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VEGAN HERBAL CHOCOLATE FORMULATION IN THE TREATMENT OF ALZHEIMER'S DISEASE

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ABSTRACT

The present research work aimed to formulate a chocolate drug comprising herbal medicines effective in Alzheimer's disease. A novel chocolate formulation comprising the combination of Brahmi, Shakhpushpi & Ginkgo Biloba was evaluated for learning and memory-enhancing activity in rodents by the ethanol-induced cognitive impairment method. Furthermore, the herbal chocolate was evaluated for organoleptic properties, hardness & drug content determination, blooming test, dimension, melting point, stability & shelf life.

Keywords: Herbal medicated chocolate, Alzheimer's disease, Brahmi, Shakhpushpi, Ginkgo Biloba

INTRODUCTION:

Herbal medicinal products have existed globally having a long ancient history. These ancient herbal medicines were used traditionally as therapeutic agents in different countries like China, Greece, Egypt & India; whereas, these medicinal herbs are rooted in the religious practices of Native Americans and African countries. The traditional Ayurvedic medicinal system in India has incorporated herbals as one of its most effective mitigate components, recorded in Vedas and Samhitas **[1-3]**.

Various researchers worked on extracting and modifying the active ingredients from herbal medicines. In the early 19th century, because of the advancement in chemical analytical methods, herbal medicinal products were switched from raw to

synthetic. This started a decline in the demand and supply of herbal medicines. However, synthetic medicinal agents are more expensive and have several unwanted side effects.

Late in the 19th century, the demand for medicines was transitioned back to herbal medicinal products obtained from natural sources. Herbal formulations have received special importance and have currently gained global attention. After the market expansion in developed countries in the last few years, there has been a tremendous increase in the usage of herbal medicinal products. As per the World Health Organization (WHO) report, 80% of the worldwide dwellers depend on traditional herbal medicines for health management. The research activity in herbal medicines is boosted by development in the isolation method, purification techniques, and identification and characterization of formulations. The term "herbal drug" represents the parts of a plant like flowers, leaves, roots, barks, seeds, stems, etc., utilized for the preparation of medicinal products. Each & every part of the herbal plant is completely utilized for manufacturing different herbal formulations such as Kwatha (Decoction), Phanta (Hot infusion), Hima (Cold infusion), Arka (Liquid Extract), Churna (Powders), Guggul (Resins and balsams), Taila (Medicated oil) etc. **[4-7]**.

Chocolate is prepared from tropical Theobroma cacao tree seeds. These beans contain more than 300 health beneficiary compounds like flavonoids, flavonols, polyphenols & catechins etc. Anthocyanidin and epicatechins are potent antioxidants. It is reported in literature that free radicals can cause damage to DNA and other cell components. These chemicals play an important role in accelerating aging and contributing to heart disease, cancer and other diseases. Chocolates are reported to have health benefits due to its antioxidant properties, particularly, dark chocolate loaded with high cocoa content is powerful antioxidant. Chocolates have a number of health benefits like lowering LDL levels, reducing the risk of cardiovascular diseases **[8-10]**.

Chocolate has organoleptic characteristics to mask the unpleasant flavour with some active drugs. Chocolate is a semisolid suspension form. Chocolate is composed of small solid particles from sugar and Cocoa made in the fat phase. The most important compound of chocolate is Cocoa Butter a mixture of different triglycerides solid in below 25^0C temperature but liquid in body temperature. The herbal chocolate formulation for the treatment of cough,

immune booster, and the treatment of malnutrition in children **[11-13]**.

Medicated Chocolate is manufactured from a Chocolate base and a suitable drug component is added to it. The drug component is released from the Chocolate base. Medicated Chocolate has more patient compliance, it is more appealing to children. Cocoa powder is found in vitamin A, phenolics, and all Nutrient etc **[14-15]**.

Nowadays, the concept of "Vegan chocolate" become more popular in developed & developing countries, chocolate is made up of raw materials devoid of animal origin. Specifically, dark chocolate is highly demanded because of the absence of any dairy (animal) product. Vegan dark chocolate normally contains more than 50% cacao content along with dried fruits like Almonds. Vegan dark chocolates have more nutritional benefits. Average dark chocolate containing an 80-85% cocoa content can provide 98% of the recommended daily intake of manganese. Vegan dark chocolates are antioxidants and may reduce the risk of cardiac problems **[16-18]**.

Shankhapushpi is effective as brain tonic and help in improving memory. Shankhapushpi has excellent therapeutic effects in number of neurological problems such as stress, anxiety, neurosis. It is also useful in rejuvenation therapy and effective as a psycho-stimulant and tranquillizer. The aqueous extract of Shankhapushpi is very useful in lipid disorders, hypertension, hypotension, peptic ulcers, hypothyroidism. Various studies on Shankhapushpi have also proved that it is beneficial for enhancing beauty and helps in nourishing different layers of skin **[19-20]**.

Brahmi has application in various disease conditions such as irritable bowel syndrome, improving memory, anxiety, attention deficit-hyperactivity disorder, Alzheimer disease, allergic conditions, stress, arthritis, gout and other inflammatory diseases, gastric ulcers and irritable bowel syndrome **[21-23]**.

As per literature reports, the leaf extract of Ginkgo biloba is very effective in age-mediated memory dysfunction, Alzheimer's disease, dementia, improve cognitive function and social functioning. In clinical studies, it was found that patients containing Ginkgo biloba leaf extract appear to be equivalent to clinical improvements in patients taking donepezil and tacrine **[24-25]**.

Vitamins and minerals play an important role in brain development, immune function, growth, and other cellular functions in the body. Specific micronutrients also play a role in preventing disease in humans. Becadexamin was used as a source of

vitamins and minerals in the present herbal chocolate composition.

MATERIALS AND METHODS:

Collection and authentication of polyherbal plant material:

The powders of Shankhapushpi, Brahmi & Ginkgo biloba were identified and procured from the local market in Mumbai and authenticated by Alarsin Ayurvedic Pharmaceuticals, Mumbai.

Preparation of Herbal Extract:

The collected powders were successfully extracted with water and ethanol as solvent using Soxhlet apparatus. The extraction was performed for 72 hours at a temperature not exceeding the boiling point of alcohol & water. An excess amount of solvent was evaporated to receive plant extracts in dry form.

Experimental animals:

SD rats of male or female sex (200-300g) were maintained for 7 days in the animal house of Chalapathi Institute of Pharmaceutical Sciences, Guntur under standard conditions of temperature (25 ± 10^0C), relative humidity (45-55%) and 12:12 light: dark cycle. The animals were fed with standard rat pellets and water ad libitum. The animals were allowed to acclimatize to laboratory conditions 48 hours before the start of the experiment. 5 rats/group were used in all sets of experiments.

Ethical committee approval: All the protocols were approved by the Institutional Animal Ethical Committee (IAEC) and conducted according to the Committee for Control and Supervision of Experimental Animals (CPCSEA) at Chalapathi Institute of Pharmaceutical Sciences, Guntur.

Preparation of Herbal Chocolate Formulation: The hydroalcoholic extract of Brahmi, Shankhpushpi & Ginkgo Biloba having a concentration of 100mg/ml & 200mg/ml respectively was incorporated in chocolate formulations (F1-F3). Along with active ingredients, the chocolate base consists of dark chocolate, Becadexamin capsule & Vanilla essence as the vehicle as shown in **Table 1**.

Table 1: The formulations of herb extract Chocolate formulations

Sr. No.	Ingredients	Conc. in mg		
		F1	F2	F3
1	Extract of Brahmi	200mg	100mg	100mg
2	Extract of Shakhpushpi	200mg	100mg	100mg
3	Extract of Ginkgo Biloba	200mg	100mg	100mg
4	Becadexamin	400 mg	200mg	100mg
5	Vanilla Essence	0.1 ml	0.1 ml	0.1 ml
6	Dark chocolate base	qs 15gm	qs 8gm	qs 8gm

Method of preparation of Herbal chocolate Formulation:

Placed the required quantity of dark chocolate base in a porcelain dish and melted it in a water bath at about 50℃. The required quantity of Vanilla essence was added to a molten chocolate base. The herbal extract of Brahmi, Shankhpushpi & Ginkgo biloba was incorporated into the chocolate base with continuous stirring. The entire content of Becadexamin capsule was emptied into the chocolate base at a low temperature. The whole mass of herbal extract loaded with a dark chocolate base was transferred into a suitable chocolate mould and refrigerated for 1 hour. Finally, de-mould the chocolate after it completely solidifies.

Evaluation of Herbal chocolate formulations [26-27]:

Organoleptic properties: The organoleptic properties of the herbal chocolate formulations F1-F3 were studied by evaluating the colour, aroma, taste, and texture.

Hardness: The hardness of chocolate formulations (F1-F3) was measured by Monsanto Hardness Tester.

Dimension: The dimension of the herbal chocolate formulation was measured by Vernier's calipers.

Blooming test: A blooming test was performed to evaluate the stability of chocolate comprising CNO towards bloom formation. A bloomed chocolate is characterized by the absence of the initial gloss of the surface, appearing more or less white. Furthermore, the bloom can have different appearances, from a uniform dull grey to a marble aspect, as well as from small individual white points to large white spots on the chocolate. Various factors are responsible for the bloom formation, such as improper processing conditions, formulation, and temperature. The bloom formation is characterized by its plurality of shape and formulation conditions. The formation and shape of the bloom are affected by various parameters such as the quality of fat and emulsifier, the presence and kind of center on which the chocolate is coated. The chocolate sample was tr to treatment cycles, change in temperature for 1 hour, and 18 ℃ for 11 hours. Followed by observation of the test sample to check the bloom formation.

Stability & Shelf Life: The precise shelf-life divination helps to control food waste. Besides a loss in texture, an important factor of filled chocolate products during storage is the fat bloom formation. Common tests for chocolate formulations are isothermal storage and storage with cycling temperature. The applicability of common tests compared to real storage conditions is difficult, and the determination of shelf-life

is indefinite. This is caused by the different temperature dependencies of the two main effects of fat bloom formation in filled chocolates, mainly migration and crystallization. While filling fat migration is always faster at higher temperatures, the crystallization of the fat on the surface, which becomes visible as fat blooms, might have different temperature optima. The physical stability test was conducted by keeping the chocolate formulation sample in a closed container for 3 months at 28°C After the one-month interval, a test sample of chocolate was examined for physical appearance and drug degradation.

Melting Point: A small sample of the chocolate formulations was placed in an empty beaker, on top of a Bunsen burner. A thermometer was used to check the temperature of the melting chocolate sample in the beaker. The starting point is stated as the onset temperature (T-onset), it is the temperature at which the entire chocolate sample melts is the peak temperature (T-peak), & the point and which the chocolate no longer melts or starts to solidify again is the end set temperature (T-endset). The fat content & the fat composition along with the particle size governed the melting behavior of dark chocolate.

Drug content determination: The drug content of herbal chocolate was evaluated by TLC (Thin Layer Chromatography) method. An extract of Brahmi, Shakhpushpi, Ginkgo Biloba was selected as a control & test as the melted chocolate sample. TLC plates were prepared using silica G and plates were activated for ½ Hr. Spotting was performed on the control and test plates using capillary. Both plates were run in the mobile phase. The mobile phase was selected as Toluene: Ethyl acetate: Methanol with a ratio of 8:4:2. Both control & test plates were dried and visualized using an iodine chamber. Finally, compare the RF value of test & control and determine the drug content.

Ethanol-induced cognitive impairment: Ethanol is a potent neurotoxin that can change behavioral and cognitive performance in humans. Its main mechanism of action by impairing hippocampus-dependent learning and memory functions. Various clinical studies proved that free-radical-mediated oxidative stress plays a crucial role. The brain is highly susceptive to oxidative stress due to high levels of polyunsaturated fatty acids (PUFAs) and catecholamines, large amounts of oxygen (O2) in relatively small masses, and conjunction with low antioxidant activities. In addition, certain regions of the central nervous system (CNS) like hippocampus and cerebellum, may be more susceptive to oxidative stress because of their low endogenous antioxidant,

concerning other brain regions. Studies proved that acetaldehyde dehydrogenase play important role in the formation of reactive oxygen species (ROS) by converting cytotoxic acetaldehyde produced from the oxidation of ethanol to acetate. It has been proved that oxidative stress is due to ethanol inducing the synthesis of CYP2E1. It also enhances the ratio of NADH/NAD, responsible for the reduction of ferric ions (Fe3+) to ferrous ions (Fe2+)) which causes lipid peroxidation by generating hydroxyl radicals.

Experimental design: The learning and memory-enhancing activity of the herbal chocolate formulations F1-F3 comprising the aqueous extract of Brahmi, Shakhpushpi, Ginkgo Biloba was conducted using the ethanol-induced cognitive impairment. It has been showed that 60% Ethanol is utilized to induce dementia-like condition in the dose 2.5 mg/kg administered i.p for 15 days. The test animals were randomly selected and divided into five groups having five rats in each as follows:

Group I: Inducing Group- Ethanol (2.5 mg/kg was administered i.p for 15 days).

Group II: Standard Group -Donepezil hydrochloride (2.5 mg/kg 7 was administered orally for 15 days) + Ethanol.

Group III: Test-I – F1 herbal chocolate formulation (100mg/kg was administered orally for 15 days) + Ethanol**.**

Group IV: Test -II -F2 herbal chocolate formulation (100mg/kg was administered orally for 15 days) + Ethanol.

Group V: Test-III-F3 herbal chocolate formulation (100mg/kg was administered orally for 15 days) + Ethanol.

RESULTS AND DISCUSSION:

Physicochemical Properties of the Herbal Chocolate Formulation:

Organoleptic properties: Organoleptic properties of the F1-F3 formulations were evaluated for color, aroma, taste, and texture. It was observed that all formulations exhibit a Dark brown color, smoky chocolate aroma, slightly sweet taste, and smooth & even texture.

Table 2: Physical Evaluation of Chocolate Formulations F1-F3

Sr. No.	Parameters	F1	F2	F3
1	Colour	Dark Brown	Dark Brown	Dark Brown
2	Aroma	Smoky Chocolate	Smoky Chocolate	Smoky Chocolate
3	Taste	Slightly sweet	Slightly sweet	Slightly sweet
4	Texture	Smooth and Even with No visible spots or Discoloration	Smooth and Even with No visible spots or Discoloration	Smooth and Even with No visible spots or Discoloration

Hardness & Dimension:

Table 3: Hardness and dimension of Chocolate Formulations F1-F3

Sr. No.	Parameters	F1	F2	F3
1	Hardness	6.1 Kg per sq. cm	6.4 Kg per sq. cm	6.2 Kg per sq. cm
2	Length	7.2 cm	7.1 cm	7.1 cm
3	Width	2.1 cm	2.2 cm	2.1 cm

Blooming test:

Table 4: Blooming test of Chocolate Formulations F1-F3

Sr. No.	Parameters	F1	F2	F3
1	Blooming test	No	No	No

Stability & Shelf Life: It was observed that no change in physical appearance and drug degradation in all the three chocolate formulations.

Table 5: Stability & Shelf-life determination of Chocolate formulation F1-F3

Sr. No.	Period	F1	F2	F3
1	One month	Stable	Stable	Stable
2	Two months	Stable	Stable	Stable
3	Three months	Stable	Stable	Stable

Drug content determination:

1) Distance traveled by the solvent of control = 5cm.

2) Distance traveled by the solute of control = 0.8cm.

3) Distance traveled by the solvent of the test = 4.5 cm.

4) Distance traveled by the solute of the test = 1.2cm.

RF value = the distance traveled by solute ÷ The distance traveled by the solvent

1) RF value of control = 0.8÷5 =0.16

2) RF value of test = 1÷4.3 =0.23

When the RF values of the control and test are compared, both values are approximately nearby; therefore, we can determine drug content.

Melting point: The lighter the chocolate is characterized by a lower melting point & white and milk chocolate is characterized by a melting point of about 30-32°C. Dark chocolate exhibits a melting point of about 32-35°C and can be heated to 45°C safely.

Table 6: Melting point values of the chocolate formulations F1-F3

Sr. No.	Temperature Stages	F1	F2	F3
1	T-onset	34°C	33°C	35°C
2	T-peak	42°C	44°C	44°C
3	T-endset	44°C	44°C	45°C

Effect of the Herbal Chocolate Formulations F1-F3 on Behavioural Parameters:

Animals administered with ethanol [2.5 mg/kg] alone for 15 days showed an increase in transfer latency in seconds on 1^{st},7^{th} and 15^{th} days.

Table 7: Effect of the herbal chocolate formulations F1-F3 on ethanol-induced cognitive impairment.

Sr. No.	Group	Treatment	Transfer latency (In seconds)		
			1st Day	7th Day	15th Day
1	I	Ethanol	23.9±2-20	26.8±3.40	27.02±4.02
2	II	Standard + Ethanol	6.02±1.36	5.6±1.25	3.4±0.72
3	III	F1 formulation + Ethanol	7.2±1.24	6.68±2.48	3.4±0.54
4	IV	F2 formulation + Ethanol	6.6±1.42	5.24±1.60	2.6±0.94
5	V	F3 formulation + Ethanol	6.8±0.27	5.18±1.04	2.8±1.28

CONCLUSION: It was concluded based on the result demonstrated that the herbal chocolate formulations F1-F3 comprise the extract of Shankhapushpi, Brahmi & Ginkgo biloba along with Becadexamin, Vanilla essence in a dark chocolate base F1-F3 exhibits cholinesterase inhibitor mechanism at an effective dose of 100 mg/kg against ethanol-induced cognitive impairment in rats. The chocolate formulation F1 contains 200mg extract of Shankhapushpi, Brahmi & Ginkgo biloba expressed a comparatively significant effect produced to donepezil hydrochloride as standard in the searching of transfer latency in sec (i.e. learning and memory activity). Transfer latency was measured after administration of ethanol on 1st day, 7th day & 15th day. This effect is ascribed to its ability to enhance the levels of the acetylcholine that are declined in the Alzheimer's disease.

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