Pharmacy Formulas. 2022. Vol. 4, no. 2. P. 10-19
PHARMACEUTICAL SCIENCES
Experimental article
DOI: https://doi.org/10.17816/phf112225

Evaluation of the effect of *Cassia fistula* L. extracts on the muscle contraction intensity using an *ex vivo* model

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ABSTRACT. *Cassia fistula* L. is a *Fabaceae* representative that has long been used in traditional medicine. The purpose of this study is to evaluate the effect of *Cassia fistula* L. herbal extracts in the form of solutions of a certain concentration on the smooth muscles of the intestine of the animal. The design of the experiment involved the identification of raw materials, their drying, grinding, extraction of soluble fractions, purification of aqueous, ethereal (diethyl ether) and ethanol extracts and their testing on the tissues of the ileum of the domestic chicken (*Gallus gallus domesticus* L.) extracted *ex vivo*.

The ethereal, alcoholic and aqueous extracts of *Cassia fistula* L. fruits showed to exhibit high relaxation activity compared to the control relaxation stimulants, whereas the leaf extracts showed a more modest relaxing activity. A similar situation was observed in testing extracts of young shoots, with aqueous extracts showing even more modest results, while alcohol and ethanol extracts of young shoots performed better than the corresponding leaf extracts, and the most modest results in terms of a dose sufficient for a physiological response was demonstrated by root extracts.

The initial assessment of the activity of *Cassia fistula* L. extracts makes it possible to identify as the most promising for further chemical study the pools of substances concentrated in the ethanol fruits extract exhibiting the minimum effective dose, in the ether extracts of fruits and bark demonstrating the shortest reaction time, and in the aqueous extracts of young shoots and cortex showing the highest percentage increase in the activity compared to the control.

KEYWORDS: *Cassia fistula* L.; biologically active substances; medicines; plant raw material; aqueous extracts of the plant; ileum of domestic chicken; traditional medicine; chamber for isolated tissues

ABBREVIATIONS:

GABA – gamma-aminobutyric acid; ITB – isolated tissue bath; Collad. – Louis Théodore Frederic Colladon, 1792–1862; DC. – Augustin Pyramus de Candolle, 1778–1841; G. Don – George Don, 1798–1856; Kunth – Carl Sigismund Kunth, 1788–1850; L. – Carl Linnaeus, 1707–1778; Pers. – Christiaan Hendrik Persoon, 1761–1836; Roxb. –William Roxburgh, 1751–1815; Willd. – Carl Ludwig von Willdenow, 1765–1812; BAS — biologically active substances.

INTRODUCTION

Cassia fistula L. is a dicotyledonous plant belonging to the Fabaceae family that [1], has long been used in traditional medicine [2–7]. This plant has been known for a long time, as a result of which many synonyms have accumulated in the botanical literature (*Bactyrilobium fistula* Willd., *Cassia bonplandiana* DC., *C. excelsa* Kunth, *C. fistuloides* Collad., *C. rhombifolia* Roxb., *Cathartocarpus excelsus* G. Don, *C. fistula* Pers., *C. fistuloides* (Collad.) G. Don, *C. rhombifolius,* G. Don).

This species is native to India but is also cultivated in many tropical and subtropical countries. It grows under warm and dry climates, on well-drained soils, and distributed throughout subtropical deciduous forests. The tree originates from India, but is distributed in many tropical and subtropical areas such as Andaman Islands, Angola, Antigua Barbuda, Argentina, Australia, Bangladesh, Barbados, Belize, Bhutan, Brazil, Brunei, Cambodia, Cayman Islands, China, Colombia, Costa Rica, Cuba, Dominican Republic, East Timor, El Salvador, Ethiopia, Fiji, French Guiana, Grenada, Guadeloupe, Guatemala, Guyana, Haiti, Hawaii, India, Indonesia, Iran, Iraq, Java, Kalimantan, Kenya, Laccadive Islands, Laos, Lesser Sunda Island, Malawi, Malaysia, Malaysia, Maldives, Martinique, Mexico, Moluccas, Myanmar, Nepal, Nicaragua, Niue, Pakistan, Panama, Papua New Guinea, Peru, Philippines, Puerto Rico, Rodriguez, Ryukyu Is, Sev. Marianas, Seychelles, Singapore, South Africa, Sri Lanka, St. Lucia, St. Vincent, Sulawesi, Sumatra, Suriname, Taiwan, Tanzania, Thailand, Tonga, Uganda, United States, Venezuela, Vietnam, Zimbabwe [8].

Cassia fistula L. is a medium-sized tree up to 15 m high, without thorns; its crown is sparse and small; leaves are alternate, paired pinnate, 15–40 cm long, with an axis of 10–30 cm, without glandules, with a bare petiole up to 10 cm long. The color of the leaves is dark-green with a shiny upper side and an opaque matte lower side. Flowering goes from spring to summer. Flowers with yellow petals, linear or caudate bracts, drooping, 3 to 5 cm in diameter with thin pedicels 6 cm long; form hanging axillary not very branched clusters from 20 to 40 cm long; each bunch bears from 15 to 60 flowers. The fruit is a cylindrical bean, hanging and hairless, 20–60 cm long and 2 cm wide, rounded at the ends, dark brown or black when ripe. Fruit ripening is slow, from December to March. Each legume may contain no less than 40 and no more than 100 seeds. Seeds are obovate or ellipsoidal, 7 to 10 mm long, 6 to 7 mm wide, smooth, reddish-brown, surrounded by sweetish mucus of dark color and dense consistency [9,10].

Medicinal products from flowers, fruits and seeds of this plant have a pronounced antifungal, antioxidant, antimicrobial, anti-inflammatory, antitumor, hepatoprotective, and hypoglycemic effect [11, 12]. Medicinal raw materials of *Cassia fistula* L. are used in traditional medicine for tumors of the intestines, endocrine system, throat, liver, burns, constipation, convulsions, diarrhea, dysuria, epilepsy, leprosy, skin and venereal diseases [13–15].

The purpose of the present study is to evaluate an effect of *Cassia fistula* L. raw material extracts in the form of solutions of a certain concentration on chicken ileum tissue in order to identify their physiological activity.

An experiment design meant identification of the plant raw material, its drying, grinding, extraction of soluble fractions, purification of aqueous, ethereal and ethanol extracts and their testing on the tissues of the ileum of domestic chicken extracted *ex vivo*.

Further, the reaction of the experimental tissue to the effect of extracts and control relaxation stimulants was determined.

For the present research, the material of *Gallus gallus* L. (Indian chicken, banking chicken, or domestic chicken, hereinafter referred to as "*Gallus gallus domesticus* L.") was selected.

Experimental studies were performed on animals preapproved by the Institutional Animal Ethics Committee (Ref: SGRS/IAEC/05-2017-2018).

In the course of the study, we obtained qualitative and quantitative results on the contraction of smooth muscles in the tissues of the ileum of the chicken, which allowed us to evaluate the effect of exposure to aqueous extracts of *Cassia fistula* L. materials and determine the prognosis of the pharmacological profile of the drug.

MATERIAL AND METHODS

Plant raw material

Plant raw material of *Cassia fistula* L. was collected in several districts of the Pune district (Tamini, Mulshi, Cantonment Board, Pashan, Chandani, Chowk, Tamini Ghat) in different seasons of the year. Identification was carried out at the Botany Department of the Agarkar Research Institute (ARI), Pune. Various parts of the plant were collected in separate plastic bags. The collection was carried out as follows: the bark was cut with a knife and a mallet; leaves of all sizes were separated from the stem; fruits were collected fully ripe and intact.

Drying of plant raw material

Drying of plant raw material was carried out first in a natural way in the air in the shade, and then in the sun. After this primary treatment, drying was carried out using an infrared lamp continuously for 7–8 days. The completely dried samples were then ground into powder using a mixer or a mortar and pestle.

Grinding of plant raw material

The particle size of the dried leaves was reduced first by manual grinding and then placed in a mortar and pestle to grind into a fine powder. The fruits were immediately crushed in a mortar. Before grinding into a fine powder, the seeds present in the fruits were separated (because no seed extracts were used in the experiment). The bark particle size was reduced with mortar and pestle. Dried young shoots were first crushed with a mortar and pestle, and then using a mixer.

The crushed powder of different parts of plants was packaged for storage in separate plastic containers with markings containing data on the place of collection and drying temperature.

Weighing samples for extraction

The extraction was carried out in flasks containing 250 ml of the extracting agent. The weight of crushed leaves

was 15 g, fruits – 50 g, bark – 40 g, young shoots – 27 g. Weights of the above samples were subsequently used for extraction.

Extraction

The extraction was performed using a Soxhlet extractor using various solvents (diethyl ether, ethanol and water) which were collected in a 250 ml beaker [16, 17].

Separation and purification

Ethanol, water, and diethyl ether extracts obtained from samples of various parts of *Cassia fistula* L. were purified by evaporating the solvents to dryness for 40–45 min on a water bath at the temperature of 100 °C to obtain purified powders. [17].

Experiments in the isolated tissue bath (ITB)

An *ex vivo* isolated preparation of chicken ileum has been used for a long time in India for testing biologically active substances. Its advantage is the absence of damage to the life of experimental animals [18]. This method reveals the responses provided by many different receptors to the effects of various extracts.

Tissue and organs placed in the ITB were oxygenated with carbogen and stored in Ringer's solution with lactic acid. The studied extracts and control specimens were injected directly into the chamber at a temperature of 37 °C. The contact time was maintained in accordance with the standard protocols. A five-minute time cycle was observed, i.e. 30 s baseline recording, 90 s contact (drug response) and subsequent 3 washes at 1 min intervals [19, 20].

ITB was equipped with its own sensor and precise positioning device for quick adjustment and measurement based on an analog-to-digital converter. Extraction of signals and recording of input data were carried out in real time. The obtained data were processed and analyzed. When analyzing the results, the dynamics of the indicators of the contractile activity of the intestinal segments was evaluated in comparison with the background values.

Based on the tissue responses to the extracts impact, the dose-response curves were built, which made it possible to primarily estimate the activity of the extracts and determine its pharmacological profile.

RESULTS AND DISCUSSION

Chicken ileum tissue was used to study neuromuscular (smooth muscle) stimulation by *Cassia fistula* L. extracts. Solutions of various extracts (volume 250 ml) and control relax-stimulators of neuromuscular contacts (caffeine, diazepam) were introduced into the ITB, and the increment in the length of the intestinal segment placed in the ITB was measured during induced relaxation of smooth muscles.

Neuromuscular tissue responses varied depending on the drug dose (Table 1) and exposure time (Fig. 1). At the

Table 1.

Administered doses of *Cassia fistula* L. extracts and control neuromuscular stimulators associated with physiological response

Табл. 1.

Введенные дозы экстрактов *Cassia fistula* L. и контрольных стимуляторов нервно-мышечных контактов, сопряженные с физиологическим ответом

Extracts origin	Substances, ml				
	Control		Ether extract	Ethanol extract	Aqueous extract
	Caffeine	Diazepam			
Fruits	167	88	141	28	160
	160	78	145	30	155
	155	75	142	25	165
	170	90	140	30	161
	171	85	142	31	162
	155	85	142	28	160
Leaves	167	88	59	115	232
	160	78	60	117	225
	155	75	61	115	230
	170	90	58	113	225
	171	85	59	116	230
	155	85	59	115	228
Young shoots	167	88	90	71	330
	160	78	102	65	300
	155	75	100	62	332
	170	90	92	68	328
	171	85	94	63	327
	155	85	98	70	325
Roots	167	88	81	254	315
	160	98	82	260	318
	155	75	80	277	320
	170	90	79	264	329
	171	85	77	270	324
	155	85	76	268	326

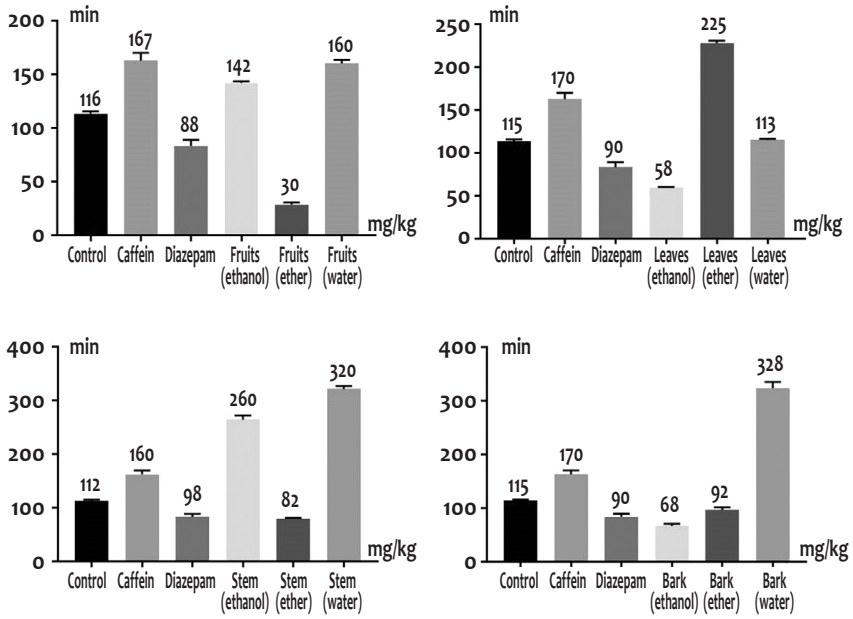

Fig. 1. Physiological response time corresponding to the optimal value of the effective dose of extracts
Рис. 1. Время физиологического ответа, соответствующее оптимальному значению эффективной дозы экстрактов

same time, extracts of raw materials obtained from various parts of the plant also demonstrated different biological activity. Ether, ethanol and aqueous extracts of the fruits showed a generally high relaxing activity in comparison with control relaxation stimulants – their dose sufficient for a physiological response varied from 28 ml (ethanol extract) to 165 ml (aqueous extract), while the minimum for a physiological response dose of caffeine was 155 ml, and diazepam – 75 ml. Leaves extracts exhibited a more modest relaxing activity compared to control stimulants (the effective dose range was 58–232 ml), and aqueous extracts were already inferior in activity to control stimulants (the dose varied from 225 to 232 ml). A similar situation was observed during testing of extracts of young shoots, with aqueous extracts showing even more modest results (effective dose ranged from 325–330 ml), while alcohol and ethanol extracts of young shoots performed better than the corresponding leaf extracts and limits. The effective dose variations are limited here to 62–102 ml. The most modest results in terms of a dose sufficient for a physiological response were demonstrated by root extracts: the effective dose of aqueous extracts varied within 315–329 ml, ethanol – 254–277 ml, and ethereal – 76–82 ml. The ethanol extract of *Cassia fistula* L. fruits (25–31 ml) showed the best result in relation to the effective dose.

The reaction of the experimental tissue to the action of extracts and control relaxation stimulants did not manifest itself immediately, but after a certain time (Fig. 1), usually within 1–4 hours. At the same time, the best results (i.e., the shortest reaction time) were again shown by the ether extracts of fruit and bark, under the influence of which a physiological response was obtained in less than 1 hour.

The ethanol extracts from fruit, leaves, shoots, and bark, although they had a lower effective dose (Table 1), showed rather significant exposure time, exceeding that demonstrated by the control drugs – caffeine and diazepam.

Perhaps, even more significant than effective dose and exposure time is the strength of the physiological response. The Figure 2 shows the protocol of the experiment, which makes it possible to evaluate the magnitude of elongation of the intestinal segment in response to the influence of both control relax stimulants and the tested extracts of *Cassia fistula* L.

The most significant responses, i. e. an increase in length by 20 mm, was recorded when the tissue was exposed to an aqueous fruit extract. The ether and ethanol extracts of the leaves, the ether extract of the fruits, the ether and ethanol extracts of the shoots also had a high activity.

In Table 2, the results of the study of the dose-effect relationship are presented for each type of extract in order of increasing force of the reaction of the experimental tissue. This allows us to identify the most promising extracts for further study: aqueous, ethanol and ether extracts of fruits and alcoholic and ether extracts of leaves.

One more parameter should be considered, namely the increase in the biological activity of extracts in comparison with the control (solvent without a pool of extracted substances). It allows one to evaluate the immobilizing ability of a pool of metabolites with any given solvent and should also be taken into account when selecting the most promising compositions for in-depth biochemical and biomedical research. These data are presented in Table 3 and Figure 3.

By this parameter, it is possible to distinguish aqueous extracts of young shoots and bark, which indirectly may indicate a noticeable content of hydrophilic glucans in this raw material as well as an ethanol extract of young shoots.

The control substance diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one) was selected by us as a muscle relaxant and GABA receptor agonist that promotes myocytes relaxation and brake signaling in autonomic nervous system. Caffeine (1,3,7-trimethyl-1H-purine-2,6(3H,7H)-dione), acting on the calcium channels

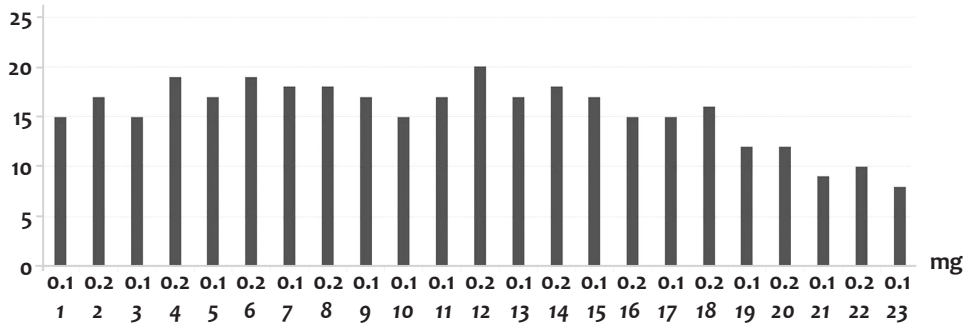

Fig. 2. Dependence of amplitude of muscle relaxation (mm) on the dose of control preparations and extracts of *Cassia fistula* L. (mg), namely, aqueous (abbreviation A in the legend), ethanol (EN) and ether (E) ones of various parts of the plant, namely, fruits (abbreviation F in the legend), leaves (L), shoots (S), bark (B): *1* – EL; *2* – EL; *3* – AL; *4* – ENL; *5* – ENL; *6* – EF; *7* – EF; *8* – ENF; *9* – ENF; *10* – AF; *11* – AF; *12* – ES; *13* – ES; *14* – ENS; *15* – ENS; *16* – AS; *17* – AS; *18* – EB; *19* – EB; *20* – AB; *21* – AB; *22* – ENB; *23* – ENB

Рис. 2. Зависимость величины мышечного расслабления (мм) от дозы контрольных препаратов и экстрактов *Cassia fistula* L. (mg) – водного (аббревиатура A в легенде), этанолового (EN) и эфирного (E) различных частей растения – плодов (аббревиатура F в легенде), листьев (L), побегов (S), коры (B): *1* – EL; *2* – EL; *3* – AL; *4* – ENL; *5* – ENL; *6* – EF; *7* – EF; *8* – ENF; *9* – ENF; *10* – AF; *11* – AF; *12* – ES; *13* – ES; *14* – ENS; *15* – ENS; *16* – AS; *17* – AS; *18* – EB; *19* – EB; *20* – AB; *21* – AB; *22* – ENB; *23* – ENB

Table 2.

Extracts of *Cassia fistula* L., grouped in order of increasing strength of the physiological response

Табл. 2.

Экстракты *Cassia fistula* L., сгруппированные в порядке возрастания силы физиологического ответа

Extract	Drug dose	Elongation at relaxation (mm)
Bark (ethanol)	0.1 mg	8
Bark (aqueous)	0.1 mg	9
Bark (aqueous)	0.2 mg	10
Bark (ethanol)	0.2 mg	10
Bark (ether)	0.1 mg	12
Bark (ether)	0.2 mg	12
Leaves (ether)	0.1 mg	15
Leaves (aqueous)	0.1 mg	15
Shoots (ethanol)	0.2 mg	15
Shoots (aqueous)	0.1 mg	15
Shoots (aqueous)	0.2 mg	16
Shoots (ether)	0.2 mg	17
Shoots (ethanol)	0.1 mg	17
Fruits (ethanol)	0.1 mg	17
Fruits (aqueous)	0.1 mg	17
Shoots (ether)	0.1 mg	17
Shoots (ethanol)	0.1 mg	17
Fruits (ether)	0.1 mg	18
Fruits (ether)	0.2 mg	18
Shoots (ether)	0.2 mg	18
Leaves (ether)	0.2 mg	19
Leaves (ethanol)	0.2 mg	19
Fruits (aqueous)	0.2 mg	20

Table 3.

Comparative activity of *Cassia fistula* L. extracts (% increase in activity compared to control)

Табл. 3.

Сравнительная активность экстрактов *Cassia fistula* L. (% прирашения активности в сравнении с контролем)

Extracts	%
Fruits (aqueous)	10
Leaves (aqueous)	15
Shoots (ethanol)	17
Bark (aqueous)	21
Shoots (aqueous)	21

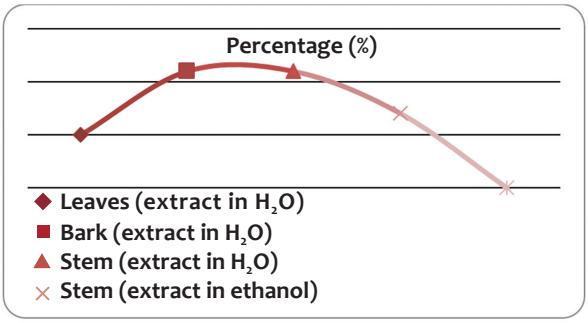

Fig. 3. Graph of the activity of extracts of *Cassia fistula* L. (% increase in activity compared to the control)

Рис. 3. График активности экстрактов *Cassia fistula* L. (% прирашения активности в сравнении с контролем)

of myocytes and endotheliocytes, also promotes muscle relaxation.

In our experiments, a pronounced muscle relaxant effect, and in some cases exceeding that of diazepam and caffeine, was found in extracts of *Cassia fistula* L. Until the extracted substances are fractionated and identified, we can limit ourselves to the following assumption.

It is known that extracts of *Cassia fistula* L. have a prominent proapoptotic activity towards malignant epithelia (cancer) rather than nervous or muscular tissues, and it has been experimentally shown that apoptosis triggered by these extracts proceeds along a mitochondria-dependent pathway [21, 22]. This suggests that the main targets of bioactive substances pools extracted from *Cassia fistula* L. can be growth factor receptors associated with cell survival reactions rater than receptors associated with the maintenance of the membrane potential. Many ramified glucans with their plastic structure often act as growth factor receptor antagonists, making them insensitive to paracrine signaling. To verify this assumption, a proper chemical study of the extracts of *Cassia fistula* L. is necessary.

The primary estimation of the activity of *Cassia fistula* L. extracts carried out here allows us to single out as the most promising for further study 1) ethanol extract of fruits, showing the minimum effective dose, 2) ether extracts of fruits and bark, showing the shortest reaction time, and 3) aqueous extracts of young shoots and cortex showing the highest percentage increase in activity in comparison with the control.

CONCLUSIONS

1. The dicotyledonous plant *Cassia fistula* L., widely used in traditional medicine, has recently attracted the attention of pharmacologists, and in the present work, in the course of experiments on muscle tissue, a primary assessment of aqueous, ethanol and ether extracts of this plant was carried out.

2. The ether, ethanol and aqueous extracts of *Cassia fistula* L. fruits showed generally high relaxation activity in comparison with the control relaxation stimulants, leaves extracts showed more modest relaxation activity, a similar situation was observed during testing extracts of young shoots, and aqueous extracts showed even more modest results, while the ether and ethanol extracts of young shoots performed better than the corresponding leaves extracts, and the root extracts showed the most modest results in terms of a dose sufficient for a physiological response.

3. The shortest time preceding the physiological response of the experimental tissue was shown by ether extracts of fruits and bark, under the influence of which the physiological response was obtained in less than 1 hour; ethanol extracts of fruits, leaves, shoots and bark show a rather significant exposure time, exceeding that demonstrated by the control drugs caffeine and diazepam.

4. The highest percentage increase in the activity compared to the control was given by the aqueous extracts of young shoots and bark, which indirectly may indicate a noticeable content of hydrophilic glucans in this raw material, and also by the ethanol extract of young shoots.

5. The primary estimation of the activity of *Cassia fistula* L. extracts allows us to identify as the most promising for further chemical study the pools of substances concentrated in the ethanol extract of fruits, which exhibits the minimum effective dose, in ether extracts of fruits and bark, which demonstrate the shortest reaction time, and in aqueous extracts of young shoots and bark, demonstrating the highest percentage increase in activity in comparison with the control.

6. The mechanism of action of *Cassia fistula* L. extracts will become clear after metabolomic profiling of the corresponding raw material, but taking into account the already discovered proapoptotic and antiproliferative activity of *Cassia* extracts, the active BAS of this plant should be considered as nonspecific muscle relaxants aimed at the growth and survival receptors rather than at the receptors of inhibitory mediators and membrane depolarization. A sufficient fraction of biologically active substances of *Cassia fistula* L. is likely to be composed of hydrophilic glucans and heteroglycans.

Acknowledgements

The authors are thankful to Principal Dr. Nitin Ghorpade, A.M. Mahavidyalaya, Hadapsar, Principal Dr. Chavan., SGRS College of Pharmacy, for their kind permission to conduct the experiments for this research work. First of all, we would like to thank the Head of the Department of Chemistry for his valuable guidance. Above all, thanks to Miss. Bhagyashree Vyapari and Miss. Prachali Chavan for their real assistance in the experimental work in the laboratory.

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The authors declare no conflicts of interests.

The article was submitted June 15, 2022; approved after reviewing June 29, 2022;
accepted for publication July 30, 2022.

Tanzania Journal of Science 47(3): 877-891, 2021
ISSN 0856-1761, e-ISSN 2507-7961

In Vivo Antidiabetic Activities of Polyherbal Extracts against Streptozotocin-Nicotinamide Induced Type 2 Diabetic Mice Model

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Received 9 May 2021, Revised 11 Jul 2021, Accepted 20 Jul 2021, Published Aug 2021
DOI: https://dx.doi.org/10.4314/tjs.v47i3.1

Abstract

Diabetes mellitus is a chronic metabolic disorder and rapidly increasing cases of diabetes throughout the world are major concerns in both developed and developing countries. Currently available oral antidiabetic medicines have limitations in efficacy and safety, which in conjunction with the emergence of diabetes mellitus as a global epidemic have aided the popularity of alternative therapies to manage the disease safely and more efficiently. Herbal medicines are accepted as alternative therapies for blood sugar control. *Gymnema sylvestre*, *Boerhavia diffusa*, *Tinospora cordifolia*, and *Argyreia nervosa* are some potent herbs used for the management of diabetes. Mixing of these plants in different ratios may produce synergistic antidiabetic actions which may have greater antidiabetic activity. Hence, the present study aimed to determine the anti-diabetic activities of polyherbal extracts in streptozotocin-nicotinamide induced type 2 diabetic mice. The administration of polyherbal extract orally at doses of 100, 200 and 400 mg/kg significantly decreased the blood glucose levels compared to the control diabetic mice. The polyherbal extract significantly lowered the elevated total cholesterol, triglycerides and low-density lipoprotein levels, while increased the high-density lipoprotein indicating antihyperlipidemic activity. The present study reveals that polyherbal extract at a dose of 400 mg/kg body weight resulted in a significant decline ($p < 0.001$) in blood glucose level.

Keywords: Polyherbal extracts, diabetes mellitus, Oral glucose tolerance test, Streptozotocin-Nicotinamide.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycaemia caused by reduced insulin secretion, decreased glucose utilization and increased glucose production (Undale et al. 2014, Mouri and Badireddy 2019). Glucose homeostasis is a balance between hepatic glucose production and peripheral glucose uptake and utilization. Insulin is the most important regulator of glucose homeostasis (Simon and Wittmann 2019, Yari et al. 2020). Diabetes mellitus is classified into general categories like type 1, type 2, gestational diabetes, among other specific types of diabetes. Type 1 diabetes is caused by B-cell destruction, usually leading to absolute insulin deficiency. Type 2 diabetes is a result of progressive insulin secretory defects in conjunction with insulin resistance. Gestational

diabetes mellitus (GDM) is a diabetes type usually diagnosed in the second or third trimester of pregnancy and is not clearly evident in diabetes. Other uncommon diabetes types include monogenic diabetes syndromes such as neonatal diabetes, maturity-onset diabetes of the young (MODY), diabetes resulting from diseases of the exocrine pancreas (such as cystic fibrosis), and drug or chemical induced diabetes such as in the treatment of HIV/AIDS or after organ transplantation (American Diabetes Association, https://www.diabetes.org/diabetes). Diabetes mellitus is a serious metabolic disease that has significant impacts on the health, quality of life and life expectancy of patients (Sancheti and Seo 2009). According to the reports of the World Health Organization, the rapid increase of diabetes in India is indicative of a potential epidemic with over 62 million individuals currently diagnosed and future projections indicating a rapid increase in numbers (Wild et al. 2004). In 2000, India had the highest number of people with diabetes mellitus (31.7 million) followed by China (20.8 million) and United States (17.7 million) in second and third places. The global prevalence of diabetes is predicted to double from 171 million in 2000 to 366 million in 2030 with India seeing the highest increase with numbers nearing 80 million (Wild et al. 2004).

There are various synthetic drugs used for the treatment of hyperglycaemia. The synthetic drugs have various side effects such as causing hyperglycaemia at higher doses, dermatological reactions, liver problems, nausea, vomiting, generalized hypersensitivity reactions, lactic acidosis and diarrhoea. These anti-diabetic drugs also cause weight gain which may further contribute to the progression of type 2 diabetes. The secondary complications arising from the use of synthetic drugs lead to limitations in their uses and potentially create issues in the management of diabetes (Chaudhuri and Sharma 2016). So, there is a need to develop safe and economic alternative treatments for diabetes mellitus. Therefore, there is considerable interest in the field of medicinal plants due to their natural origins and fewer side effects (El-Shafey et al. 2013). The World Health Organization also recommended and encouraged the practice of herbal medicines especially in countries where access to the conventional treatments of diabetes is inadequate.

The pharmacological activity of a single plant is less when compared to polyherbal formulations that contain combinations of various plants. Polyherbal formulations produce synergistic actions which are more potent and also diminish the concentrations of individual herbs, thereby reducing adverse effects (Shah et al. 2019).

In the present study, polyherbal formulations which consisted of extracts of *Gymnema sylvestre*, *Boerhavia diffusa*, *Tinospora cordifolia*, and *Argyreia nervosa* were used to determine hypoglycaemic activities. The herbs used in the formulations are reported in traditional medicines to treat diabetes mellitus and their active phytoconstituents like gymnemic acids, gymnemosides a, b, c, d, e, and f and gymnemosaponins (Kanetkar et al. 2007, Mishra et al. 2014), punarnavine-1, β-sitosterol (Sharma et al. 2019), tinosporin (Galani et al. 2010), quercetin and kaempferol (Gosh 1984) etc. Their details are given in Table 1. Therefore, in the present investigation, the polyherbal formulations were evaluated for hypoglycaemic activity in streptozotocin-nicotinamide (STZ-NIC) induced diabetes in mice.

Table 1: Phytoconstituents of polyherbs used for polyherbal extract preparation

Sr.No.	Plant name	Phytochemical constituents
1	*Gymnema sylvestre* R. Br.	Gymnemic acid I-IV, Gymnemasaponins, Gymnemosides a, b, c, d, e, and f, Kaempferol 3-O-β-D-glucopyranosyl-(1-4)-α-L-rhamnopyranosyl-(1-6)-β-D-galactopyranoside, Stigmasterol, Conduritol A and Quercitol (Kanetkar et al. 2007)
2	*Boerhavia diffusa* L.	Punarnavine-1 & 2, Boeravinone A-I, 9-O-Methyl-10-hydroxy coccineone E, 10-dimethyl boeravinone C, Coccineone E, β-Sitosterol β-Sitosterol-D-glucoside, β-Sitosteryl oleate, Sitosteryl palmitate, Liriodendrin, Stringarsionol and Mono-D-glucoside and β-D-glucoside (Mishra et al. 2014)
3	*Tinospora cordifolia* (Thunb.) Miers	Tinosporide, Furanolactone diterpene, Furanolactone clerodane diterpene, furanoid diterpene, Tinosporaside, Giloinsterol, ß-Sitosterol, 20a- Hydroxy ecdysone, Giloin, Tinosporan acetate, Tinosporal acetate, Tinosporidine, Heptacosanol, Octacosanol, sinapic acid, Tinosponone, and 2- phytoecdysones (Sharma et al. 2019)
4	*Argyreia nervosa* (Burm.f.) Bojer	1-Triacontanol, Epifriedelinol Acetate, Epifriedelinol and β-Sitosterol, N-Triacontanol, B-Sitosterol, P-Hydroxycinnamoyl Octadecanolate, Caffeic acid, p-Hydroxycinnamate, Hexadecanyl p-hydroxycinnamate, L-Ester coumarin and Tetradecanyl palmitate (Galani et al. 2010, Gosh 1984)

Materials and Methods

Plant materials

The leaves of *Gymnema sylvestre* (Asclepiadaceae), whole plant of *Boerhavia diffusa* (Nyctaginaceae), stem and leaves of *Tinospora cordifolia* (Menispermaceae**)** and whole plant of *Argyreia nervosa* (Convolvulaceae) were collected from local regions around Purandar town in Maharashtra and Pune University campus. Freshly collected plants were pressed in a herbarium press and dried plant specimens mounted on herbarium sheet. All the information provided and herbaria were submitted to the Botanical Survey of India (BSI) Herbarium in Pune for authentication; the corresponding voucher numbers are VP-11, VP-10, VP-9 and VP-7. The plant parts were washed with distilled water to remove dirt and soil, and then shade dried.

Preparation of extracts

The selected parts of plants were washed and dried under shade for 20 days. The dried plants were pulverized using a mechanical grinder and powdered for further studies and extracted with 80% absolute ethanol using Soxhlet apparatus for 6 hours. The extracts were evaporated to dryness (resinous material) under reduced pressure at 60 °C and stored at 4 °C until use. The polyherbal formulation was developed by combining the dried extracts of the plant extracts (Mandlik 2008, Maurya et al. 2011).

Preparation of polyherbal mixture

Polyherbal mixture was prepared by mixing individually extracted powders of plants *Gymnema sylvestre*, *Boerhavia diffusa*, *Tinospora cordifolia*, and *Argyreia nervosa*. The concentration of each plant in the plant polyherbal extract mixture was decided according to their potent antidiabetic activity at that concentration. *Gymnema sylvestre* shows potent antidiabetic activity at a dose of 400 mg/kg (Aralelimath and Bhise 2012), and the average weight of the experimental animal is 27 g, so, 10.8 mg of *Gymnema sylvestre* was used in the polyherbal mixture. *Boerhavia diffusa* shows potent antidiabetic activity at a dose of 200 mg/kg (Malhotra et al. 2014), so 5.4 mg of *Gymnema sylvestre* was used in the polyherbal mixture. *Tinospora cordifolia* shows potent antidiabetic activity at a dose of 400 mg/kg (Puranik et al. 2010), so 10.8 mg of *Gymnema sylvestre* was used in the polyherbal mixture. *Argyreia nervosa* shows potent antidiabetic activity at a dose of 500 mg/kg (Kumar and Alagawadi 2010), so 13.5 mg of *Gymnema sylvestre* was used in the polyherbal mixture. All these powders were mixed well to make polyherbal mixtures, and this prepared polyherbal mixture was administered to an animal with water at different concentrations (100, 200 and 400 mg/kg).

Chemicals and reagents

Streptozotocin, nicotinamide and glucose were obtained from Sigma. Other chemicals and reagents used in the study were of analytical grade.

Determination of in vivo antidiabetic activity

Animals: Swiss albino mice bred in the animal house facility of PDEA's SGRS College of Pharmacy were used. The animals were maintained under controlled temperature, humidity and light cycle as prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Standard animal pellet diet and water were provided *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) (SGRS/ IAEC/ 07/2018-19) and experiments were conducted according to the guidelines of the CPCSEA.

Experimental design

Acute oral toxicity tests of the polyherbal extract

An acute oral toxicity study was carried out according to OECD guidelines 423. In this method pre-specific fixed doses of the test substances were used, i.e., 5 mg/kg, 50 mg/kg, 300 mg/kg, 2000 mg/kg, 5000 mg/kg and the mortality rates due to these doses were observed. Generally, the female was used for this study and each dose Group consisted of 3 animals.

Effect of polyherbal formulation on oral glucose tolerance test (OGTT)

The oral glucose tolerance test was performed in overnight fasted (12-14 hrs) mice. The mice were divided into six Groups (n = 6).

Group I: served as normal control mice, administered drinking water daily.

Group II: had glucose control mice.

Group III: mice were administered standard drug metformin (250 mg/kg).

(The dose of metformin administered to mice in this study was calculated according to a clinically relevant human dose based on body surface area. Metformin 250 mg/kg)

Group IV: mice were administered polyherbal extract of 100 mg/kg.

Group V: mice were administered polyherbal extract of 200 mg/kg.

Group VI: mice were administered polyherbal extract of 400 mg/kg.

Glucose (2 g/kg) was fed to mice of groups II to VI, 30 minutes before the administration of the extracts and standard drug. Blood was withdrawn from the retro-orbital sinus after 0, 30, 60, 90 and 120 minutes of extract and standard drug administration, and the plasma obtained after centrifugation at 3000 rpm was estimated for fasting plasma glucose levels glucometer (Turner 1965, Gosh 1984, Zhang et al. 2002, Chaudhuri and Sharma 2016).

Induction of diabetes mellitus (non-insulin dependent diabetes mellitus)

In overnight fasted mice, streptozotocin (STZ) was injected (50 mg/kg in 0.1 mL citrate buffer pH 4.5 i.p.) 15 min after nicotinamide (NIC) injection (120 mg/kg in 0.1 mL normal saline) in all the groups except for Group I which was the non-diabetic control i.e., Normal control. Animals were fed with glucose solution (5%) for 12 hrs to avoid hypoglycaemia. Hyperglycaemia was confirmed after 3 days. Mice having serum glucose >250 mg/dl were selected for the study (Birgani et al. 2018).

Evaluation of antidiabetic activity

The animals were divided into six Groups (I-VI) of six mice each as mentioned previously and Groups III to VI were treated with test drugs. The fasting glucose levels were determined on days 0, 7, 14, 21 and 28 of extract administration. During the experimental period, the mice were weighed daily and the mean changes in body weights were calculated.

Estimation of biochemical parameters

On day 28, the animals were sacrificed by cervical dislocation to determine the biochemical parameters. triglycerides (TGL), low density lipoprotein (LDL), cholesterol and high-density lipoprotein (HDL) were determined (Rakieten et al. 1963, Nagja et al. 2017, Sheikh et al. 2015).

Statistical analysis

Results were analysed statistically using the One-Way ANOVA method followed by Bonferroni Test (multiple comparison test). The minimum level of significance was set at $p < 0.05$. The data are presented as the mean ± SEM.

Results

Acute oral toxicity according to OECD guidelines 423

In acute oral toxicity study, oral administration of the polyherbal extracts at the doses of 5 mg/kg, 50 mg/kg, 300 mg/kg, 2000 mg/kg, 5000 mg/kg body weight did not induce any changes in behaviour and no mortality was observed during the study. There was no significant difference in the body weight and food consumption when compared to the normal control group. On the 7^{th} day, macroscopic pathology observations revealed no visible lesions in any animals after sacrifice. The observed data suggested that the oral LD_{50} value of polyherbal extract is greater than 2000 mg/kg body weight. Therefore, polyherbal extract can be safely used as a dose up to 2000 mg/kg body weight for therapeutic use.

Selected doses: 100 mg/kg, 200 mg/kg, 400 mg/kg.

Effects of polyherbal extracts on body weight

The effects of the polyherbal extracts on the bodyweight of animals were checked, the results of which are presented in Table 2. The body weight measured in grams in Group II (Disease control) was observed to be 20.17 ± 0.5426 g on the 28^{th} day. Significantly ($p < 0.001$) decreased level of bodyweight in Group II (Disease control) was observed as compared to Group I (Normal control). In Groups III, IV, V and VI significant ($p < 0.001$) increase in body weight was observed as compared to Group II (Disease control). In Group VI, significant ($p < 0.01$) increase in body weight as compared to Group III (Standard control). All data were subjected to the One-Way ANOVA method followed by Bonferroni's multiple comparisons test and given in Figure 1.

Table 2: Effects of polyherbal extracts on body weight of STZ-NIC induced diabetic mice

S.No.	Treatment Group	Body weight (g)				
		0 Day	**7th Day**	**14th Day**	**21th Day**	**28th Day**
I	Normal Control	27 ± 0.5164	26.5 ± 0.4282	26.5 ± 0.3416	26.83 ± 0.4779	27.17 ± 0.4014
II	Disease (Diabetic) control	28.33 ± 0.3073 ^{NS}	26.33 ± 0.3333^{NS}	24.5 ± 0.4282^{NS}	23.17 ± 0.7032*	20.17 ± 0.5426***
III	Standard Treated	27.33 ± 0.8433^{NS}	26.37 ± 1.022^{NS}	27.5 ± 0.8062^{NS}	26.17 ± 0.9804^{NS}	25.67 ± 0.8028***
IV	Polyherbal extract treated I 100 mg/kg	30.5 ± 0.9916^{NS,NS}	28.67 ± 0.9545^{NS,NS}	27.5 ± 0.8062^{NS,NS}	26.50 ± 0.5627^{*,NS}	25 ± 0.5164^{**,NS}
V	Polyherbal extract treated II 200 mg/kg	30 ± 0.4472^{NS,NS}	29.17 ± 0.4773^{NS,NS}	28.5 ± 0.6708^{**,NS}	27.67 ± 0.5578^{**,NS}	27.17 ± 0.8333^{***,NS}
VI	Polyherbal extract treated III 400 mg/kg	28.33 ±1.145^{NS,NS}	28 ±1.211^{NS,NS}	27.33 ±1.054^{NS,NS}	27.50 ±1.057^{**,NS}	29.17 ±0.8724$^{***,\#\#}$

Notes: All values are expressed as mean ± SEM. N = 6. All data are subjected to One Way ANOVA followed by Bonferroni's multiple comparisons test; here Group II is compared with Group I and Groups III, IV, V and VI are compared with Group II. *p < 0.05, **p < 0.01, ***p < 0.001, NS: Not Significant. Multiple Comparison: Groups IV, V, VI are compared with Group III. # p < 0.05, ## p < 0.01, ### p < 0.001, NS: Not Significant.

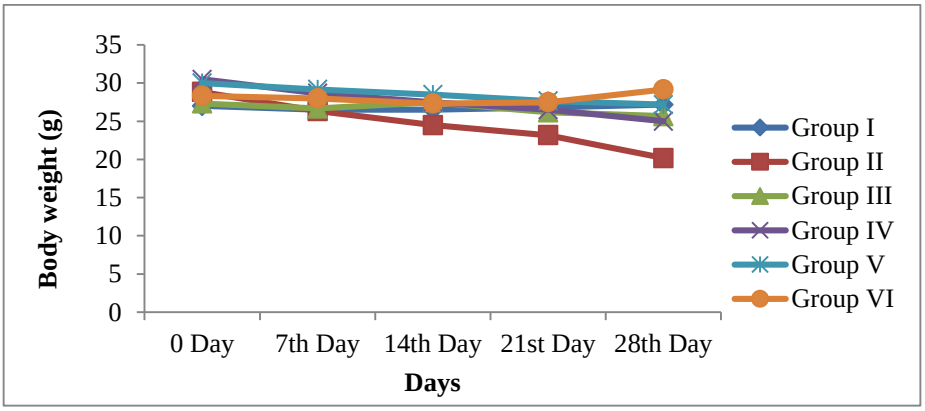

Figure 1: Effects of polyherbal extracts on body weights in STZ-NIC induced diabetic mice; values are expressed as mean ± SEM, n = 6. Where, Group I: Normal control, Group II: Disease control, Group III: Standard control, Group IV: polyherbal extract treated 100 mg/kg, Group V: polyherbal extract treated 200 mg/kg, Group VI: polyherbal extract treated 400 mg/kg.

Effects of the polyherbal extracts on oral glucose tolerance

The effects of the polyherbal extracts on the blood glucose level measured with Glucopoint One-touch glucometer in Group II (Disease control) were observed to be 320.2 ± 9.513 mg/dl at 120 min. All the readings are given in Table 3 and significant ($p < 0.001$) increase in blood glucose level in Group II (Disease control) was observed as compared to Group I (Normal Control). In Group VI polyherbal extract-treated III 400 mg/kg, a significant ($p < 0.001$) decrease in blood glucose level at 60, 90 and 120 min was observed as compared to Group II (Disease control). In a similar comparison against Group III (Standard control) treated with metformin (250 mg/kg, p.o.), Group VI polyherbal extract-treated III 400 mg/kg showed a significant ($p < 0.001$) decrease in blood glucose level at 90 min and 120 min. The results suggest the potential effects of polyherbal extract-treated at dose of 400 mg/kg at 90 and 120 mins of treatment (Figure 2).

Table 3: Effects of polyherbal extracts on oral glucose tolerance test in STZ-NIC induced diabetic mice

S.No	Treatment Group	Plasma glucose concentrations (mg/dl)				
		0 min	30 min	60 min	90 min	120 min
I	Normal Control	93.67 ± 1.145	129.2 ± 1.537	146.7 ± 1.453	129.3 ± 2.108	94.33 ± 0.8819
II	Disease (Diabetic) control	326.0 ± 8.839***	350.7 ± 10.7***	371.2 ± 9.41***	349.2 ± 10.03***	320.2 ± 9.513***
III	Standard Control	313.3 ± 9.510^{NS}	329.0 ± 9.29^{NS}	309.3 ± 9.59***	292.2 ± 7.026***	263.8 ± 9.888***
IV	Polyherbal extract treated I 100 mg/kg	324.3 ± 8.804^{NS}	342 ± 8.021^{NS}	354.0 ± 6.127$^{NS,\#\#}$	343.5 ± 6.339$^{NS,\#\#\#}$	329.8 ± 6.041$^{NS,\#\#\#}$
V	Polyherbal extract treated II 200 mg/kg	326.2 ± 9.228^{NS,NS}	334.3 ± 8.920^{NS,NS}	330.5 ± 9.66$^{**,\ NS}$	324.8 ± 9.428$^{NS,\#}$	317.2 ± 9.407$^{NS,\#\#}$
VI	Polyherbal extract treated III 400 mg/kg	310.0 ± 8.14^{NS,NS}	328.5 ± 11.95^{NS,NS}	295.8 ± 7.300^{***,NS}	249.2 ± 3.745$^{***,\#\#\#}$	199.7 ± 0.9888$^{***,\#\#\#}$

Notes: All values are expressed as mean ± SEM, n = 6. All data are subjected to One Way ANOVA followed by Bonferroni's multiple comparisons test; here Group II is compared with Group I and Groups III, IV, V and VI are compared with Group II. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, NS: Not Significant. Multiple Comparison: Groups IV, V and VI are compared with Group III. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, NS: Not Significant.

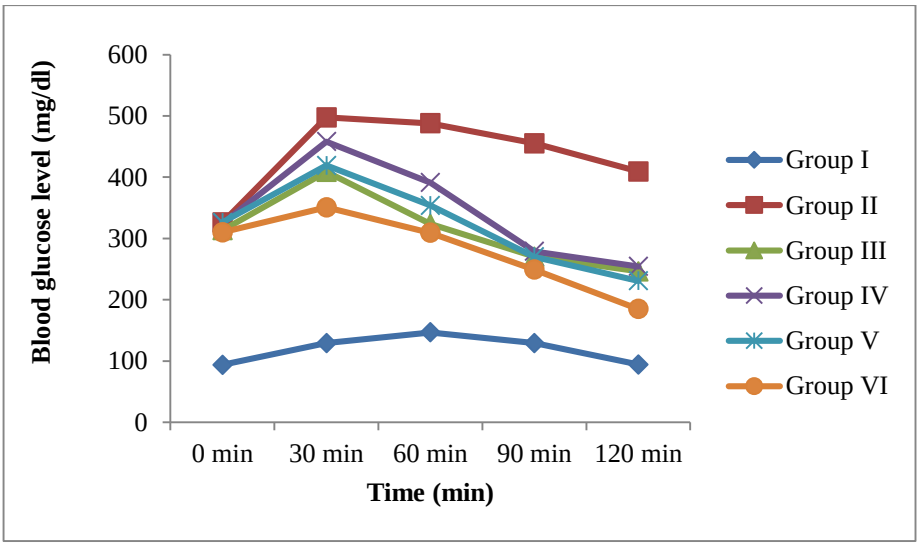

Figure 2: Effects of polyherbal extracts on oral glucose tolerance test in STZ-NIC induced diabetic mice; values are expressed as mean ± SEM, n = 6. Where, Group I: Normal control, Group II: Disease control, Group III: Standard control, Group IV: polyherbal extract treated 100 mg/kg, Group V: polyherbal extract treated 200 mg/kg, Group VI: polyherbal extract treated 400 mg/kg.

Effects of the polyherbal extracts on non-insulin-dependent diabetes mellitus

In the sub-acute study conducted in the STZ-NIC induced diabetic mice, the blood glucose level in Group II (Disease control) was found to be 316.7 ± 6.800 mg/dl (Table 4). Significant ($p < 0.001$) increase in blood glucose level in Group II (Disease control) was observed as compared to Group I (Normal control). At the end of 28 days of treatment schedule, significant ($p < 0.001$) decrease in the blood glucose level was observed in Groups III, IV, V and VI compared to Group II (Disease control). In Group VI, significant ($p < 0.01$) decrease in blood glucose level at 28 days was observed as compared to Group III (Standard control) treated with metformin (250 mg/kg, p.o.). While in Groups IV and V, no significant decrease in blood glucose level at 28 days was observed as compared to Group III. The results suggest the potential effects of polyherbal extract-treated at dose of 400 mg/kg at 14, 21 and 28 days of treatment (Figure 3).

Table 4: Effects of polyherbal extracts on blood glucose level in STZ-NIC induced diabetic mice

Sr. No	Treatment Group	Fasting plasma glucose concentration (mg/dl)				
		Day 0	**7th Day**	**14th Day**	**21st Day**	**28th Day**
I	Normal Control	96 ± 0.6831	96.17 ± 0.4014	96.33 ± 0.3333	97.50 ± 0.4232	97.67 ± 0.4216
II	Disease (Diabetic) control	309.5 ± 7.018***	311.5 ± 6.662***	316.7 ± 7.592***	314.2 ± 7.609***	316.7 ± 6.800***
III	Standard Control	325 ± 7.043^{NS}	316.7 ± 7.592^{NS}	221.3 ± 10.93***	165.6 ± 8.036***	101.3 ± 1.874***
IV	Polyherbal extract treated I 100 mg/kg	325 ± 7.043^{NS,NS}	289.5 ± 6.893$^{NS,\#}$	238.0 ± 6.061^{***,NS}	196.3 ± 3.964$^{***,\#\#}$	146.8 ± 3.745^{***,NS}
V	Polyherbal extract treated II 200 mg/kg	329 ± 7.033^{NS}	287.7 ± 4.197$^{NS,\#}$	232.5 ± 5.464$^{***,\#}$	164.2 ± 4.534^{***,NS}	112.3 ± 2.060$^{***,\#\#\#}$
VI	Polyherbal extract treated III 400 mg/kg	323.8 ± 5.231^{NS}	283.3 ± 5,783*,$^{\#\#}$	218.7 ± 9.050$^{***,\#\#}$	156.0 ± 3.416$^{***,\#\#}$	100.8 ± 1.352$^{***,\#\#}$

Notes: All values are expressed as mean ± SEM, n = 6. All data are subjected to One Way ANOVA followed by Bonferroni's multiple comparisons test; here Group II is compared with Group I, and Groups III, IV, V and VI are compared with Group II. *p < 0.05, **p < 0.01, ***p < 0.001, NS: Not Significant. Multiple Comparison: Group IV, V and VI are compared with Group III. # p < 0.05, ## p < 0.01, ### p < 0.001, NS: Not Significant.

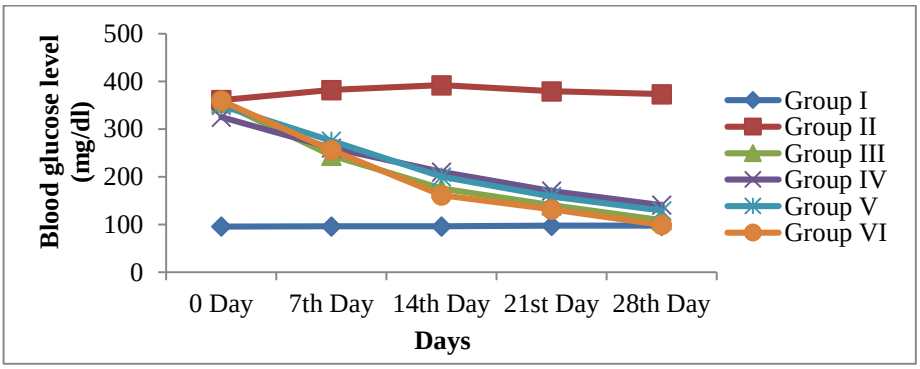

Figure 3: Effects of polyherbal extracts on blood glucose level in STZ-NIC induced diabetic mice; values are expressed as mean ± SEM, n = 6. Where, Group I: Normal control, Group II: Disease control, Group III: Standard control, Group IV: polyherbal extract treated 100 mg/kg, Group V: polyherbal extract treated 200 mg/kg, Group VI: polyherbal extract treated 400 mg/kg.

Effects of polyherbal extracts on lipid profile

The levels of serum lipids are usually elevated in DM and such an elevation represents a risk factor for coronary heart disease. This abnormally high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat deposits due to the action of insulin. The regulation of lipid profiles in different groups of mice is exhibited in Table 5. A significant increase in TGL, total cholesterol and LDL, and a decrease in HDL was observed in Group II Disease control mice

compared with Group I Normal control mice (Table 5). Groups IV, V and VI polyherbal extract-treated and Group III standard drug-treated mice showed significantly decreased TGL, total cholesterol, LDL, and increased HDL compared to Group II Disease control mice. The results of the study indicate the anti-hyperlipidaemic activity of the polyherbal extracts. The Group VI polyherbal extract-treated at dose of 400 mg/kg produced extreme anti-hyperlipidemic activity compared with Groups IV and V polyherbal extract-treated at doses of 100 mg/kg and 200 mg/kg (Figure 4).

Table 5: Effects of polyherbal extracts on lipid profile in STZ-NIC induced diabetic mice

Sr. No.	Treatment Groups	Lipid profile mg/dl			
		Cholesterol	**Triglycerides**	**LDL**	**HDL**
I	Normal Control	73.50 ± 2.078	85.50 ± 0.3416	51.83 ± 0.3074	69.07 ± 0.2108
II	Disease (Diabetic) control	126.2 ± 1.078***	200.00 ± 0.3651***	194.8 ± 0.5426***	24.0 ± 0.3651***
III	Standard Control	82.33 ± 0.8433***	86.17 ± 1.621 ***	51.27 ± 0.3073***	71.33 ± 0.3333***
IV	Polyherbal extract treated I 100mg/kg	95.50 ± 1.839 $^{***,###}$	95.83 ± 0.3073$^{***,###}$	58.33 ± 0.4126$^{***,###}$	74.50 ± 0.6191$^{***,###}$
V	Polyherbal extract treated II 200 mg/kg	90.33 ± 0.5578$^{***,###}$	87.33 ± 0.6146 $^{***,###}$	54.83 ± 0.3073$^{***,###}$	68.17 ± 0.1666 $^{***,###}$
VI	Polyherbal extract treated III 400 mg/kg	71.17 ± 0.4773$^{***,###}$	83.33 ± 0.4216^{***,ns}	52.00 ± 0.3651$^{***,###}$	59.00 ± 0.3651$^{***,###}$

Notes: All values are expressed as mean ± SEM, n = 6. All data are subjected to One Way ANOVA followed by Bonferroni's multiple comparisons test; here Group II is compared with Group I and Groups III, IV, V and VI are compared with Group II. *p < 0.05, **p < 0.01, ***p < 0.001, NS: Not Significant. Multiple Comparison: Groups IV, V and VI are compared with Group III. # p < 0.05, ## p < 0.01, ### p < 0.001, NS: Not Significant.

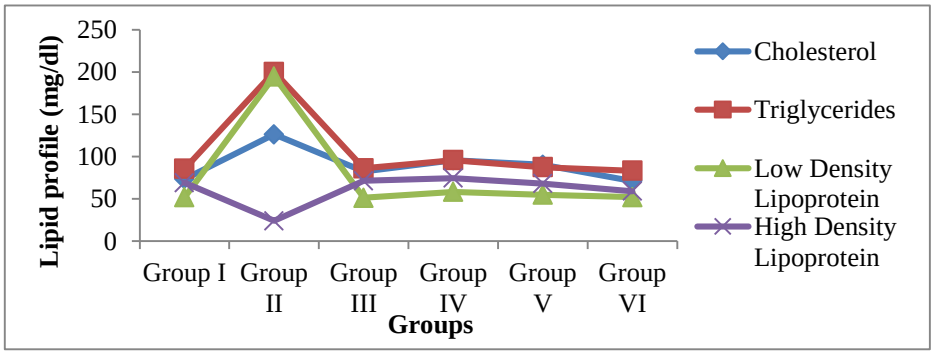

Figure 4: Effects of polyherbal extracts on lipid profile in STZ-NIC induced diabetic mice; values are expressed as mean ± SEM, n = 6. Where, Group I: Normal control, Group II: Disease control, Group III: Standard control, Group IV: polyherbal extract treated 100 mg/kg, Group V: polyherbal extract treated 200 mg/kg, Group VI: polyherbal extract treated 400 mg/kg.

Histopathologic analysis of pancreas

At the end of the study, the histopathology of the pancreas was done; results are shown in Figure 5. In Group II (Disease control), the histopathologic analysis of the pancreas revealed severe congestion, a huge decrease in the number of islets of Langerhans and β cells, and fibrosis and inflammatory cell infiltration into the islets of Langerhans. Group III (Standard control) treated with drug metformin showed moderate congestion with a comparatively lower decrease in the number of islets of Langerhans and β cells and lymphocytic infiltration of mild nature. Groups IV and V, i.e., polyherbal extracts at doses of 100 and 200 mg/kg, respectively also showed moderate congestion with a moderate decrease in the number of islets of Langerhans and β cells and mild lymphocytic infiltration. While the Group VI polyherbal extract at the dose of 400 mg/kg showed mild congestion and mild decrease in the number of islets of Langerhans with normal β cell population indicating a significant amount of recovery.

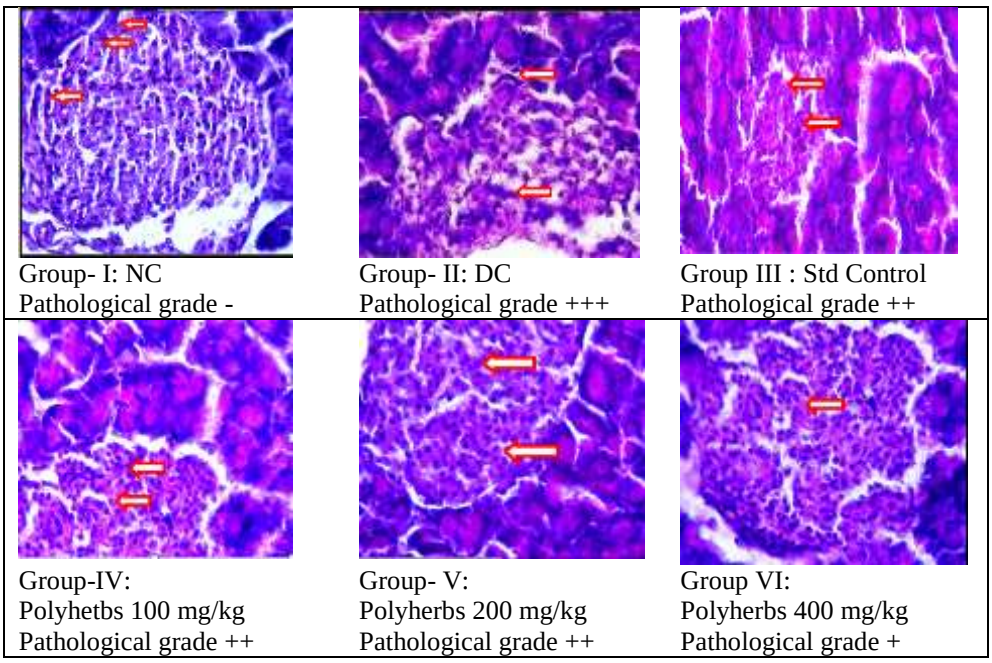

Figure 5: Pathological grades; Grade: -- no injury; Grade: +++ severe injury; Grade: ++ moderate injury; Grade: + mild injury.

Figure 5: Group I Normal control show "-" pathological grade which indicates no injury in tissue. The histopathologic analysis of pancreas revealed severe congestion, huge decrease in the number of islets of Langerhans and β cells, and fibrosis and inflammatory cell infiltration into the islets of Langerhans in Group II Disease control which shows +++ pathological grade indicating severe injury. Group III Standard drug metformin treatment shows ++ pathological grade, i.e., moderate congestion with moderate decrease in the number of islets of Langerhans and β cells, and mild lymphocytic infiltration. Groups IV and V, i.e. polyherbal extracts at doses of 100 and 200 mg/kg, respectively also shows ++ pathological grade indicating moderate congestion with moderate decrease in the number of islets of Langerhans and β cells and mild lymphocytic infiltration. While the group VI of polyherbal extract at 400 mg/kg showing + pathological grade indicates mild congestion and mild decrease in the number of islets of Langerhans with normal β cell population, thereby indicating significant amount of recovery.

Discussion

Various synthetic drugs are used for the treatment of hyperglycaemia, like insulin and oral hypoglycaemic agents. These synthetic drugs are associated with side effects which lead to limitations in their use and create issues in the management of diabetes. So, there is a need to develop safe and economic alternative treatments for diabetes mellitus. As such, there is considerable interest in the field of medicinal plants due to their natural origins and fewer side effects. In recent times, herbal formulations have gained greater importance mainly due to their efficacy and easy availability, as well as fewer side effects when compared to synthetic drugs. These advantages have led the people to move toward herbal preparations for disease treatment and prevention, as they are claimed to display synergistic, potentiate, and agonistic/antagonistic actions and the mixtures of species in them show better therapeutic effects than individual species on their own (Petchi et al. 2014, Mohapatra et al. 2016). Srivastava et al. (2012) noted that the concept of polyherbalism has been highlighted in Sharangdhar Samhita, Ayurvedic literature dating back to 1300 AD. Also, they highlighted any single herb concentration of polyherbal formulation is reduced which is also responsible for reducing the adverse effects. In the present study, polyherbal formulations containing extracts of leaves of *Gymnema sylvestre*, whole plant of *Boerhavia diffusa*, stem and leaves of *Tinospora cordifolia*, and whole plant *Argyreia nervosa* were selected for the antidiabetic study. The individual plants have already been determined to have antidiabetic activity. The polyherbal formulations were formulated using the ethanolic extracts of the leaves of *Gymnema sylvestre*, the whole plant of *Boerhavia diffusa*, stem and leaves of *Tinospora cordifolia*, and whole plant *Argyreia nervosa*. The antidiabetic activity of the individual plants has been proven. The leaves of the *Gymnema sylvestre* showed significant antidiabetic activity against STZ-induced diabetes at a dose of 400 mg/kg (Aralelimath and Bhise 2012), *Boerhavia diffusa* shows potent antidiabetic activity at dose of 200 mg/kg (Malhotra et al. 2014), *Tinospora cordifolia* shows potent antidiabetic activity at a dose of 400 mg/kg (Puranik et al. 2010), *Argyreia nervosa* shows potent antidiabetic activity at dose of 500 mg/kg (Kumar et al. 2010). The administration of polyherbal mixture exhibited significant antidiabetic effects and shows synergistic and potential actions against diabetes. The concentrations of the extracts were calculated from the references mentioned in the present study and worked for polyherbal extracts. The findings of the present study indicate the significant effects of the polyherbal extracts on streptozotocin-nicotinamide induced type 2 diabetic animals (mice).

Streptomyces achromogenes is a source of STZ, which is an antibiotic. Structurally, is a glucosamine derivative of nitrosourea. The nitrosourea moiety of this derivative is responsible for beta cell toxicity, while deoxyglucose moiety helps transport across the cell membrane. STZ causes alkylation or breakage of DNA strands and a consequent increase in the activity of poly-ADP-ribose synthetase, an enzyme depleting NAD in beta cells finally leading to energy deprivation and death of beta cells (Srinivasan and Ramarao 2007).

Recently, Undale et al. (2014) stated that a new animal model of type 2 diabetes has been produced by the combination of STZ and NIC administration in adult rats. The rats administered NIC (230 mg/kg, ip) 15 min before STZ (65 mg/kg, iv) have shown to develop moderate and stable non-fasting hyperglycaemia without any significant changes in plasma insulin levels. As NIC is an antioxidant, it exerts a protective effect on the cytotoxic action of STZ by scavenging free radicals and causes only minor damage to pancreatic beta-cell mass-producing type 2 diabetes. Therefore, this model has been found to be an advantageous tool for the investigation of insulinotropic agents in the treatment of type 2 diabetes (Undale et al. 2014).

The principle objective in the treatment of diabetes mellitus is to decrease the elevated blood glucose to normal physiological level to prevent further micro and macrovascular complications. In this study, metformin was used as a standard drug. Metformin is accepted as a first-line antidiabetic agent for the management of type 2 diabetes. It is suited for the treatment irrespective of body weight, age, severity of hyperglycaemia and provides an agreeable pharmacological base for therapy in conjunction with other antidiabetic agents (Scarpello and Howlett 2008). Metformin has lower mortality and cardiovascular risks as compared to other insulin secreting agents such as glimepiride, glibenclamide, glipizide and tolbutamide in patients with type 2 diabetes mellitus. Metformin also does not produce hyperglycaemia since it does not stimulate insulin secretion when administered alone in patients with type 2 diabetes mellitus (Wright et al. 2006). As shown in Table 1, reduction in body weight observed in diabetic mice might be the result of degradation of structural proteins and fats due to deficiency of carbohydrates for the energy metabolism and significant increase in body weight of diabetic mice treated with polyherbal extracts showed the blood glucose stabilization effect which in turn prevents the loss of body weight.

Glucose tolerance test is a standard procedure that determines how rapidly exogenous glucose can be cleared from the blood. Insulin is important for regulating the uptake of glucose from the blood by cells. Soni et al. (2018), explained impairment of glucose tolerance, which means takes a longer time to clear a given amount of glucose divulges problems with the maintenance of glucose homeostasis (insulin resistance, carbohydrate metabolism, diabetes, etc).

As seen in Table 2, in Group VI mice treated with a polyherbal extract at the dose of 400 mg/kg, a significant ($p < 0.001$) decrease in blood glucose level at 90 min and 120 min was observed as compared to Group-III treated with standard drug metformin. The results indicated that the polyherbal extract at the dose of 400 mg/kg increases the clearance of the glucose from the blood which might be due to enhanced uptake of glucose from the cells which is regulated by insulin.

The diabetes was induced in mice after administration of streptozotocin-nicotinamide. As shown in Table 3, a consistent decrease in the blood glucose level was observed in the groups treated with metformin (Group III) and polyherbal extract-treated Groups IV, V and VI. In Group VI, polyherbal extracts at the dose of 400 mg/kg, a significant ($p < 0.001$) decrease in blood glucose level at 28 days was observed as compared to Group III treated with metformin (250 mg/kg, p.o.). While in Groups IV and V no significant decrease in blood glucose level at 28 days was observed as compared to Group III. The results suggest the potential effects of polyherbal extract-treated at dose of 400 mg/kg at 14, 21 and 28 days of treatments. The possible mechanism by which polyherbal extract brings about its hypoglycemic action in diabetic mice maybe by potentiating the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form.

Dyslipidaemia is a secondary complication accompanied by long term effects of diabetes and had been discussed many times during the past decades. During diabetes, the levels of TGL, cholesterol and LDL increase and the HDL levels decline significantly. In the diabetic model, abnormal concentrations of serum lipids might be due to a disturbance in hormone-sensitive enzyme lipase. The lipase enzyme concentration allows the mobilisation of free fatty acids from the peripheral fat repository because of insulin deficiency (Soni et al. 2018). The administration of polyherbal extract significantly restored cholesterol, TGL, LDL and HDL levels to their normal values in diabetic mice, which might be due to its lipid-lowering activity. Therefore, this polyherbal extract also plays a significant role against diabetes-associated complications.

Histopathology of the pancreas of STZ-NIC diabetic animals showed a severe decrease in

the number of islets of Langerhans and β cells, with fibrosis and inflammatory cell infiltration into the islets of Langerhans. Polyherbal extract and metformin treatment to the animals reduced the severity of the histopathologic changes caused by STZ.

Conclusion

Chemically active principles present in the studied plants may be responsible for the observed antidiabetic effect of the polyherbal extracts. The hypoglycaemic effect produced by the polyherbal formulations may be due to the glycosides, flavonoids, tannins, and saponins present in the extracts. From the present study, it could be concluded that the administration of polyherbal formulations containing extract of *Gymnema sylvestre*, *Boerhavia diffusa*, *Tinospora cordifolia* and *Argyreia nervosa* exhibited significant antidiabetic effects by controlling the blood glucose levels. Additionally, the polyherbal formulations decreased total cholesterol, triglycerides and LDL, with an increase in HDL at the end of the treatments. The polyherbal extract-treated Group VI (400 mg/kg) exhibited maximum antidiabetic activity compared to the polyherbal extract-treated Group IV (100 mg/kg) and Group V (200 mg/kg). The significant hypoglycaemic activity of the polyherbal formulation might be due to the varied mechanism of action of each herbal drug present in the formulations. Hence, the developed polyherbal formulation might prove to be a potential safe alternative for the existing anti-diabetic synthetic drugs.

Acknowledgement

The authors are thankful to Pune District Education Association for providing animal house facilities during study. The author Dr. Patil is thankful to the DS Kothari Fellowship program, University Grants Commission and Savitribai Phule Pune University for the financial support during the post-doctoral research, this research idea emerged during the study.

Ethics Approval and Ethical statement

The experimental protocols were approved by the Institutional Animal Ethical Committee of PDEA's Seth Govind Raghunath Sable College of Pharmacy, Saswad (Ref. No. SGRS/IAEC/07/2019-20).

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The material used for Bioresorbable Implants

Material able to serve a cause after which disappearing in the human body is no magic folklore however primarily based totally on years of rigorous medical evidence tested clinical data, and huge business use. With over 5 many years of scientific use as substances for orthopedic programs, those styles of substances, referred to as bioresorbable substances, hold to discover use in novel programs inclusive of sutures, screws, stents, scaffolds, or even artificial skin. Their persevered improvement may be attributed to improvements in novel synthesis techniques, processing technologies, implant layout improvement, and revolutionary surgical techniques. Bioresorbable substances are capable of being degraded in physiological environments into products that might be metabolized into non-poisonous degradation merchandise or very well bio-absorbed.[20]

Uses of bioresorbable implant

Strong, elastic, bioresorbable implants might be beneficial in cartilage repair, vascular grafts, sinusitis remedy, and the treatment of pediatric conditions. Essential challenge of clinical implants used to deal with those tissues is the shortage of substances that mimic the strength and elasticity of the local tissue.[21]

- **NEUROETHICS**

Neuroethics is a subject that research the moral, legal, and societal implications of neuroscience. Advances in our knowledge of the brain and capacity to display and modulate brain characteristic can increase unresolved moral questions, consisting of the ones associated with non-public identity, consciousness, and autonomy. For example, deep brain stimulation remedies may also alleviate signs and symptoms of Parkinson's disease.

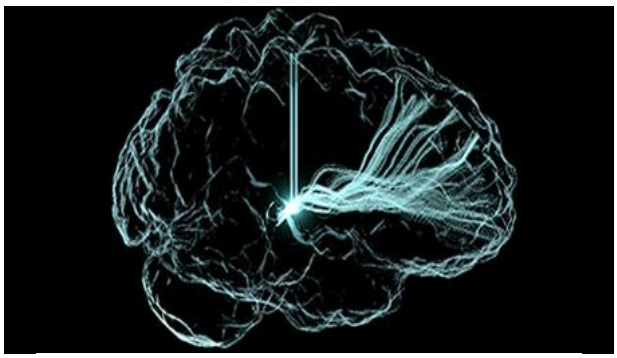

Figure no. 4: Neurological disorders and stroke.

Neuroethics can work with neuroscientists to discover and address the moral questions that arise in neuroscience studies. As such, neuroethics can strengthen neuroscientific studies and contribute to how research is designed, conducted, interpreted, and applied. NINDS participates in numerous cross-company applications that support alliances between neuroethics and neuroscientists.[22]

DISCUSSIONS

The creation of brain implants has brought about a renaissance in present day neurosurgery and has certainly brought about the refinement withinside the remedy for complicated motor troubles like Parkinson'sDisease, Alzheimer's disease, intense epilepsy, and brain seizures. This evaluation has taken into consideration a variety of therapeutics throughout journals and the effects they have got yielded. However, the powerful manipulation of neuropsychiatric complications springing up for the duration of and after the treatment is the need of the hour. Future demanding situations in the utilization of bio-electronics device for the control of complicated neurological issues have to encompass improven understanding of the symptoms, unswerving and well-timed medication, thereby averting iatrogenic troubles as ways as possible. Every exercise technique has to pave manner for the patient's neural ethics and his/her 'brain privacy'. Contrivances like reminiscence chips, implants to circulation tune without delay into the brain, implants to govern the idea system and IQ in human beings must be delivered into use most effective after meticulous assessment in their professionals and cons.[1]

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