RESEARCH ARTICLE

Polyherbal Formulation Development for Parkinson's Disease: Comprehensive Quality Control and Toxicological Evaluation

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ABSTRACT

Background and objectives: In this study, extracts from eight herbal medicines *Withania somnifera, Nardostachys jatamansi, Convolvulus pluricaulis, Mucuna pruriens, Centella asiatica, Bacopa monnieri, Tinospora cordifolia,* and *Ginkgo biloba* were combined to create a potential medication. **Methods:** Quality Control study had been done according guideline or procedure drafted by CCRAS, Ministry of AYUSH, Govt. of India, Oral Acute and Repeated Dose 28-day Toxicity Study had been done according to OECD 423 and 407 guidelines. **Results**: The polyherbal formulation's physiochemical characteristics, phytochemical content, safety profile, and possible impacts on physiological parameters were all carefully assessed. The formulation was establish to be safe at a single administration dose of 2000 mg/kg and safe at repeated doses up to 1000 mg/kg over the course of 28 days. **Conclusion**: This study highlights the polyherbal formulation's potential for therapeutic application in the cure of Parkinson's disease by offering insightful information on its toxicological properties and quality control.

Keyword: Polyherbal formulation, HPTLC, Phytochemical, Physiochemical, toxicity study.

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Conflict of interest: None

INTRODUCTION

A neurodegenerative condition that affects millions of individuals worldwide is Parkinson's disease. While there is no known treatment for Parkinson's disease. There are a lot of botanical substance that are available in nature which have potential effect in Parkinson’s disease like *Withania somnifera, Nardostachys jatamansi, Convolvulus pluricaulis, Mucuna pruriens, Centella asiatica, Bacopa monnieri, Tinospora cordifolia,* and *Ginkgo biloba*. These drugs are also scientifically reported and proven as anti Parkinson drug. *Withania somnifera having* bioactive constituents Withanolides, such as withanolide A, withanolide D, and Anaferin which exhibit numerous therapeutic effects, including anti-Parkinsonian properties.[1] *Nardostachys jatamansi having* 11-ethoxyviburtinal, kanshone H, nootkatone, nardosinone, and nardosinone, dehydrocostus lactone is the major active component has significant anti-Parkinsonian potential.[2] Convolvulus pluricaulis have cinnamic acid, ascorbic acid, and vitamin E having neuroprotective effects; indicate its potential for use in Parkinson's disease.[3] *Bacopa monnieri* contains glutamic acid, alanine, aspartic acid, bacoside A & B, bacogenin A, bacopaside I, serine, cucurbitacin B, bacopasaponin C, bacosine, and stigmasterol chemical constituents and it exhibits anti-Parkinsonian effects by falling α synuclein aggregation, preventing dopaminergic neurodegeneration, & restoring the lipid content in model organisms.[4, 5] Seeds of *Mucuna pruriens* have been consumed in conventional Indian medicine for the treatment of Parkinson's illness due to their high levodopa content, which is a potent precursor of the neurotransmitter dopamine.[6] *Centella asiatica* contains bioactive compounds such as asiaticoside, madecassoside, hydrocotylin, kaempferol, chlorogenic acid and it exhibits neuroprotective, antioxidant, anti-inflammatory, and antiulcer properties, making it a potential therapeutic candidate for neurodegenerative disorders, including Parkinson's disease.[7] *Tinospora cordifolia* is rich in metabolites such as alkaloids, diterpenoid, glycosides, phenolics, lactones, aliphatic compounds, and polysaccharides. It has been reported to have antioxidant, anti-inflammatory, and immunomodulatory properties, which could be beneficial in addressing neurodegenerative conditions like Parkinson's disease.[8] *Ginkgo biloba* contains active natural compound such as ginkgolides flavonoids, and terpenoids which is act and Anti Parkinsonism effect.[9]

Objective of this study to develop a potential drug with the combination of extracts of eight herbal drugs for Parkinson's disease, ensuring quality control and conducting thorough toxicological evaluations are essential steps. Quality control measures are required to guarantee the consistency, efficacy, and safety of the formulation, while toxicological evaluations are crucial for assessing its potential adverse effects on human health. This study aims to grant an overview of the importance of quality control and toxicological evaluation in the context of a polyherbal formulation developed for Parkinson's

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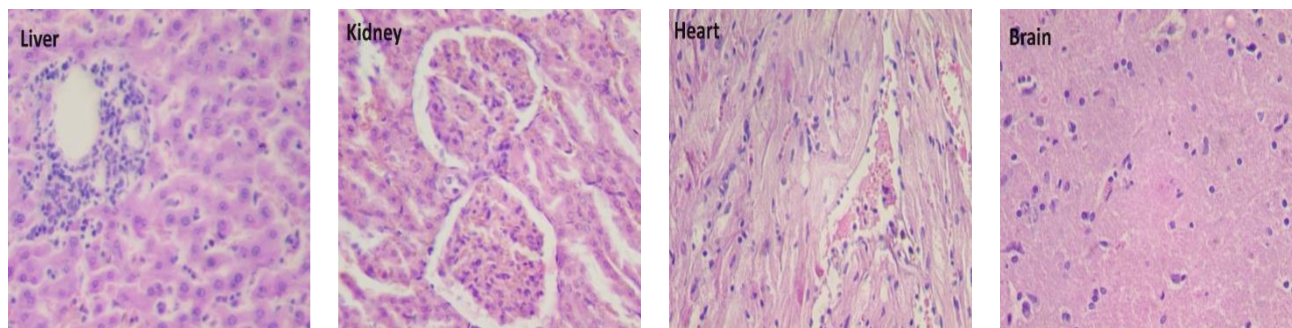
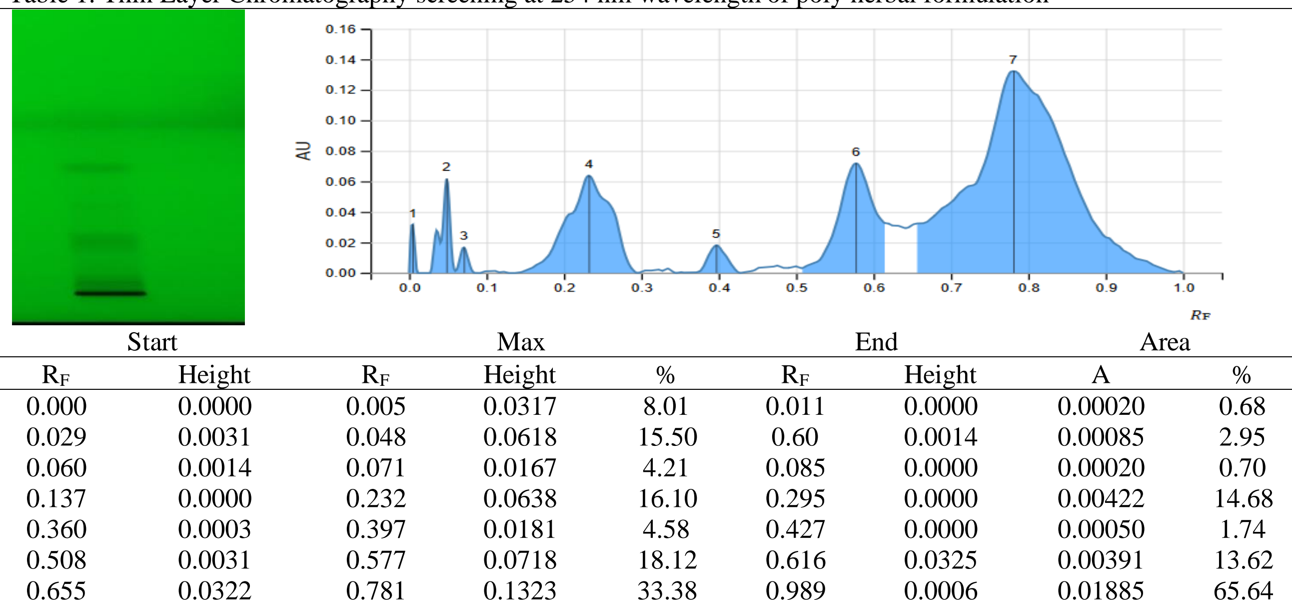


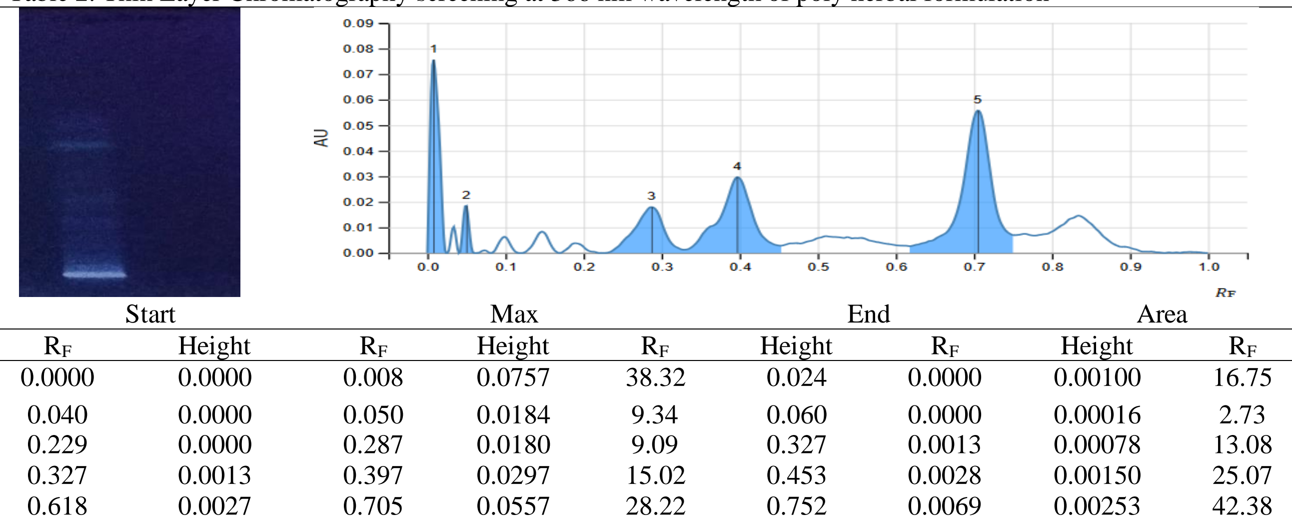
Figure 1: Histopathology of Vital Organs (Liver, Kidney, Heart, Brain) Normal histoarchitecture in the transverse section of organs was observed (H&E, 40x).

Table 1. Thin Layer Chromatography screening at 254 nm wavelength of poly herbal formulation



Start		Max			End		Area	
R_F	Height	R_F	Height	%	R_F	Height	A	%
0.000	0.0000	0.005	0.0317	8.01	0.011	0.0000	0.00020	0.68
0.029	0.0031	0.048	0.0618	15.50	0.60	0.0014	0.00085	2.95
0.060	0.0014	0.071	0.0167	4.21	0.085	0.0000	0.00020	0.70
0.137	0.0000	0.232	0.0638	16.10	0.295	0.0000	0.00422	14.68
0.360	0.0003	0.397	0.0181	4.58	0.427	0.0000	0.00050	1.74
0.508	0.0031	0.577	0.0718	18.12	0.616	0.0325	0.00391	13.62
0.655	0.0322	0.781	0.1323	33.38	0.989	0.0006	0.01885	65.64

Table 2. Thin Layer Chromatography screening at 366 nm wavelength of poly herbal formulation



Start		Max			End		Area	
R_F	Height	R_F	Height	R_F	Height	R_F	Height	R_F
0.0000	0.0000	0.008	0.0757	38.32	0.024	0.0000	0.00100	16.75
0.040	0.0000	0.050	0.0184	9.34	0.060	0.0000	0.00016	2.73
0.229	0.0000	0.287	0.0180	9.09	0.327	0.0013	0.00078	13.08
0.327	0.0013	0.397	0.0297	15.02	0.453	0.0028	0.00150	25.07
0.618	0.0027	0.705	0.0557	28.22	0.752	0.0069	0.00253	42.38

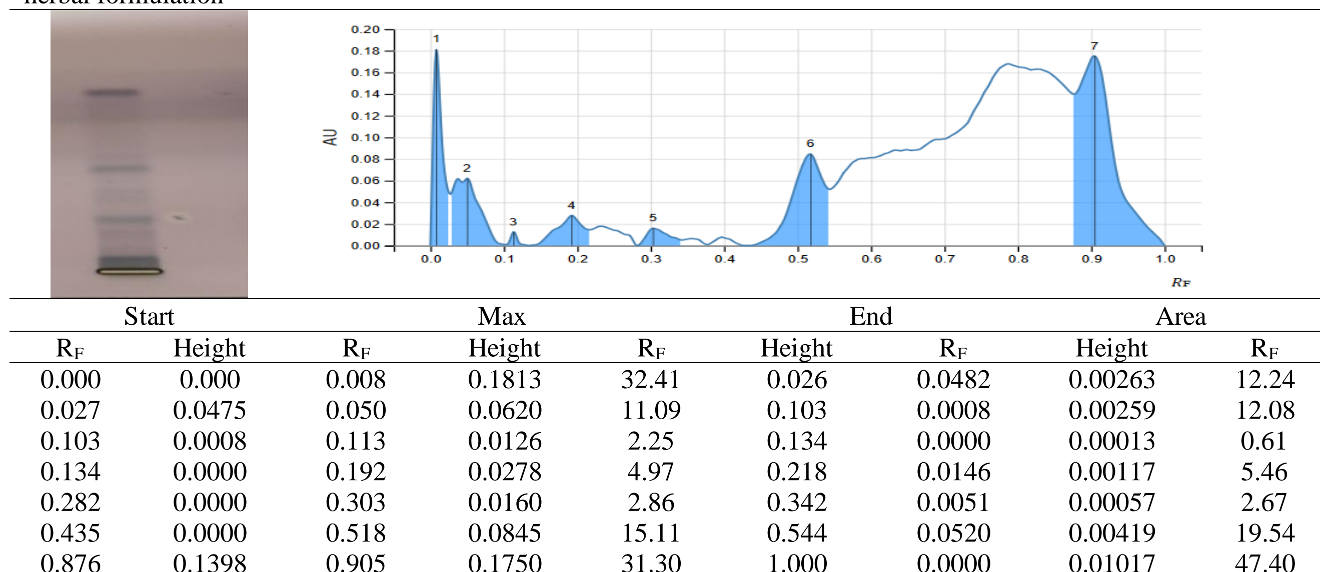
disease. It will explore the significance of these processes in ensuring the efficacy and safety of the formulation, as well as their role in promoting its potential clinical application.

MATERIAL AND METHODS

Material

Withania somnifera, Nardostachys jatamansi, Convolvulus pluricaulis, Mucuna pruriens, Centella asiatica, Bacopa monnieri, Tinospora cordifolia, and *Ginkgo biloba* were the eight test samples that were obtained and authenticated

Table 3. Thin Layer Chromatography screening at 542 nm wavelength after derivatization with p-Anisaldehyde of poly herbal formulation



Start		Max			End		Area	
R_F	Height	R_F	Height	R_F	Height	R_F	Height	R_F
0.000	0.000	0.008	0.1813	32.41	0.026	0.0482	0.00263	12.24
0.027	0.0475	0.050	0.0620	11.09	0.103	0.0008	0.00259	12.08
0.103	0.0008	0.113	0.0126	2.25	0.134	0.0000	0.00013	0.61
0.134	0.0000	0.192	0.0278	4.97	0.218	0.0146	0.00117	5.46
0.282	0.0000	0.303	0.0160	2.86	0.342	0.0051	0.00057	2.67
0.435	0.0000	0.518	0.0845	15.11	0.544	0.0520	0.00419	19.54
0.876	0.1398	0.905	0.1750	31.30	1.000	0.0000	0.01017	47.40

Table 4: Compared weight changes, food consumption rates, and water consumption averages per day in three groups over a period of 14 days.

Groups	Observation day	Weight changes (%)	Food consumption rat average of per day (gm)	Water consumption average of per day(ml)
Group 1	7th day	4.06±0.261	8.23±1.178	16.00±15.528
	14th day	6.52±0.308	8.30±1.735	14.67±1.764
Group 2	7th day	3.95±0.313	7.59±1.031	15.67±1.202
	14th day	6.38±0.610	8.13±0.241	16.33±1.856
Group 3	7th day	4.37±0.303	9.62±1.354	16.67±0.882
	14th day	7.11±0.068	8.47±1.555	14.67±2.028

Table 5: Compared the hematological parameters of three groups.

Name of Test	Group 1 Mean±SEM	Group 2 Mean±SEM	Group 3 Mean±SEM	Reference Value
RBC(x10 /mm^3)	8.50±0.282	8.72±0.554	8.75±0.306	7 - 10
PCV(%)	40.72±0.581	40.32±0.882	40.67±1.920	36 - 48
Hb(g/dl)	13.89±1.179	14.52±0.980	13.32±0.916	11 - 18
WBC(X10^3/mm^3)	11.42±1.690	14.33±0.869	12.75±0.436	6 - 17
Neutrophils(%)	22.62±4.522	27.21±3.314	25.11±1.736	9 - 34
Lymphocytes(%)	72.16±4.739	67.23±3.235	69.36±1.580	65 – 85
Eosinophils(%)	1.82±0.257	2.35±0.675	2.49±0.331	0 - 6
Monocytes(%)	3.40±0.358	3.22±0.504	3.04±0.596	0 - 5
Basophils(%)	0.00±0.000	0.00±0.000	0.00±0.000	0 - 1.5
Platelets(X10^3/mm^3)	690.00±36.171	669.00±47.816	699.00±81.586	500-1300

by BMRL, Rajasthan, a GMP-certified Ayurvedic manufacturing company.

According to the ancient *Ayurvedic* texts, all individual plant samples and the formulation are harmless. Since the suggested therapeutic dose of nontoxic plant-based substances is 3 to 5 gm, identical ratios of each sample were taken to construct the formulation.

Each of the eight ingredients weighed out individually to provide 200 grams, which were then put in a round bottom flasks and put together in a soxhlet assembly to perform a six-hour soxhlet extraction using distilled water as the solvent. Ingredient dried extracts were produced and weighed. Eight distinct sample extracts were extracted, thoroughly combined, and 200 g of formulation was created.

Animals

Nineteen female and ten male albino wistar rats, weighing between 150 and 200 grams, with six individuals in each group, were kept in cages with three rats each, each with a 12-hour light & dark cycle, regulated temperature, and unrestricted access to food and water. The Institutional Animal Ethical Committee of Bilwal Medchem and

Table 6: Compared the biochemical parameters of three groups.

Name of Test	Group 1 Mean±SEM	Group 2 Mean±SEM	Group 3 Mean±SEM	Reference Value
Protein(g/dl)	6.91±0.137	7.00±0.312	6.31±0.277	5.6 - 7.6
Albumin(g/dl)	3.29±0.200	3.22±0.231	3.50±0.597	2.8 - 4.8
Globulin(g/dl)	2.69±0.148	2.58±0.337	2.47±0.119	1.8 - 3 2
Glucose(mg/dl)	101.09±4.658	101.55±7.697	101.22±4.606	50 - 135
Urea nitrogen(mg/dl)	18.42±0.395	18.95±0.753	17.42±0.301	15 - 21
Creatinine(mg/dl)	0.35±0.035	0.46±0.064	0.49±0.110	0.2 - 0.8
Bilirubin(mg/dl)	0.40±0.027	0.42±0.052	0.40±0.007	0.2 - 0.55
Cholesterol (mg/dl)	50.62±3.028	53.32±3.667	58.85±1.668	40 - 130

Table 7: Compared weight changes, food consumption, and water consumption over a period of 28 days.

Groups	Observation day	Weight changes (%)	Food consumption rat average of per day (gm)	Water consumption average of per day (ml)
Male	7th day	4.26±0.325	8.15±2.065	16.24±2.487
	14th day	6.14±0.415	7.19±1.874	15.85±3.658
	21th day	9.74±0.245	9.45±2.154	15.74±2.658
	28th day	12.65±0.358	7.32±1.685	16.74±1.874
Female	7th day	4.85±0.316	8.29±1.398	14.95±1.952
	14th day	6.75±0.274	7.95±2.148	19.65±2.047
	7th day	10.15±0.458	9.69±1.749	14.32±3.658
	14th day	13.28±0.394	7.59±1.958	15.85±2.198

Table 8: Compared the hematological parameters in 28 days repeated dose toxicity study.

Name of Test	Group (Male) Mean±SEM	Group (Female) Mean±SEM	References Value
RBC(x10 /mm³)	8.79±0.336	8.25±0.327	7 - 10
PCV(%)	42.67±1.121	128.73±85.667	36 - 48
Hb(g/dl)	14.76±0.269	14.97±0.444	11 - 18
WBC(X10³/mm³)	11.19±1.168	10.54±0.628	6 - 17
Neutrophils (%)	26.71±2.304	25.70±1.177	9 - 34
Lymphocytes(%)	70.96±1.044	69.94±0.980	65 – 85
Eosinophils(%)	3.02±0.430	3.95±0.452	0 - 6
Monocytes(%)	3.00±0.518	2.83±0.387	0 - 5
Basophils(%)	0.00±0.000	0.00±0.000	0 - 1.5
Platelets(X10³/mm³)	759.60±49.328	789.60±56.597	500-1300

Research Laboratory Private Limited (BMRL/IAEC/22/2/2) gave its prior consent to all procedures.

Methods

Loss on drying

The test sample that had not been dried before was weighed at around 10 g and put in a tared evaporating dish. I used a hot air oven set to 105 degree Celsius for five hr. to dry it. Drying and weighing should be done every hour until the discrepancy between two successive weight readings is less than 0.25%. The weight was deemed constant if there was a variation of no more than 0.01 g between two successive weigh-ins after a 30-minute drying and 30-minute cooling interval in a desiccator. Ascertain the percentage of loss concerning 10 grams of the specimen under examination.

Alcoholic Extractive Value

For 24 hours, macerate 5 g of the coarsely ground, air-dried test sample in a closed flask with 100 ml of alcohol. Shake the mixture regularly for the first 6 hr., and then leave it alone for the left behind 18 hours. Filter as soon as possible, taking care to avoid solvent loss. Next, evaporate twenty-five milliliters of the filtrate in a shallow dish with a flat bottom until it achieves a constant weight, and then weigh it. Dried at a temperature of 105 degree Celsius. Calculate the extractive % of alcohol in respect to the test sample that has been dried. found the methanol-soluble extractive by substituting methanol for alcohol.

Aqueous Extractive Value

For 24 hours, macerate 5 g of the coarsely ground, air-dried test sample in a closed flask with 100 ml of distilled water. Shake the mixture regularly for the first 6 hr., and then leave it alone for the left behind 18 hours. Filter as soon as possible, taking care to avoid solvent loss. Next, evaporate twenty-five milliliters of the filtrate in a shallow dish with a flat bottom until it achieves a constant weight, and then weigh it. Dried at a temperature of 105 degree

Table 9: Compared the biochemical parameters of 28 days repeated dose toxicity study. The parameters measured included Protein, Albumin, Globulin, Glucose, Urea nitrogen, Creatinine, Bilirubin, Cholesterol.

Name of Test	Group (Male) Mean±SEM	Group (Female) Mean±SEM	Reference Value
Protein (g/dl)	6.66±0.199	6.54±0.241	5.6 - 7.6
Albumin (g/dl)	4.08±0.194	3.78±0.246	2.8 - 4.8
Globulin (g/dl)	2.49±0.164	2.63±0.137	1.8 - 3 2
Glucose (mg/dl)	106.30±4.452	103.94±4.122	50 - 135
Urea nitrogen(mg/dl)	16.92±0.881	17.69±0.956	15 - 21
Creatinine (mg/dl)	0.45±0.051	0.51±0.027	0.2 - 0.8
Bilirubin (mg/dl)	0.40±0.023	0.38±0.045	0.2 - 0.55
Cholesterol (mg/dl)	104.97±4.648	101.27±4.941	40 - 130

Table 10: Mean and SEM value of Weight of Organ (Gram) of rats for Group 1 (Female)

Marking	Liver Mean±SEM	Kidney Mean±SEM	Heart Mean±SEM	Brain Mean±SEM
Male	9.1565±0.2019	2.5203±0.0484	1.2360±0.0267	1.9319±0.0596
Female	8.7874±0.2175	2.2575±0.0400	1.6859±0.1612	2.1091±0.1588

Celsius. Calculate the extractive percentage that is soluble in water relative to the test sample that is dried.

Total ash

2 to 3 g of the powdered sample should be burned in a silica crucible at a temperature no elevated than 600 degree celsius until the sample is carbon-free. After cooling, weigh it. In the event that carbon-free ash could not be produced using this procedure, the burned mass was put out, the remains was assemble on ashless filter paper, the remains and filter paper were burned together, the filtrate was added, the evaporated material was dried, and the mixture was ignited at a temperature not to exceed 600 degree celsius. Find the proportion of ash in the test sample that has been dried.

Acid-insoluble ash

I filled the crucible with all of the ash and then added 25 milliliters of diluted hydrochloric acid. Whatman 41 ashless filter paper was used to collect the insoluble particles, and the filtrate washed in hot water until it reached a neutral pH. I burned it until it achieved a steady weight after placing the filter paper with the insoluble material back into the original crucible and letting it dry on a hot plate. I immediately weighed the residue after allowing it to cool for thirty minutes in the proper desiccator. Ascertain the reference dry test sample's acid-insoluble ash %.

Water-Soluble Ash

I filled the crucible with all of the ash and added 25 milliliters of distilled water. Whatman 41 ashless filter paper was used to collect the insoluble particles, and the filtrate washed in hot water until it reached a neutral pH. I burned it until it achieved a steady weight after placing the filter paper with the insoluble material back into the original crucible and letting it dry on a hot plate. I immediately weighed the residue after allowing it to cool for thirty minutes in the proper desiccator. Ascertain the reference dried test sample's water-soluble ash percentage.[10]

Analysis of Primary and Secondary Metabolite Qualitatively Phytochemical testing is used to identify the primary (carbohydrate, protein, and amino acid) and secondary (alkaloids, glycosides, tannin, saponin, and phenolic compounds) metabolites. The alcoholic and aqueous extracts were used to look for primary and secondary metabolites in test samples.

Molisch's Test: After adding two milliliters of test solution and two milliliters of Molisch's reagent, and giving it a gentle shake, one milliliter of concentrated H2SO4 was poured out the test tube's surface and permitted to stand for a minute. A purple ring at the link of the two layers indicated the existence of carbohydrate.

Benedict's test: Sugars were reduced using this technique, which mainly used copper sulfate and sodium hydroxide. Four milliliters of the drug's aqueous solution were combined with one milliliter of Benedict's solution, and the mixture was heated to almost boiling. As the concentration of simple sugar rose, colors such as green, yellow, orange, red, or brown were generated in the test solution as a result of the production of cuprous oxide.

Fehling solution test: Usually used to lower sugar levels, this test consists of two solutions blended in-situ. Fehling solution A includes 0.5% $CuS0_4$, and Fehling solution B is composed of $C_4H_4O_6KNa{\cdot}_4H_2O$. Two milliliters of the drug's aqueous solution were added after one milliliter each of Fehling A and B solutions were blended. The mixture was next brought to a boil in a water bath for five to ten minutes.

Dragondroff's Reagent: Two milliliters of the test solution and two milliliters of bismuth subnitrate and potassium iodide solution, or Dragondroff's reagent, were added to a test tube. The presence of alkaloids was demonstrated by the materials' capacity to produce an orange precipitate.

Wagner Test: The presence of alkaloids was indicated by the production of a reddish-brown precipitate after a few drops of Wagner's reagent (diluted iodine solution) were added to two milliliters of test solution.

Hager test: Picric acid was dissolved in a saturated aqueous solution. After the test filtrate was treated with this reagent, an orange-yellow precipitate was formed, indicating the presence of alkaloids.
Ninhydrin test: This test was used to identify proteins with free amino groups and alpha-amino acids. The characteristic deep blue or light blue color is caused by the synthesis of a mixture between two ninhydrin molecules and the nitrogen of free amino acids.
Biuret test: The mixture was mixed with one milliliter of 4% sodium hydroxide solution, five milligrams of residue, and a drop of 1% $CuSO_4$copper sulfate solution. The proteins were indicated by the emergence of a violet or pink tint.
Xanthoprotic test: A small portion of the test material was treated with two milliliters of water and five milliliters of strong HNO_3. The presence of proteins was shown by the emergence of a yellow hue.
Foam test: A tiny amount of the sample was added to a test tube together with a small amount of water and sodium bicarbonate, and the mixture was shaken vigorously. Saponins were identified by a continuous, identifiable froth that looked like honeycomb.
Borntragor's Test: The ethanol extract was mixed with 1 milliliter of C_6H_6 and 0.5 milliliter of diluted NH_4OH solution. This produced a reddish-pink hue, which indicated the presence of glycoside.
Phenolic compound test: Two milliliters of $FeCl_3$ solution were added to the sample extract after it had been heated in water. After that, the mixture was left to be observed to see if any green or blue hue developed, which would suggest the presence of phenolic chemicals.
Salkoweski reaction: 5 mg of extract, 2 ml of $CHCl_3$, and 2 ml of concentrated H_2So_4 were introduced from the side of the test tube. The test tube was shaken for a few minutes. Redness was an indication that steroids were present.
Lead acetate: The test filtrate was mixed with a 10% w/v solution of basic $PbS0_4$ in distilled water. Precipitate formation suggested the presence of tannins.
Potassium dichromate: The filtrate was put to a solution of $K_2Cr_2O_7$ for this test. The presence of tannins was indicated by the black tint.
High-Performance Thin Layer Chromatography One technique for determining and separating the various chemical components present in a test sample is chromatography. The stationary phase, mobile phase, and visualization phase of the chromatography process were all finished. Phase in station: 10 x 20 cm, silica gel 60 F_{254}. $C_6H_5CH_3$ (12 ml): $C_4H_8O_2$ (8 ml) is the mobile phase. R_f Value: The difference in the travel distances from the origin line of the solvent and solute. Visualized under 366, 540, and 254 nm. Derivatization Anisaldehyde sulphuric acid are used.[11-15]

Oral Acute Toxicity Study

An oral acute toxicity study was done according to OECD Guideline 423 at dose levels of (Group 1) 50, (Group 2) 300, and (Group 3) 2000 mg/kg. Each dose level contained 3 female Wistar rats and observed behavioral, hematological, Protein, Albumin, Globulin, Glucose, Urea nitrogen, Creatinine, Bilirubin, Cholesterol.[16]

Repeated Dose 28-day Oral Toxicity Study

Repeated Dose for 28-day oral toxicity study was done according to OECD Guideline 407 at a fixed dose of 1000 mg/kg. The test formulation group and another normal control group contained the same number of animals and observed behavioural, haematological, Protein, Albumin, Globulin, Glucose, Urea nitrogen, Creatinine, Bilirubin, Cholesterol and histopathological changes in the liver, kidneys, brain, and heart.[17]

RESULT AND DISCUSSION

This study was done to evaluate quality and safety of developed poly herbal formulation**.** The physiochemical tests revealed values for loss on drying, aqueous extractive value, alcoholic extract value, total ash, acid insoluble ash, and water-soluble ash.
The sample's ability to retain water was demonstrated by the loss-on-drying test, which revealed a moisture content of 8.95%. Important components were present, as evidenced by the extractive values of 98.65% for the water-soluble extract and 56.65% for the alcoholic-soluble extract, which are directly related to the drug's strength or potency. The amount of inorganic and earthy stuff measured by the ash value was 1.65%, with the water-soluble ash being 0.12% and the acid-insoluble substance being 1.19%. These numbers show that the sample contains earthy materials and inorganic salts. Primary metabolites (carbohydrates, proteins, fats, etc.) and secondary metabolites (alkaloids, glycosides, tannins, etc.)was distinguished by using the qualitative phytochemical test. The Molisch test for the aqueous extract of *poly herbal formulation* is positive, indicating the presence of mono-, dis-, and polysaccharides. The sample's Aqueous and ethanolic extract yielded a positive test result in Benedict test, indicating the incidence of sugars with a free aldehyde or ketone group. Fehling test was positive which indicate that reducing sugar was present in aqueous extract. The aqueous and alcoholic extract was found to contain alkaloids as a consequence of both the Dragondroff and Wagner tests yielding positive results. Since both extracts include amino acids, the ninhydrine test yields a positive result. Additionally, proteins were present, as evidenced by positive results from the xenthoprotic test in the ethanolic extract and the Biuret and Millon assays in the aqueous extract. The aqueous extract of the material passed the foam test, indicating the presence of saponine. Both the sample's alcoholic and aqueous extracts passed the borntrager's test, indicating the presence of glycosides. Both the sample's alcoholic and aqueous extracts passed the phenolic test, indicating the presence of phenolic chemicals. Tannin was present in the test sample, as indicated by the positive results of the lead acetate, $FeCl_3$ and Potassium dichromate tests. Seven chemical constituents were visible in T.L.C. at a wavelength of 254 nm; the corresponding relative frequencies (R_f values) were 0.005, 0.048, 0.071, 0.232, 0.397, 0.577 and 0.781. five chemical componentswere seen at a wavelength of

366 nm, and the Rf value was found to be 0.008, 0.050, 0.287, 0.397, 0.705. Seven chemical constituents were observed after derivatization and visualization at 540 nm wavelength. The R_f values of these constituents were determined to be 0.008, 0.050, 0.113, 0.192, 0.303, 0.518, 0.905 (Table 1, 2 & 3).

Oral Acute Toxicity Study

In Oral Acute Toxicity that was made according to OECD 423 Guideline at dose level of 50, 300, 2000 mg per kg the result was found that no any behavioral abnormality like eye, skin, fur, sleep, mucous membrane, are normal and lethargy, coma, salivation, convulsions, tremors, diarrhea, mortality, morbidity was not present and changes in food water intake, weight heamotology and biochemical observation were in normal range in each dose level 50, 300, 2000 mg/kg during observation till 14 day. It indicates that developed test polyherbal formulation found safe in single doe up to 2000 mg/kg (Table 4, 5 & 6).

28 days repeated Dose Toxicity Study

The impact of a polyherbal formulation on Liver, Kidney, Heart and Brain induced histological alterations of experimental rats is depicted in Figure 1. In the Repeated dose toxicity study which was done according to OECD 407 guideline at fixed dose level 1000 mg/kg and compare with normal control was found that no any behavioral abnormality like eye, mucous membrane, skin, fur, sleep are normal and coma, convulsions, salivation, lethargy, tremors, diarrhea, mortality, morbidity was absent and changes in food water intake, weight, heamotology and biochemical observation and no any artitrectrual changes was observed in cellular structure of Liver, Kidney, Brain, Heart. It Indicate that developed formulation was safe in repeated dose up to 1000 mg/kg (Table 7, 8, 9 & 10), (Figure 1).

CONCLUSION

The polyherbal formulation sheds light on its physiochemical properties, phytochemical composition, safety profile, and potential effects on physiological parameters. These findings are crucial for understanding the quality control and toxicological aspects of the polyherbal formulation, which was found safe at a dose of 1000 mg per kg in a single administration and safe in a 28-day repeated dose up to a dose of 1000 mg per kg.

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