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In-silico and *in-vitro* toxicological evaluation of *Tiktakam kashayam*

Research Article

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Abstract

Inflammatory bowel disease is a chronic disease characterized by gastro intestinal tract inflammation. Antinflammatory drugs help to decrease the inflammation of the digestive tract but having lot of side effects. Many ayurvedic formulations shows promising effect for IBD, but there is no scientific evidence for its toxicity; hence "*Tiktakam Kashayam*" ayurvedic formulation is selected for the study and evaluated by *in-silico and invitro studies. Insilico* study is done by using PASS online software. *In-vitro* heavy metal analysis and cytotoxicity was evaluated by MTT assay. *Insilico* studies shows anti-inflammatory activity and also having anti-neoplastic activity especially colon cancer. Some important toxicities like hepatotoxicity shown by the compound azadirachtin present in *Azadiracta indica*, neurotoxic shown by the compound biflorne present *Inoldenlandia corymbosa,* Ototoxicity shown by the compound sweroside, reproductive dysfunction produced by the compound luteolin present in *Gentiana kuro*. Neurotoxic by kobusone, respiratory failure by swertiamarin, ototoxicity by sweroside present in the plant *swertia chirayata*. Parkinsonism like effect produced by the compound piperine and pipperitin by *Piper longum*. *In-vitro* heavy metal analysis shows lead and mercury below the detection limit, cadmium not detected and arsenic is present below the approved range. The ayurvedic formulation "*Tiktakam Kashayam*" possess anti-inflammatory activity and will be beneficial for the treatment of inflammatory bowel disease

Key Words: Ayurvedic formulation*, Piper longum, Azadiracta indica,* Inflammatory bowel disease*,* Hepatotoxicity.

Introduction

Inflammatory bowel disease (IBD) is a chronic and life-long disease characterized by gastro intestinal tract inflammation (1). It is a group of chronic idiopathic inflammatory disease of the gastrointestinal tract with symptoms evolving in a relapsing and remitting manner (2). It occurs in genetically susceptible individuals after an exaggerated immune response to a normal stimulus such as food and intestinal flora (3).

Inflammation anywhere along the digestive tract interferes with this normal process. IBD can be very painful and disruptive. In rare cases, it may even be life threatening (4). This inflammatory condition encompasses two major forms (5), known as:

1. Ulcerative colitis(UC)
2. Crohn's disease (CD)

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The highest rates of IBD are assumed to be in developed countries, and the lowest are considered being in developing regions; colder-climate regions and urban areas have a greater rate of IBD than those of warmer climates and rural areas. Internationally, the incidence of IBD approximately 0.5-24.5 cases per 100,000 person-years for Ulcerative colitis and 0.1-16 cases per 100,000 person years for Crohn's disease. Overall, the prevalence for IBD is 396 cases per 100,000 person annually (6). Last comparative population study in Asia found that the incidence of IBD varied across Asia ranging from 0.54 per 100,000 to 344 per 100,000 persons (7). A review of IBD reported that the prevalence of Crohn's disease in North America was 319 per 100,000 persons. Prevalence rates of ulcerative colitis were 249 per 100,000 persons, in North America and 505 per 100,000 persons in Europe (6). The risk of developing ulcerative colitis in children of migrants from low incidence to high- incidence countries is similar to non-immigrants (8). Time trend analyses showed statistically significant increase in the 100,000 person-year in Asia and the Middle East. Time trend analyses showed statistically significant increase in the incidence of IBD over time. Regional differences in UC and CD have been reported from India. The first prevalence figure of 42.8/100,000 patients for UC from Haryana and in 2003, reported a similar prevalence of 44.3/100,000 while screening 51,910 people from

Punjab. Both these population-based studies are, however, from select North Indian population and do not in any way represent the true burden of IBD in the Indian subcontinent. But these figures are only one-third to one-sixth of the population-based studies from Canada, North America or the UK. In a national survey from India in, UC was equally prevalent in the northern and southern States of the country. Inflammatory bowel disease (IBD) burden is increasing at a rapid pace in India and other Asian countries and with total population into consideration, India is projected to have among the highest IBD burden across the globe, despite having lower prevalence as compared to the West (6). The Crohn's and colitis foundation of America (CCFA) estimates that around 3.1 million people in the United States have IBD. Many diseases are included under the umbrella term IBD. The two most common ones are Ulcerative colitis and Crohn's disease (4). Ulcerative colitis usually presents with bloody diarrhoea and it is diagnosed by colonoscopy and histological findings. Ulcerative colitis can be debilitating and can sometimes lead to life threatening complication (9). Types of ulcerative colitis include Ulcerative proctitis, Proctosigmoiditis, Left sided colitis, Pancolitis (10). Crohn's disease is a chronic relapsing inflammatory bowel disease. It is characterized by a transmural granulomatous inflammation which can affect any part of the gastrointestinal tract, most commonly the ileum, colon or both**.** A recent study in which all new CD cases in Finland between 2000 and 2007 were included revealed an overall incidence rate of 9.2 per 100 000 inhabitants (11)**.**

Ayurvedic formulation

Ayurveda, the traditional Indian medicinal system remains the most ancient yet living traditions with sound philosophical and experimental basis. It is a science of life with a holistic approach to health and personalized medicine. It is known to be a complete medical system that comprised physical, psychological, philosophical, ethical, and spiritual health (12, 13). In Ayurveda, each cell is considered to be inherently an essential expression of pure intelligence hence called self-healing science. In addition, to the self-healing concept, the use of herbal treatment is equally important in this Indian traditional system of medicine. According to the World Health Organization, about 70–80% of the world populations rely on nonconventional medicines mainly of herbal sources in their healthcare. Public interest for the treatment with complementary and alternative medicine is mainly due to increased side effects in synthetic drugs, lack of curative treatment for several chronic diseases, high cost of new drugs, microbial resistance, and emerging diseases, etc (13). According to Indian mythological concept Ayurveda originated from Brahma, the God of Creation. Hindu myth says that, Brahma wants to ease the sufferings of his creation by transferring the knowledge of Ayurveda to deities. Dhanvantari was one of those deities, who then transferred this knowledge of science to modern world. Dhanvantari is considered as "Father of Ayurveda". The last of the 'Great Three' of Ayurveda Astanga hrdaya was composed by Vagbhata. Every living and non-living entity is constituted by five primordial principles or *mahabhoota Prithvi, Jela, Theja, Agni* and *Vayu*. The tissues of the body are also composed of these five elements and their derivatives. This identity of composition is the central principle of ayurvedic therapeutics which mandates the choice of drugs and food without causing side effects (14).

Ayurvedic formulations for IBD (14)

- **Tiktakam kashyam**
- Guluchayadi kashayam
- Nyagrodhadhi kashayam
- Mahatiktakam kashayam
- Laksha choornam
- Lodhra choornam
- Setubandham choornam
- Astakashari gulika

Tiktakam kashayam

Tiktakam kashayam is a Ayurvedic formulation for IBD having the following ingredients is shown in the table no1 and its chemical constituents is listed in table no 2.

Table.1- Ingredients of Tiktakam Kashayam

SL. No	Officinal part	Botanical name	Parts used	Quantity
1	Patola	*Trichosanthes lobata* Wall.	Preliminary Leaf	1.425g
2	Nimba	*Azadirachta indica,* A.Juss	Stem bark	1.425g
3	Katuka	*Neopicrorhiza Scrophulariiflora* (Pennell) D.Y.Hong	Root	1.425g
4	Darvi	*Berberis aristata* DC	Root	1.425g
5	Patha	*Cyclea peltata* (Lam.) Hook.f. & Thomson	Root	1.425g
6	Duralabha	*Tragia involucrata* L.	Root	1.425g
7	Parpata	*Hedyotis corymbosa* (L.) Lam.	Preliminary Leaf	1.425g
8	Trayamana	*Gentiana kurroo* (Royle)	Root	1.425g
9	Musta	*Cyperus rotundus* L.	Root	1.425g
10	Bhunimba	*Swertia chirayita (*Roxb.)	Preliminary Leaf	1.425g
11	Kalinga	*Wrightia antidysenterica* R. Br.	Seed	1.425g
12	Kana	*Piper longum* L.	Fruit	1.425g
13	Chandana	*Santalum album L.*	wood	1.425g

Table.2 Composition and Constituents Present In Tiktakam Kashayam Ayurvedic Formulation

Sl.No	Plant Name	Constituents
1	*Trichosanthes lobata (patola)*	1. Octanoic acid 2. Dodecanoic acid 3. Octadecane 4. Enoic acid 5. Hexanoic acid 6. Quinazolin-8-one 7. Ilicic acid 8. Pentadecanoic acid 9. Oxaspiro 10. Benzene acetic acid 11. 3 Beta-cucurbita-5,24-dien-3-ol 12. Alpha carotene 13. Beta carotene 14. Lycopene 15. Lutein 16. Ascorbic acid 17. Beta- sitosterol
2	*Azadirachta indica (Nimba)*	1. Nimbn 2. Quercetin 3. Ascorbic acid 4. Azadirachtin 5. Salanin 6. N-hexacosanol 7. Beta-sitosterol 8. Nimbidin 9. Nimbinene 10. Nimbolide 11. Nimbolinin 12. Nimbandiol 13. Meliantriol 14. Gedunin 15. Azadiradione 16. Azadirone 17. Scopoletin 18. Rutin 19. Myricetin 20. Vilasinn 21. Behenic acid
3	*Neopicrorhiza scrophularflora (katuka)*	1. Picroside IV 2. Specioside 3. Verminoside 4. Aucubin 5. Abeloside A 6. Sweroside 7. Picrorhizaoside 8. Cinnamic acid 9. Scrophuloside 10. 7-Hydoxy coumarin 11. Hebitol III 12. Galic acid 13. Isoferulic acid 14. Vanillic acid 15. Hexacosanol
4	*Berberis aristata (Darvi)*	1. Taxilamine 2. Palmatine 3. Oxyberberine 4. Tetrahydrodropimatine 5. Jatrorhizine 6. Pakistanine
5	*Cyclea peltata (patha)*	1. Cyclea peltin 2. Cycleadrine 3. Cycleonorine 4. Cycleohomine chloride 5. Cycleocurine
6	*Tragia involucrata (Duralabha)*	1. 2,4-dimethyl hexane 2. 2,4-dimethyl heptane 3. 2-methyl nonane 4. Friedelan-3-one 5. Rutin 6. Quercetin 7. Stigmasterol
7	*Hedyotis corymbosa* (parpata)	1. Geniposide 2. Asperuloside 3. Rutin 4. Aspenicosidic acid 5. Scendoside methyl ester 6. Oleanolic acid 7. Y-sitosterol 8. 6-alpha hydroxy geniposide 9. Iridoid glycoside 10. Biflorine
8	*Gentiana kurro (trayamanna)*	1. Sweroside 2. Gentiopicroside 3. Amarogentin 4. Robinetino 5. Luteolin 6. Apigenin 7. Swertiamarin
9	*Cyperus rotundus*	1. 2-cyperone 2. Cyperone 3. Cyperotundone 4. Cyperol 5. Beta selinene 6. Isocyperol 7. Sitosterol 8. Valerenal 9. Sugeonyl acetate 10. 2-copaene 11. Kobusone 12. Isokobusone 13. Sugenol 14. Beta caryophyllene 15. Sugetnol
10	*Swertia chirayita (bhunimba)*	1. Gentiopicrin 2. Sweroside 3. Swertiamarin 4. Amerogentin
11	*Wrightia antidysenterica (kalinga)*	1. Stigmasterol 2. Campesterol 3. Indigo 4. Indirubin 5. Isatin 6. Methyl anthranilate 7. Rutin 8. Cyclo arthrenone 9. Alpha amyrin 10. Beta amyrin 11. Beta sitosterol 12. 14-alpha methyl zymosterol
12	*Piper longum (kana)*	1. Piperine 2. Pipperitin 3. Piperlongumin 4. Piplartin 5. Guineensine 6. Pellitorine 7. Brachystamide B
13	*Santalum album (chandhana)*	1. Alpha santalene 2. Beta santalene 3. Epi beta santalene 4. Z-alpha santalol 5. Z-alpha trans bergamotol

Based on the above complete review *In-silico* toxicological evaluation of the compounds present in "Tiktakam Kashayam". *In-vitro* toxicity evaluation by Heavy metal analysis and MTT Assay.

Materials and Methods

Materials and Instruments

All the chemicals were purchased from Thermo fisher scientific, MTT staining solution from Sigma Aldrich, Bilirubin kit, Urea kit, Creatinine kit, Glucose kit from AGAPPE diagnostics Ltd. Eosin, OG6, EA36 from Merck. Inductively coupled plasma mass spectrometer from Agilent Technologies, Micro digestion system from MARS-6, Micro plate from Thermo fisher scientific, Centrifuge from REMI, Refrigerator from Whirlpool, Electronic weighing balance from WENSAR, Semi auto analyzer and Micro pipettes from MISPAVIVA.

Methods

In- Silico Toxicological evaluation

PASS is a computer aided software tool that predicts the biological activity spectrum of different compounds based on their structure. The prediction result depends on the analysis of structure activity-relationships for more than 250,000 biologically active substance including drugs, drugs- substances, leads and toxic substances. PASS online has the ability to predict over 4000 different kinds of biological activities that include therapeutic effects, toxic effects, adverse effects, enzyme interaction, mechanism of action etc. This is one of the most successful programs giving early indications about compound that it might be useful. The biological activity spectrum of a chemical compound is the collection of various types of biological activities that indicates the output of its interaction with different biological molecules. Pa (probability "to be active") evaluates the possibility that the predicted compound belongs to the category of active compounds. Pi (probability "to be inactive") evaluates the possibility that the predicted compounds belongs to the category of inactive compounds (15).

Steps involved in pass:

Step I: Navigation of the PASS online web page:

Enter the words "PASS Prediction" in any of web browsers and it allow the direct access to the pass online. A free registration can be done and after logging, use the prediction page of the online program for prediction of component of interest.

Step II: Drawing the structure of molecule:

PASS online prediction tool uses the 2D structure of the components, which forms the basis for the prediction as an input hence the structure can be drawn using Chemsketch version 12 and uploaded in the PASS website as a (*.mol) file or it can be-directly drawn on the website using JAVA that uses a drawing program called Marvin Sketch.

Step III: Prediction output

The input structure which was drawn in the second step its activities are now compared with the structures with known activities present in the database of the program.

Bayesian approach being the principal of estimation, the prediction tool with predicts Pa: Pi ratio of the input substance. The output is given in the form different biological activities in descending of their probability ratios (16).

In-vitro toxicity stidues

a. Heavy metal analysis (17)

Standard Preparation

Preparation of 1ppm stock solution

1ppm stock solution is prepared by pipette 1ml of 1000 ppm standard solution to a 100 ml standard flask and make up to the mark using ultrapure water.

Preparation of working standard solution

0.5ppb, 5ppb, 50ppb, 100ppb,200ppb, 250ppb and make up to the mark using HPLC water.1ml of 20%extrapure concentrated HNO3 were added to the standard flask prior to make up to mark.

Sample preparation

0.25g to 0.5g sample is weighed accurately in to MDS (Microwave digestion system) digestion tube. Add 5.0ml conc. HNO3 (extra pure), and 0.5 ml HCl (extra pure), and 1.0 ml H2O2 (extra pure), and allow 15 min self-digestion. Tighten the cap and keep for digestion in the MDS. After digestion quantitatively transfer the contents into 50 ml tube and make upto50 ml using extra pure water.

Calculation

Element (µg/l) =concentration from calibration graph (µg/l) x dilution factor.

MTT assay (18)

Protocol-Cytotoxicity

The cells were seeded a 96- well plate flat-bottom micro plate at 37°C in 95% humidity and 5% CO_2 for overnight. Different concentrations (100, 50, 25, 12.5, 6.25, 3.125µg/ml) of samples were treated.The cells were incubated for another 48 hours. The wells were washed twice with PBS and 20µL of the MTT staining solution was added to each well plate and plate was incubated at 37 °C. After 4h, 100µL of DMSO was added to each well to dissolve the formazan crystals, and absorbance was recorded with a 570 nm using micro plate reader(1).

Formula

Surviving cells (%) = mean OD of test compound/ mean OD of negative control x100

Using graph pad prism version 5.1, we calculate the IC 50 of compounds.

Results and Discussion

In-Silico Toxicity studies

Table 3: Pharmacological and toxicological activity of plant constituents

Plants	Plant constituents	Pharmacological activity			Toxicological activity		
		Pa	Pi	Activity	Pa	Pi	Activity
Trichosanthes lobata (patola)	**a. Lutein**	952	2	CYP2J substrate	915	9	Diarrhea
		913	5	Antineoplastic	894	9	Ocular toxicity
		895	2	Radioprotector	892	12	Conjunctivitis
		815	4	Anti-ulcerative	886	13	Toxic
	b. Ascorbic acid	948	2	Vasoprotector	900	3	Allergic contact dermatitis
		928	3	Antioxidant	892	3	Hyperglycemic
		845	1	Chemoprotective	895	11	Diarrhea
		779	8	Antiinflammatory	842	11	Tachycardiac
	c. Lycopene	812	3	Antioxidant	893	7	Anemia
		783	1	Antiviral (Rhinovirus)	885	13	Conjunctivitis
		753	5	Dermatologic	861	4	Skin irritation, high
		754	10	Antiinflammatory	856	4	Hyperglycemic
Azadirachta indica (Nimba)	**a. Nimbin**	936	4	Antineoplastic	Nil	Nil	Nil
		895	3	Prostate cancer treatment	Nil	Nil	Nil
		880	4	Antineoplastic (colorectal cancer)	Nil	Nil	Nil
	b. Azadira chtin	939	4	Antineoplastic	918	9	Toxic
		891	4	Antiinflammatory			
		741	8	Antifungal	752	27	Hepatotoxic
		702	6	CYP2A11 substrate			
	c. Gedunin	935	4	Antineoplastic	791	29	Toxic
		777	4	CYP3A4 inhibitor			
Neopicrorhiza scrophularflora (katuka)	**a. Isoferulic acid**	944	4	Membrane integrity agonist	839	7	Urine discoloration
		915	3	JAK2 expression inhibitor	819	4	Irritation
		903	1	Aryl sulfotransferase inhibitor	812	4	Hypercholesterolemic
		903	2	Preneoplastic conditions treatment	809	3	Ulcer, gastric
	b. Hexacosanol	965	2	Sugar-phosphatase inhibitor	958	9	Toxic, respiration
		958	2	Alkenylglycerophosphocholine hydrolase inhibitor	940	4	Acidosis, metabolic
		952	1	Carboxypeptidase Taq inhibitor	940	5	Euphoria
		951	2	Alkylacetyl glycerophosphatase inhibitor	926	3	Eye irritation, moderate
	c. Gallic acid	955	2	Arylacetonitrilase inhibitor	939	3	Hematemesis
		954	2	Chlordecone reductase inhibitor	934	3	Ulcer, aphthous
		950	2	Dehydro-Lgulonate decarboxylase inhibitor	887	3	Gastrointestinal hemorrhage
		950	3	Testosterone 17 beta dehydrogenase (NADP+) inhibitor	878	9	Acidosis, metabolic
Berberis aristata (Darvi)	**a. Taxilamine**	823	27	Aspulvinone dimethylallyltransferase inhibitor	698	25	Gastrointestinal hemorrhage
		729	41	Gluconate 2- dehydrogenase (acceptor) inhibitor	674	48	Fibrillation, atrial
		709	25	Antineoplastic	588	32	Hyperuricemia
	b. Oxyberberine	777	7	Antidyskinetic	519	54	Fibrosis, interstitial
		774	4	MAP kinase stimulant	487	91	Psychomotor impairment
		773	22	Antineurotic	472	79	Depression
		716	10	Neurotransmitter uptake inhibitor	481	105	Gastrointestinal hemorrhage

	c. Palmatine	944	2	Male reproductive disfunction treatment	392	131	Pseudoporphyria
		870	2	Antiprotozoal	299	92	Piloerection
Cyclea peltata (patha)	**a. Cyclea peltine**	918	1	Histamine release stimulant	931	5	Hypotension
		897	1	Oxygen scavenger	892	4	Panic
		862	2	Leukopoiesis stimulant	882	6	Toxic, vascular
		860	8	5-Hydroxytryptamine release stimulant	843	13	Neurotoxic
	b. Cycleonorine	949	3	Spasmolytic	978	4	Hypotension
		935	1	Histamine release stimulant	977	4	Toxic, vascular
		886	1	Oxygen scavenger	929	5	Tremor
		825	3	Muscle relaxant	896	8	Dyskinesia
	c. Cycleohomine chloride	868	2	Histamine release stimulant	946	5	Hypotension
		833	2	Oxygen scavenger	850	9	Toxic, vascular
		812	3	Muscle relaxant	848	12	Neurotoxic
		791	15	5-Hydroxytryptamine release stimulant	835	8	Tremor
Tragia involucrata (Duralabha)	**a. 2,4 - dimethyl hexane**	943	3	Phobic disorders treatment	883	9	Shivering
		936	3	Acrocylindropepsin inhibitor	861	12	Pure red cell aplasia
		936	3	Saccharopepsin inhibitor	857	21	Toxic, respiration
		936	3	Chymosin inhibitor	840	4	Skin irritation, corrosive
	b. 2,4 -dimethyl heptanes	942	3	Chymosin inhibitor	905	5	Pure red cell aplasia
		942	3	Saccharopepsin inhibitor	894	7	Shivering
		942	3	Acrocylindropepsin inhibitor	903	17	Toxic, respiration
		936	3	Phobic disorders treatment	862	3	Skin irritation, corrosive
	c. 2-methylnonane	959	2	Chymosin inhibitor	922	4	Shivering
		959	2	Acrocylindropepsin inhibitor	917	5	Pure red cell aplasia
		959	2	Saccharopepsin inhibitor	913	2	Skin irritation, corrosive
		947	3	Polyporopepsin inhibitor	924	15	Toxic, respiration
***Hedyotis corymbosa* (parpata)**	**a. Geniposide**	988	1	Hepatoprotectant	984	3	Inflammation
		961	2	Hepatic disorders treatment	863	17	Toxic
		955	1	Cholesterol antagonist	707	21	Hypercholesterolemic
	b. Biflorne	915	3	Anaphylatoxin receptor antagonist	880	13	Diarrhea
		914	2	Cardioprotectant	795	28	Toxic
		918	7	Membrane integrity agonist	713	27	Neurotoxic
		893	5	TP53 expression enhancer	706	40	Hematotoxic
	c. Oleanolic Acid	984	2	Caspase 3 stimulant	886	7	Muscle weakness
		954	1	Transcription factor NF kappa B stimulant	852	12	Weakness
		954	1	Transcription factor stimulant	813	25	Toxic
		937	2	Chemopreventive	743	12	Hyper-cholesterolemic
Gentiana kurro (trayamanna)	**a. Swerosi de**	985	1	Antiprotozoal (Leishmania)	882	8	Inflammation
		904	9	CDP-glycerol glycerophosphotransferase inhibitor	850	17	Diarrhea
		854	7	Gluconate 2-dehydrogenase (acceptor) inhibitor	844	16	Emetic
		849	3	Antiprotozoal	820	3	Ototoxicity
	b. Amarogentin	875	3	Hepatoprotectant	895	7	Inflammation
		853	19	CDP-glycerol glycerophosphotransferase inhibitor	837	21	Toxic

	c. Luteolin	837	4	UDP- glucuronosyltransferase substrate	833	20	Diarrhea
		830	10	Gluconate 2- dehydrogenase (acceptor) inhibitor	800	22	Emetic
		978	1	Chlordecone reductase inhibitor	824	4	Genotoxic
		965	3	Membrane integrity agonist	819	16	Reproductive dysfunction
		964	3	HIF1A expression inhibitor	823	28	Shivering
		953	2	Membrane permeability inhibitor	804	17	Toxic, vascular
Cyperus rotundus (Musta)	**a. Alpha-cyperone**	915	2	Carminative	816	10	Ataxia
		845	5	Apoptosis agonist	811	4	Irritation
		839	1	Retinol dehydrogenase inhibitor	792	10	Non mutagenic, Salmonella
		826	8	CYP2C substrate	776	18	Excitability
	b. Cyperol	912	2	Carminative	875	4	Irritation
		899	5	Antieczematic	816	15	Respiratory failure
		894	5	Antineoplastic	755	15	Ataxia
		871	10	Testosterone 17 beta- dehydrogenase (NADP+) inhibitor	768	33	Toxic
	c. Kobusone	900	5	HIF1A expression inhibitor	821	24	Toxic
		881	5	Antineoplastic	795	18	Neurotoxic
		746	11	Antiinflammatory	729	33	Toxic, respiration
Swertia chirayita (bhunimba)	**a. Swertiamarin**	954	2	Antiprotozoal (Leishmania)	934	5	Inflammation
		912	8	CDP-glycerol glycerophosphotransferase inhibitor	910	7	Respiratory failure
		898	2	Hepatoprotectant	896	8	Emetic
		791	7	Antiinflammatory	881	13	Diarrhea
	b. Amerogentin	875	3	Hepatoprotectant	895	7	Inflammation
		853	19	CDP-glycerol glycerophosphotransferase inhibitor	837	21	Toxic
		837	4	UDP-glucuronosyltransferase substrate	833	20	Diarrhea
		830	10	Gluconate 2- dehydrogenase (acceptor) inhibitor	800	22	Emetic
	c. Sweroside	985	1	Antiprotozoal (Leishmania)	882	8	Inflammation
		849	3	Antiprotozoal	850	17	Diarrhea
		843	12	Benzoate-CoA ligase inhibitor	844	16	Emetic
		811	4	Hepatoprotectant	820	3	Ototoxicity
Wrightia antidysenterica (kalinga)	**a. Lupeol**	978	2	Caspase 3 stimulant	948	3	Irritation
		947	1	Transcription factor stimulant	901	7	Inflammation
		950	4	Antineoplastic	822	21	Behavioral disturbance
		907	2	Hepatoprotectant	822	24	Toxic
	b. 3,4 seco-20-(29)en-3-oic acid	882	3	Hepatoprotectant	837	11	Inflammation
		853	2	Transcription factor NF kappa B stimulant	766	33	Toxic
		853	2	Transcription factor stimulant	711	43	Toxic, gastrointestinal
Piper longum (kana)	**a. Piperine**	916	7	Membrane integrity agonist	724	8	Parkinsonism
		826	3	Carminative			
		814	4	Neurotransmitter uptake inhibitor			
		734	4	Sigma receptor agonist			
	b. Pipperitin	916	7	Membrane integrity agonist	724	8	Parkinsonism
		814	4	Neurotransmitter uptake inhibitor			
		875	8	Chymosin inhibitor			
		875	8	Acrocylindropepsin inhibitor	811	35	Twitching
		870	8	CYP2J substrate			

Santalum album (chandhana)	**a. Alpha santalene**	900	6	Testosterone 17beta- dehydrogenase (NADP+) inhibitor	713	7	Irritation
		897	3	Cardiovascular analeptic	708	17	Withdrawal
	b. Z alpha santalol	894	3	Cardiovascular analeptic	751	6	Irritation
		886	6	CYP2J substrate	702	22	Ataxia

The results obtained from *in-silico* study by using PASS online software were shown in the table No.7 plants like: *Trichosanthus lobata* Wall. constituents like lutein shown anti-ulcerative, ascorbic acid show antioxidant and anti-inflammatory, lycopene show anti-inflammatory. *Azadaricta indica* constituent nimbin produce anti-inflammatory, anti-neoplastic (colon cancer), gedunin shown anti-neoplastic. *Berberis aristata* constituent taxilamine shown anti-neoplastic. *Cyprus rotundus* plant constituent kobusone shown anti-inflammatory and anti- neoplastic. *Swertia chirayata* plant constituent swartiamarin shown anti-inflammatory. *Wrightia antidysenterica* plant constituent lupoel shown anti-neoplastic activity. The result obtained from *in-silico* study support the ayurvedic formulation "Tiktakam kashayam" used for Inflammatory bowel disease. The results of the *in-silico* toxicological studies were shown in the table No.7 by PASS online software: Some important toxicities like hepatotoxicity shown by the compound azadirachtin present in *Azadiracta indica*, neurotoxic shown by the compound biflorne present in *oldenlandia corymbosa,*Ototoxicity shown by the compound sweroside, reproductive dysfunction produced by the compound luteolin present in *Gentiana kurroa*. Neurotoxic by kobusone, respiratory failure by swertiamarin, ototoxicity by sweroside present in the plant *swertia chirayata*. Parkinsonism like effect produced by the compound piperine and pipperitin by *Piper longum*.

In-vitro Toxicity studies

Heavy metal analysis

Table 4: Heavy metal analysis

Parameters	Unit	Results	Specification	Detection Limit	Test Method
Arsenic	mg/kg	0.18	NMT 3.0	0.05	CKL/ANL/AY-008
Cadmium	mg/kg	Not detected	NMT 0.3	0.05	CKL/ANL/AY-008
Lead	mg/kg	BDL	NMT 10.0	0.05	CKL/ANL/AY-008
Mercury	mg/kg	BDL	NMT 1.0	0.05	CKL/ANL/AY-008

Heavy metal analysis results were shown in the table No.4. Heavy metals like arsenic, mercury, cadmium, lead are commonly available heavy metals in ayurvedic formulations. From the results obtained from the study shown that cadmium (NMT 0.3) is not detected in the formulation. Lead (NMT 10.0) and mercury (NMT 1.0) present below detection limit. Arsenic 0.18mg/kg present in the formulation but within the normal range.

MTT ASSAY: IC 50 value of compounds (µM/ml)

Table 5: IC 50 value of sample

Sample codes	HEK293	
	Mean	SD
Sample	44.73	0.71

Table6: Cell viability

Cell viability of HEK293			
Concentration µg/ml	Sample		
100	52.19	50.31	51.25
50	56.56	57.50	57.19
25	60.00	61.25	61.25
12.5	63.44	64.38	63.75
6.25	67.19	68.13	67.19
3.125	69.69	69.38	71.56
Negative control	100		

Figure.6-Cell viability graph

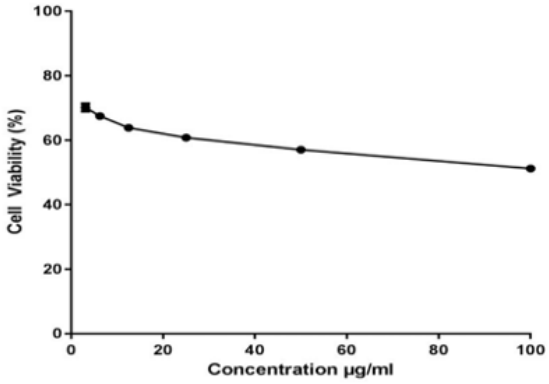
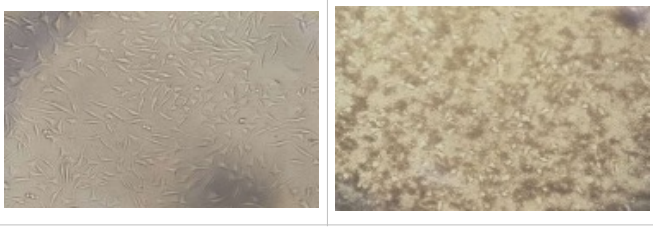


Figure.7- Microscopic picture showing cell viability

(a) Normal Cell (b) Drug Treated



The result obtained from MTT assay was shown in the table No.9 and 10. MTT assay was performed in HEK293 cells and IC 50 value of the formulation "*Tiktakam Kashayam*" shown 44.43±0.71

and graphical representation were shown in figure N0.6. The photographical representation of normal cells and drug treated were shown in figure No.7.

Conclusion

Based on present study results we concluded that *In-silico* study reports supports the ayurvedic formulation "*Tiktakam Kashayam*" having anti-inflammatory activity and also having anti-neoplastic activity especially colon cancer. *In-vitro* heavy metal analysis shows lead and mercury below the detection limit, Cadmium-not detected and arsenic is present below the approved range.

The ayurvedic formulation "*Tiktakam Kashayam*" possess anti-inflammatory activity and will be beneficial for the treatment of inflammatory bowel disease. Further studies are required to fix the dose levels to avoid the long term toxicity.

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