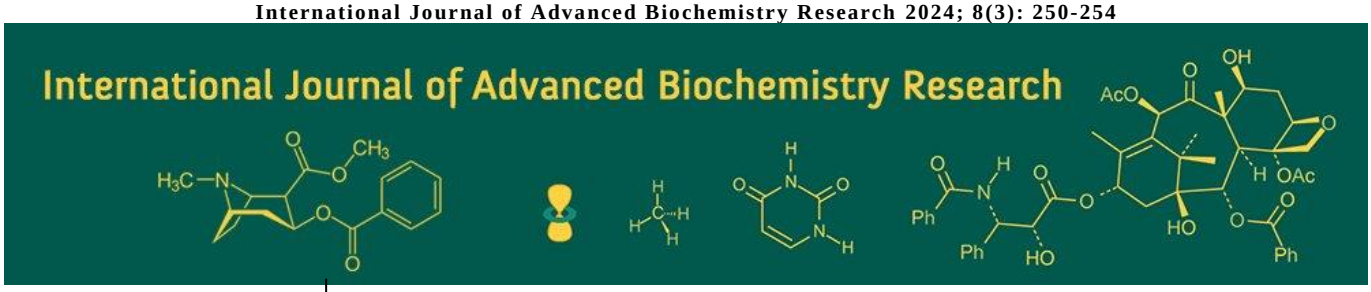

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Diabetic neuropathy protective action of polyherbal preparation

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Abstract
Background: The current investigation was planned based on the aforementioned details. The aim of the study was to enhance the screening procedure and evaluate the efficacy of specific plants as antidiabetic and reduces the diabetes originated microvascular complications like neuropathy. Once the most effective technique has been identified, the parameters used to validate the models will be used to scientifically establish the antidiabetic effect(s).The research was conducted to create an anti-diabetic polyherbal formulation from an ethanol extract of *Azadirachta indica* leaves, *Cinnamomum zeylanicum* bark, *Terminalia bellirica* fruits, and *Withania somnifera* roots.
Method: For determining the Polyherbal preparation as normoglycemic and reduces the diabetes originated neuropathy the experimental model was designed, dose determination acquired by acute toxicity method of OECD guidelines. Dose for the test group is 100 mg/Kg, 200 mg/Kg and 400 mg/Kg and compared with Glibenclamide 10 mg/Kg as standard group.
Results: Our finding explains that the polyherbal extract at different doses shows hypoglycemic. The result obtains in the form of data showed that the preparation treats neuropathy acquired due to diabetes complications. Based on these findings, it is possible that this polyherbal extract could serve as the basis for the development of a novel Antidiabetic medication.
Conclusion: Biochemical and microscopic examinations of tissue sections provide evidence to the polyherbal formulation's stated therapeutic effects. *In-vitro* antioxidant activities and *In-vivo* efficacy of the combination proves that it provides better option to treat diabetes and its micro and macro complications.

Keywords: Polyherbal formulation, antioxidant activity, antidiabetic activity, neuropathy

1. Introduction
Due to the increase of industrialization and an unexpected rise in obesity, diabetes is now a global issue. Recent studies indicate that the adult prevalence of diabetes will increase from 4% in 1995 to 6.4% in 2025 (King *et al*., 1998) [7]. There is no adequate, viable treatment for diabetes mellitus available in modern medicine. The neuropathy, associated with DM (Diabetes Mellitus) is a condition that is progressive and silent as it shows any symptoms or pathological severity during its progress. Often, they are categorized as symmetrical neuropathy and asymmetrical neuropathy (Dyck & Saunders, 1999) [2]. When the patients are undergoing neurological examinations in the clinics they are often diagnosed with diabetes or by impaired glucose tolerance which is mostly undetected previously (Singleton *et al*., 2005) [11]. Diabetic sensorimotor polyneuropathy is a type of neuropathy that often leads to pathophysiological complications like foot ulceration (Perkins & Bril, 2003) [9]
As a consequence of the adverse effects associated with the use of insulin and oral hypoglycemic drugs, patients are demanding more natural anti-diabetic remedies. There are a variety of traditional medicinal plants with hypoglycemic properties, such as *Azadirachta indica* (Dholi *et al*., 2011) [1], *Cinnamomum zeylanicum* (Udell *et al*., 2021; Verspohl *et al*., 2005) [12, 13], *Terminalia bellirica* (Gupta *et al*., 2020; Kadian *et al*., 2014) [5, 6] and *Withania somnifera* (Sarangi *et al*., 2013) [10].

2. Material and Methods
2.1 Collection of Plant material
The local market was surveyed for leaves of *Azadirachta indica*, barks of *Cinnamomum zeylanicum*, preserved fruits of *Terminalia bellirica*, and roots of *Withania somnifera*. Dr.

Naveen Kumar, head of the Christ Church College Botany Department, authenticated these commercially available raw PHP constituents. (04/2021/CCK-06)

2.2 Preparation of Extract

Before shade dry for 10 to 15 days, all of the plant materials used to produce Polyherbal powder was adequately and separately washed with water then obtained plant materials that were completely desiccated. The desiccated leaves of *Azadirachta indica*, the bark of *Cinnamomum zeylanicum*, the fruits of *Terminalia bellirica*, and the roots of *Withania somnifera* were separately subjected to fine grinding in an electric grinder. To produce the polyherbal powder, fine powders of each plant were obtained and combined in accordance with a predetermined ratio. The ratio of the polyherbal powder's basic materials is tabulated in Table No. 1.

The phytoconstituents isolation technique is based on the solvent used for the extraction technique. Normally, the selection of the solvent is done on the basis the order of acetone, petroleum ether, ethyl acetate, chloroform, methanol, ethanol, and water in terms of their relative polarities. After estimation of Percentage yield it concluded that the concentrated ethanol extract was used for its pharmacological investigation as an Antioxidant in Diabetic Neuropathy.

2.3. *In-vitro* Hydrogen peroxide (H2O2) scavenging assay (Standard used: Ascorbic acid)

1.0 ml plant extract (10- 320 µg/ml) + 2.4 ml of 0.1 M phosphate buffer solution (pH 7.4)+ 0.6 ml H2O2 solution (40 mM). It was then shaken vigorously; incubated at room temperature for 10 min. Absorbance determined at 230 nm. H2O2 scavenging activity calculated as inhibition percentage of H2O2 radical by sample and standard.

2.4. *In-Vivo* Activity

2.4.1. Animals

Wistar rats as a species and a strain gender were males and females both; Weight range from 180-240 gm and the test drug is Ethanol extract of Polyherbal Powder. Wistar rats that were in good condition were housed in a monitored environment with a constant temperature of 22±2 ^{0}C and a light/dark cycle of 12 hours each day. They received a normal pellet diet and free consumption of water. There were distinct accommodations for males and females**.** Females used were nulliparous and non-pregnant.

2.4.2. Diabetic neuropathy: Behavioral tests

A) Grip strength or Motor co-ordination activity using Rota rod apparatus:

It is used in this study for the evaluation of grip strength of the Wistar rats during the experiment. The apparatus is constructed such that it consists of a horizontal rod with either of wood or metal which is coated with rubber. This rod with dimensions; 3 cm in diameter is attached to a motor with adjusted speed of 25 rpm. Length of rod is 23cm and the rod is thus divided into three sections as that allows the simultaneous experimentation of three rats at a time. There is a provision of cage below that serves for the restriction of the experimental rats after falling from the roller. Selection of the experimental rats for the grip strength activity is done on the basis of the experimental rats being on the revolving rod for least one minute and thus is selected for the study. Oral administration of the PHP was done and each week selected experimental Wistar rats were placed on the rod of Rota rod Apparatus. The fall-off time from the rod was recorded in seconds of the rats. (Eltokhi *et al*., 2021) [3]

B) Hyperalgesia

By using Eddy's Hot Plate: Evaluation of sensory function is done by the pain threshold. Eddy's *et al* had described the procedure of Hot Plate Test which was carried out for the experimentation. Selected experimental Wistar rats were placed on the hot plate with temperature at 55±1 ^{0}C and the reaction time i.e., the time taken for paw licking was recorded as response latency. Prior and post treatment response latencies were measured and recorded. Hot plate latency provided the cut-off time 10 sec (Fan *et al*., 2014) [4]

2.4.3. Statistical Analysis

Statisticians utilized Graph Pad Prism 9. Statisticians employed one-way ANOVA and Descriptive analysis. Values represented the mean SD.

3. Result and Discussion

3.1 Scavenging of Hydrogen Peroxide: Hydrogen peroxide, although not a radical species play a role to contribute oxidative stress. The generation of even low levels of H_2O_2 in biological systems may be important. Naturally-occurring iron complexes inside the cell believed to react with H_2O_2 in vivo to generate highly reactive hydroxyl radicals and this may be the origin of many of its toxic effects (Miller *et al*., 2000) [8]. Scavenging of hydrogen peroxide of polyherbal extracts is presented in Table No.2 and Figure No. 1. The percentage of H_2O_2 scavenging activity of Polyherbal extract was found to be 74.16±0.03 % which is at 50µg/ml compared to antioxidant activity of standard ascorbic acid was 85.23±0.04 % at the same concentration. Grip strength: The results are mentioned in Table No. 3 and Figure No. 2 Grip strength and neuromuscular coordination result using Rota rod of Group V and Group VI improved at the end of the experiment. When results were compared with the responses from the Diabetes control group (Group II) of experimentation on 12th week the results were quite significant. Neuromuscular coordination test (motor coordination) result or rota rod test results are recorded an improvement of 85.83±1.941 and 101.50±1.05 in Group V and Group VI animals.

3.2 Thermal Hyperalgesia: Result of Eddy's hot plate method is mentioned in Table No. 4 and Figure No. 3. Treatment Group V and VI have shown a mean reaction latency of 5.017±0.033 and 4.992±0.059 (sec) in response on week 12. The results are significant when compared with the results of Group II (Diabetic control) from week 12.

Table 1: Constituents of Polyherbal Powder

S. No.	Name of raw material of PHP	Weight (in grams)
1	*Azadirachta indica*(leaves)	25
2	*Cinnamomum zeylanicum*(bark)	25
3	*Terminalia bellirica*(fruits)	25
4	*Withania somnifera*(roots)	25

Table 2: H_2O_2 Scavenging Activity of PHP (test) and Ascorbic Acid (standard)

S.No.	Conc.(µg/ml)	% inhibition of Ascorbic Acid	% inhibition of PHP
1.	10	43.26± 0.21	23.04± 0.05
2.	20	54.06± 0.08	37.61± 0.04
3.	30	69.23± 0.09	50.92± 0.04
4.	40	79.06± 0.04	62.18± 0.05
5.	50	85.23± 0.04	74.16±0.03

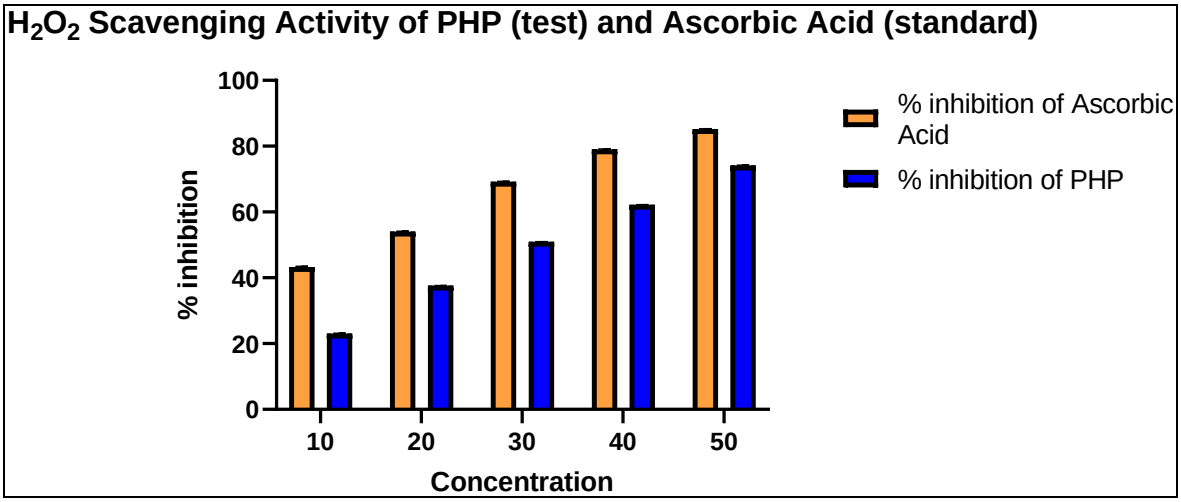

Fig 1: H_2O_2 Scavenging Activity of PHP and Ascorbic Acid

Table 3: Grip strength or Motor co-ordination activity using Rota rod apparatus

S.No.	Treatment type	Rota rod Test				
	Group type	Counts 0 Week	Counts 2nd Week	Counts 4th Week	Counts 8th Week	Counts 12th Week
1.	Normal control	123.8±1.472	125±1.414	124±1.378	125±1.095	126.3±2.582
2.	Diabetic control	124±2.449	49.83±2.927	28.17±4.309	19.33±1.366	8.33±0.816
3.	Standard	123±1.414	62.67±5.428	84.17±5.636	101.2±1.472	102.2±1.835
4.	Test 1	122.8±1.169	50.33±4.68	67.5±1.517	76.67±1.211	84.17±1.169
5.	Test 2	123.2±1.722	52.83±5.419	71±3.521	79.5±1.975	85.83±1.941
6.	Test 3	123.3±1.633	71±3.521	79.5±1.975	85.83±1.941	101.50±1.05

ANOVA summary	
F	0.1390
P value	0.9664
P value summary	ns
Significant diff. among means (P < 0.05)?	No
R squared	0.01947

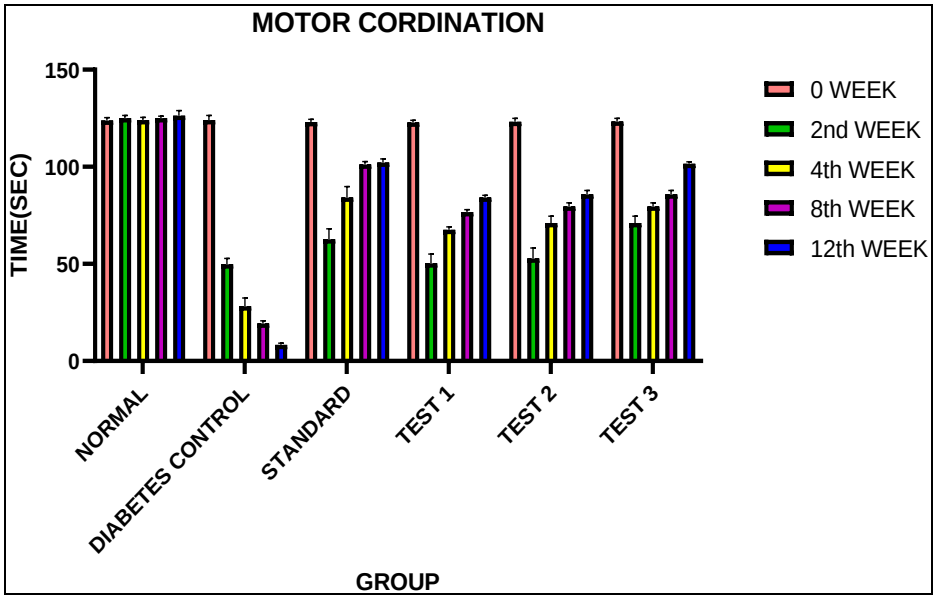

Fig 2: Grip strength or Motor co-ordination activity using Rota rod apparatus

Table 4: Thermal Hyperalgesia-Eddy's Hot plate method

S.No.	Treatment type	Thermal Hyperalgesia-Eddy's Hot plate method Response Time (s)				
	Group type	Reaction Latency 0 Week	Reaction Latency 2nd Week	Reaction Latency 4th Week	Reaction Latency 8th Week	Reaction Latency 12th Week
1.	Normal control	4.083±0.075	4.067±0.121	4.15±0.1049	4.033±0.136	4.217±0.2137
2.	Diabetic control	4.167±0.121	6.868±0.104	7.6±0.1095	8.692±0.185	9.575±0.209
3.	Standard	4.13±0.136	6.113±0.012	5.668±0.047	5.017±0.008	5.145±0.021
4.	Test 1	4.15±0.137	6.023±0.012	5.568±0.044	5.207±0.066	5.033±0.036
5.	Test 2	4.217±0.231	6.028±0.011	5.572±0.045	5.145±0.125	5.017±0.033
6.	Test 3	4.067±0.136	6.022±0.023	5.487±0.146	5.093±0.101	4.992±0.059

F	1.766
P value	0.1673
P value summary	ns
Significant diff. among means (P < 0.05)?	No
R squared	0.2203

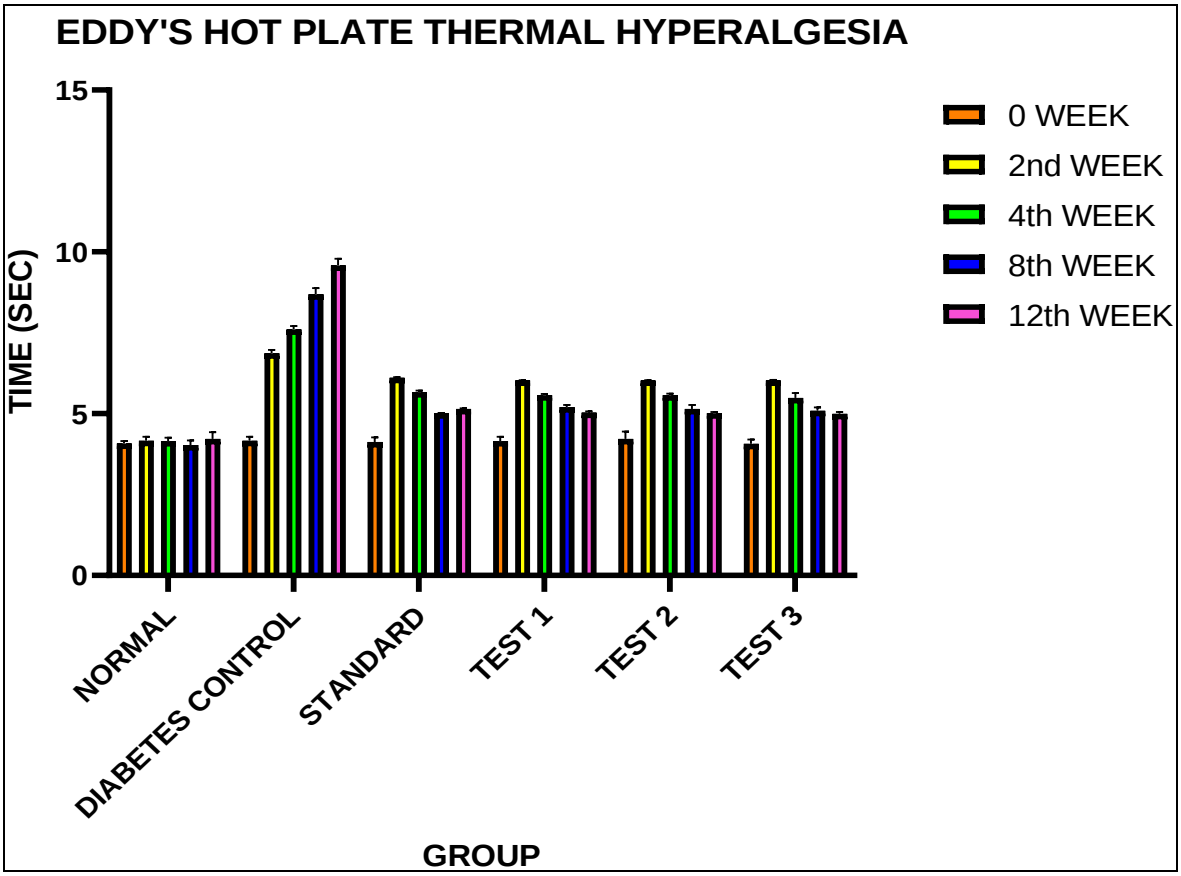

Fig 3: Thermal Hyperalgesia-Eddy's Hot plate method

4. Abbreviations

- DM: Diabetes mellitus
- gm (Grammes)
- mg (Milligrams)
- ml (milliliter)

5. Conclusion

In summary, the study involved the collection and authentication of plant materials, followed by the preparation of a polyherbal powder extract using a predetermined ratio of constituents. The extraction technique utilized concentrated ethanol extract for pharmacological investigation as an antioxidant in diabetic neuropathy. In-vitro assays revealed significant scavenging activity of hydrogen peroxide by the polyherbal extract compared to the standard ascorbic acid. In-vivo experiments conducted on Wistar rats showed improvements in grip strength and neuromuscular coordination, as well as alleviation of thermal hyperalgesia in response to the polyherbal extract treatment. Statistical analysis supported these findings, indicating the potential therapeutic benefits of the extract in managing diabetic neuropathy. Further research is warranted to explore its efficacy and safety for clinical applications.

6. Conflict of Interest: The authors report no financial or other conflicts of interest.

7. Consent for publication

Not applicable.

8. Acknowledgments

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