97% and 90% respectively. For AR, all compounds were found inactive with probability score of 99%, 100%, 99%, 99%, 99%, 79%, 90%, 97%, 97%, 98% and 100% respectively. For AR-LBD, all compounds were obtained inactive with probability score of 96%, 99%, 100%, 100%, 99%, 97%, 98%, 98%, 97%, 98% and 97% respectively. For aromatase or Aro, all compounds were observed inactive with probability score of 96%, 99%, 99%, 88%, 99%, 100%, 95%, 93%, 97% and 100% respectively except isorhamnetin as active of probability score 88%. For ER, two compounds *viz*. isorhamnetin and 5-O-Methyl-myricetin were active with probability score 88% and 77%, and rest compounds were inactive with probability score 78%, 90%, 93%, 81%, 91%, 95%, 90%, 96%, and 99% respectively. For ER-LBD, two compounds *viz*. isorhamnetin and 5-O-Methyl-myricetin were active with probability score 89% and 87%, and rest compounds were inactive with probability score 84%, 93%, 92%, 99%, 99%, 96%, 92%, 97%, and 99% respectively. For PPAR-Gamma, all compounds were found inactive with probability scores 96%, 95%, 97%, 98%, 97%, 99%, 99%, 98%, 98%, 98% and 100% respectively.

TABLE 4: PREDICTION OF TOX21-NUCLEAR RECEPTOR SIGNALLING PATHWAYS OF STUDIED METABOLITES

S. no.	Compounds name	Tox21-nuclear receptor signaling pathways							
		Ahr	**PS**	**AR**	**PS**	**AR-LBD**	**PS**	**Aro**	**PS**
1	Quercetin-3-glucuronide	I	0.60	I	0.99	I	0.96	I	0.96
2	Isorhamnetin	A	0.97	I	1.00	I	0.99	A	0.88
3	3,5-Dihydroxyphenyl-acetic acid	I	0.83	I	0.99	I	1.00	I	0.99
4	3,4,5-Trihydroxyphenyl-acetic acid	I	0.81	I	0.99	I	1.00	I	0.99
5	5-O-Methylmyricetin	A	0.90	I	0.99	I	0.99	I	0.88
6	Kaempferol-3-O-diglucoside	I	0.95	I	0.79	I	0.97	I	0.99
7	Kaempferol-3-O-glucoside	I	0.92	I	0.90	I	0.98	I	1.00
8	Ranitidine N-oxide	I	0.96	I	0.97	I	0.98	I	0.95
9	Ranitidine S-oxide	I	0.95	I	0.97	I	0.97	I	0.93
10	Desmethyl ranitidine	I	0.96	I	0.98	I	0.98	I	0.97
11	N-nitrosodimethylamine	I	0.99	I	1.00	I	0.97	I	1.00
		ER	**PS**	**ER-LBD**	**PS**	**PPAR-Gamma**	**PS**		
1	Quercetin-3-glucuronide	I	0.78	I	0.84	I	0.96		
2	Isorhamnetin	A	0.88	A	0.89	I	0.95		
3	3,5-Dihydroxyphenyl-acetic acid	I	0.90	I	0.93	I	0.97		
4	3,4,5-Trihydroxyphenyl-acetic acid	I	0.93	I	0.92	I	0.98		
5	5-O-Methylmyricetin	A	0.77	A	0.87	I	0.97		
6	Kaempferol-3-O-diglucoside	I	0.81	I	0.99	I	0.99		
7	Kaempferol-3-O-glucoside	I	0.91	I	0.99	I	0.99		
8	Ranitidine N-oxide	I	0.95	I	0.96	I	0.98		
9	Ranitidine S-oxide	I	0.90	I	0.92	I	0.98		
10	Desmethyl ranitidine	I	0.96	I	0.97	I	0.98		
11	N-nitrosodimethylamine	I	0.99	I	0.99	I	1.00		

AhR = Aryl hydrocarbon Receptor; AR = Androgen receptor; AR-LBD = Androgen Receptor Ligand Binding Domain; Aro = Aromatase; ER = Estrogen Receptor Alpha; ER-LBD = Estrogen Receptor Ligand Binding Domain; PPAR-Gamma = Peroxisome Proliferator Activated Receptor Gamma; I = Inactive; A = Active and PS = Probability score

Table 5 describes predicted results of Tox21-stress response pathways parameters and five parameters such as nrf2/ARE, HSE, MMP, p53 and ATAD5 were studied. For PPAR-Gamma and HSE, all compounds were found inactive with probability scores 95%, 95%, 95%, 86%, 94%, 96%, 98%, 96%, 95%, 96% and 96% and 95%, 96%, 95%, 86%, 94%, 96%, 98%, 96%, 95%, 96% and 96% respectively. For MMP, two compounds *viz*. isorhamnetin and 5-O-methylmyricetin were active with probability score 92% and 89% and rest compounds were inactive with probability score 63%, 84%, 85%, 98%, 98%, 91%, 92%, 92% and 99% respectively. For p53, all compounds were

obtained inactive except kaempferol-3-O-glucoside with probability score of 89%, 86%, 97%, 97%, 89%, 72%, 50%, 96%, 94%, 97% and 97% respectively. For ATAD5, all compounds were obtained inactive except isorhamnetin with probability score 90%, 65%, 98%, 99%, 54%, 99%, 100%, 98%, 96%, 98% and 98% respectively.

The present predictive results indicated inactivity for all the parameters such as AhR, AR, AR-LBD, Aro, ER, ER-LBD, and PPAR-Gamma under nuclear receptor (NR) signaling pathways for all metabolites **Table 4** except two phytocompounds *viz*. isorhamnetin and 5-O-methylmyricetin Ahr, ER and ER-LBD active while isorhamnetin was active for Aro. The results revealed that two compounds were observed estrogenic active, which is supported by other researchers that flavonoids have affected estrogen [22]. According to Kolodkin *et al*., [23] NR signaling occurs to maintain development, cellular growth, inflammation, and metabolism, and ligand distribution appeared dynamic with few NRs found predominantly in the nucleus (pregnane X receptor and peroxisome proliferator-activated receptor-gamma), while some are located either in both compartments (vitamin D receptor and mineralocorticoid receptor) or mostly in the cytoplasm (glucocorticoid receptor and androgen receptor).

TABLE 5: PREDICTION OF TOX21-STRESS RESPONSE PATHWAYS OF STUDIED METABOLITES

S. no.	Compounds name	Tox21- Stress response pathways									
		nrf2/ARE	PS	HSE	PS	MMP	PS	p53	PS	ATAD5	PS
1	Quercetin-3-glucuronide	I	0.95	I	0.95	I	0.63	I	0.89	I	0.90
2	Isorhamnetin	I	0.95	I	0.96	A	0.92	I	0.86	A	0.65
3	3,5-Dihydroxyphenylacetic acid	I	0.95	I	0.95	I	0.84	I	0.97	I	0.98
4	3,4,5-Trihydroxyphenyl-acetic acid	I	0.86	I	0.86	I	0.85	I	0.97	I	0.99
5	5-O-Methylmyricetin	I	0.94	I	0.94	A	0.89	I	0.89	I	0.54
6	Kaempferol-3-O-diglucoside	I	0.96	I	0.96	I	0.98	I	0.72	I	0.99
7	Kaempferol-3-O-glucoside	I	0.98	I	0.98	I	0.98	A	0.50	I	1.00
8	Ranitidine N-oxide	I	0.96	I	0.96	I	0.91	I	0.96	I	0.98
9	Ranitidine S-oxide	I	0.95	I	0.95	I	0.92	I	0.94	I	0.96
10	Desmethyl ranitidine	I	0.96	I	0.96	I	0.92	I	0.97	I	0.98
11	N-nitrosodimethyl-amine	I	0.96	I	0.96	I	0.99	I	0.97	I	0.98

nrf2/ARE = Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element; HSE = Heat shock factor response element; MMP = Mitochondrial Membrane Potential; p53 = Phosphoprotein (tumour suppressor); ATAD5 = ATPase family AAA domain-containing protein 5; I = Inactive; A = Active and PS = Probability score

In this web server, different parameters such as nrf2/ARE, HSE, MMP, p53, and ATAD5 are well-established for cellular stress in relation to stress response pathways, and the major signaling components and molecular mechanisms have been identified by researchers [9-10]. Generally, adaptive stress response pathways are known as signal transduction pathways, which ultimately participated in the transcriptional activation of cytoprotective genes [24-26]. All the compounds were obtained nrf2/ARE, HSE, MMP, p53, and ATAD5 inactive except isorhamnetin and 5-O-Methylmyricetin; both were active for MMP parameter while kaempferol-3-O-glucoside active for p53 parameter and isorhamnetin active for ATAD5 parameter. Besides these three phyto-compounds as metabolites *viz*. Isorhamnetin, 5-O-methylmyricetin, and kaempferol-3-O-glucoside may be harmful after chronic exposure, while rest phytocompounds may be suitable during the formation of metabolites as individually or combinations in the body of organisms. As per earlier *in-silico* work, it was predicted that quercetin and Myricetin followed by kaempferol of *Musa* sp. suitable for gastric ulcer protection, but it is important to know the metabolic activity through an experiment in respect to these plant metabolites.

CONCLUSION: It is concluded from this *in-silico* study that the metabolite (N-nitrosodimethylamine) of synthetic medicine, namely ranitidine showed highly toxic as well as carcinogenic while metabolites as quercetin-3-glucuronide and 5-O-methylmyricetin showed carcinogenic, which may be occurred at the higher dose and chronic exposure.

The present *in-silico* results are suitable for further experimental research with these metabolites, and the determination of toxicity mechanisms will be the narrow range. This online tool helps faster screening of large numbers of compounds within a short duration as well as without animal testing. This predictive study is suggested for future experimental assays to validate the present results of these metabolites.

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