Anti-Migraine Activity of Freeze Dried-Latex Obtained From *Calotropis Gigantea Linn*.

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Abstract

Migraine which is characterized by a pulsating headache affected an estimated population of 12% worldwide. Herbal products like latex derived from *Calotropis gigantea* R. Br. (Asclepiadaceae) are a representative intervention to treat migraine traditionally. However, post harvesting stability issues of latex affects its biological potential. Freeze drying has been successfully employed for the encapsulation of herbal bioactive compounds resulting in stable dried preparations. Latex derived from *Calotropis gigantea* (*C. gigantea)* was microencapsulated using chitosan by freeze-drying (FDCG) method and compared with sun rays dried latex (ADCG). Current investigation was aimed to improve the shelf life of latex by freeze drying microencapsulation technique and evaluation of its antimigraine potential. Dried latex powders (ADCG and FDCG) were evaluated in terms of phenolic content, coloring strength, first-order kinetic, color parameters (L*, a*, b*, C* and E*), moisture, water activity, solubility and hygroscopicity. Additionally, apomorphine -induced climbing behavior, *l*-5-HTP induced syndrome and MK 801 induced hyperactivity were used to evaluate the antimigraine potential of powdered latex. FDCG showed good physico-chemical properties due to its higher concentration of phenolic and flavonoid content. Moreover, FDCG significantly reduced the apomorphine -induced climbing behavior, *l*-5-HTP -induced syndrome and MK 801 -induced hyperactivity in a dose dependent manner through dopaminergic and serotonergic receptors interaction. In conclusion, method developed for shelf-life improvement of latex offered maximum protection over a period of 10 weeks with retaining its natural biological potential, thus it can be effectively utilized in the treatment or management of migraine.

Introduction

Crude drugs contain an array of bioactive compounds which have been considered as valuable and effective pharmacological agents. They are broadly classified as organized (cellular) and unorganized (acellular) drugs. Natural latex, an unorganized crude drug, is a milky fluid produced by flowering plants which contains complex mixture of bioactive compounds responsible for various biological activities. Latex produced by plants is more vulnerable to environmental degradation therefore adequate protection measures should be adopted to prevent the degradation of its bioactive compounds and to preserve its biochemical based functional properties. Various environmental factors including biotic (e.g., microbial degradation) and abiotic (e.g., radiations, heat, and oxygen) often affect physico-chemical and biological properties of latex. Due to these factors such as solubility and bioavailability in different biological fluids are also affected which can ultimately affects biological potential of latex (Tresserra-rimbau et al. 2018; Ray et al. 2016; Ballesteros et al. 2017; Yamashita et al. 2017; Kuck & Noreña 2016). Microbial degradation is one of the major causes of degradation which can affects biological activity of phytochemicals mainly phenolic compounds in latex during the storage (Srivastava et al. 2007).

Drying of the latex from the plants is considered as the most reliable method as it can remove water content to avoid microbial degradation of phytochemicals present in the extract. Additionally, drying process increases the shelf life of latex by slowing or preventing microbial growth and preventing certain biochemical reactions that might alter its organoleptic characteristics (Rahimmalek & Goli 2013). Numerous drying procedures have been recently introduced to ensure microbiological stability, reduce product degradation due to chemical reactions, facilitate storage, and lower transportation costs. Selection of the suitable drying process is critical as it affects retention of bioactive compounds and other organoleptic characters of natural product. Freeze

drying or lyophilization is the most recent procedures for the drying of natural products however their application in drying of latex hasn't been explored yet.

Freeze drying or lyophilization is an effective procedure which has been recently utilized during post collection mainly to enhance the shelf life of natural products. Freeze drying involves sublimation process, in which structural changes are minimized and bioactive compounds present in dried sample are retained (Ceballos et al. 2012). This process involves three major steps: product freezing, primary drying (removing the ice by direct sublimation under reduced pressure), and secondary drying (release of unfrozen water by desorption and diffusion) (Geidobler & Winter 2013). In addition to its merits this process also has major disadvantage as it may cause damage to the intrinsic bioactive compounds which always results in products with different characteristics. Thus, this process requires careful optimization to improve the retention bioactive compounds and prevent its successive damage (Akdaş & Başlar 2015; Salazar et al. 2018). Freezing is the initial step of freeze-drying (during which the ice is formed) which includes three main stages (nucleation, growth of ice crystals, recrystallization) (D'Andrea et al. 2014). Rate of freezing always determines final properties (productivity and quality) of the dried product, as it affects the pore size, or inherent structure, primary drying rate, and rate of nucleation (Grajales et al. 2005; Franceschinis et al. 2015). Freezing variables such as variation in pressure, nucleation temperature, surface freezing, annealing, vacuum-induced freezing, and addition of nucleating agent can be controlled to get more process benefits mainly to accelerate the subsequent ice sublimation process (Kramer et al. 2002; Oddone et al. 2014; Oddone et al. 2016; Liu et al. 2005; Rezende et al. 2018).

Bio-integrity of investigated samples with outstanding characteristics can be preserved by freeze–drying mediated encapsulation which is always performed at low temperatures. Based on nature of products, particularly in case of plant-based products such as extracts, exudates, latex, natural polysaccharides working conditions of freeze-drying varies or optimized. Moreover, mere lyophilization is not effective to extend the shelf life of extract. An effective procedure microencapsulation of dried latex by a polymer or blend of polymers is required to prevent its degradation (Carpentier et al. 2007). Freeze drying is the most suitable technique for dehydration of all heat-sensitive materials and for microencapsulation (Desai & Park 2007). Microencapsulation procedure involves the encapsulation of sample by using bio-compatible, non-toxic, and edible material to form stable capsules with several useful properties. This procedure is used to enhance the stability of encapsulated material by protecting them from adverse environmental conditions. Homogeneous or heterogeneous material can be used to develop an efficient process however selection of the encapsulation agent is usually depending on the final application of the encapsulated material (Mahdavee et al., 2014). Various encapsulating agents and their blends have reported with key characteristics such as their ability to form films, biodegradability, and resistance to gastrointestinal tract, viscosity, solids content, hygroscopicity, and cost. However, some of the basic characteristics are essential such as it should colorless and provides good protection against oxidation. Current study is designed for the long-term preservation of latex at different conditions to encapsulate latex as a core material and to further retain and protect more sensitive bioactive compounds present in it (Zhu et al. 2019; Gomes et al., 2018). Herbal treatment for headache disorders is considered as an ancient treatment which is now increasing worldwide (Levin 2012). Vast majority of episodic headaches is migraine which is now considered as most common disabling brain disorder characterized by a pulsating headache affected an estimated population of 12% worldwide (Yeh et al. 2018; Mirshekari et al. 2020; Song et al. 2021; Tirumanyam et al. 2019; Kinawy 2019). One of the most promising tools to treat

migraine patients are herbal products (D'Andrea et al. 2014). Calotropis genus plants (*C. procera and C. gigantea*) have been traditionally used to cure migraine (Ahmed et al. 2005). Leaves are externally applied to treat headache in Malaysia (Lin 2005). Leaves of *C. procera* are effective in treating migraines (Prasad 1985). *C. gigantea* (Ait.) R.Br. (Apocynaceae) commonly known, as 'Akra' is a traditional medicinal plant which is used in many ayurvedic formulations like Arkelavana. This plant is identified as ‘milkweed’ as it’s abundantly available and contains latex in its leaf and stem. It has been observed that parts of this plant are traditionally used to treat headache disorders (Pathak & Argal 2007). This milky fluid is a complex mixture of various bioactive compounds such as cardiac glycosides which show diverse biological activities (Deshmukh et al. 2009; Rajesh et al. 2005).

Nevertheless, the chemical profile and stability of latex remains under mystery. One of best ways to identify the cause behind degradation is to investigate degradation products those are degraded due to the oxidative cleavage of the double bond in the polymer backbone. Certain intrinsic and extrinsic factors such as treatment with solvents, pH and temperature variation, oxygen, light, and enzymes can affect the overall therapeutic potential of latex. Post collection immediate treatment or processing is required to overcome these problems. In this respect freeze drying gained importance as it maintain the inherent structure (i.e., pore morphology etc.) of the sample with minimal shrinkage, retain bioactive compounds and their physico-chemical properties and improve rehydration behavior of the sample. Due to their different operating procedures, they always result in products with different characteristics (Çam et al. 2014). Thus, by selecting a suitable procedure and appropriate conditions, the final product quality can be handled (Hamrouni-Sallami et al. 2011).

An objective of the present work is to improve the shelf life of freeze-dried alkali treated *C. gigantea* latex and evaluate its anti-migraine potential. For the first-time freeze-drying process with certain modifications was used for latex to limit oxidative changes of chemical metabolites. Stability studies of FDCG were performed till 10 weeks and it was compared with non-lyophilized samples, sun rays dried sample from *C. gigantea* milk (AECD). Several physico-chemical parameters such as product rehydration capacity, water activity, hygroscopicity, solubility, the total color difference (ΔEab), total polyphenols content (TPC), core phenolic content (CPC), moisture content and microencapsulation efficiency were determined to evaluate the quality of dried samples. Further anti-migraine potential of FDCG and AECG was evaluated by apomorphine induced climbing behavior, *l*-5-HTP induced syndrome and MK 801 induced hyperactivity assays.

Materials And Methods

2.1. Chemicals, reagents, and encapsulating agents

All chemicals were of analytical grade or of HPLC purity standard. Dizocilpine (MK-801), Apomorphine, 5-HTP (5-hydroxy-L-tryptophan), methysergide (MSG) and Pargyline hydrochloride were purchased from Sigma-Aldrich, India. The encapsulating agent, chitosan (CS) was procured (Central Institute of Fisheries Technology, Cochin, No. F.1 (2)/BP/Chitin/12) and the molecular weight CS 39,000 da was reduced, with 95% deacetylation). Materials were lyophilized in a Heto LyoPro 3000 (Heto-Holten A/S, Allerod, Denmark) with collector temperature − 50°, ice holding capacity 5l, refrigerant HFC type R507, analogue controller with ‘Alarm-Wait-Okay’ status indicator and temperature read-out.

2.2. Plant material

Plant material was collected in month of July (2019) from our medicinal garden (Amity university Haryana, 28.1518° N, 76.7178° E) cleaned with sterilized water to remove extraneous material. For taxonomical analysis washed plant material was kept in 4% formalin solution. Voucher specimen (AIP-CG-2019-01) of herbarium was submitted to Amity University Haryana, Gurgaon, India. Milky exudate in form of crude latex was aseptically collected from the aerial parts of collected plant material.

2.3. Extraction method

Latex was aseptically collected from the aerial parts of mature *C. gigantea* plant and then transferred to petri dishes (100 x 15 mm) to prepare dried sample under sunlight (ADCG) and freeze-dried microencapsulated latex (FDCG) of *C. gigantea*. To prepare dried latex 250 ml of milk was transferred into glass container with glass lid [L (21 cm) X W(15 cm) X H(7 cm)] and dried under sunlight (93°F- 97°F in July, Gurgaon) till 3 days. After being exposed to sunlight, it turns into a dark brown and solidified substance to yield 37 g of latex. The sample was stored at room temperature in amber flask container until further analysis.

For FDCG preparation aseptically collected fresh milk (250 ml) was immediately treated with 1% solution of sodium hydroxide (1g/100ml). This dilution of latex was mixed with 2% chitosan solution (CH) (prepared in 1% glacial acetic acid). Mixture was stirred for 15 min at 600 rpm in a rotor–stator. Solution obtained was kept overnight at − 17◦C and lyophilized in a freeze dryer, Heto LyoPro 3000 (Heto-Holten A/S, Allerod, Denmark) and T = − 50.7°C, pressure of 7.1 mbar, vacuum of 0.58 mbar for 48 h. The freeze-dried encapsulated latex was converted into powder with help of a pestle and mortar and passed through sieve. The sample was stored at room temperature in amber flask container until further analysis. All analyses were performed in triplicate (Rezende et al. 2018; Hussain et al. 2018).

2.4. Qualitative phytochemical Screening

Phytochemical screening was done to identify nature of secondary metabolites present in the dried latex. The prepared samples (ADCG & FDCG) and other fractions obtained from different solvents were investigated for the presence of various chemical constituents by using different phytochemical tests. For test sample preparation ADCG & FDCG (25 mg/mL) were dissolved in 95% methanol and ultrasoinicated for 30min. After adding specific reagents in the test solutions, color change or precipitate formation was observed by using the standard colorimetric procedures as described by Sofowora and Kennedy and Thorley (Sofowora 1993; Kennedy & Thorley 2000). For qualitative estimation of tannins and phenols, 3 mL of the test samples was treated with 60 µL of 2% $FeCl_3$ in ethanol. Blue precipitated material indicated soluble tannins and a green color indicated condensed or cachectic tannins whereas blue-red soluble phase indicated phenols. Sterols and triterpenes were determined by Liebermann-Burchard test in which test sample 250 mg/5 mL chloroform was treated with 1 mL of acetic anhydride and 60 µL sulfuric acid. Brown-red color indicated triterpenes whereas green color indicated free sterols (Jucá et al. 2013; Matos 1997). Killer killiani test was used to detect the presence of cardiac glycosides in dried latex samples.

2.5. TPC and TFC

The total phenolic content (TPC) of the samples was determined as per Folin-Ciocalteu spectrophotometric method (Kim et al. 2003; Kim et al. 2012) with slight modifications. The absorbance was measured at 765 nm and total phenolic content was expressed as the gallic acid equivalents (GAE) 100 g^{-1} dry weight (DW) of

latex. The total flavonoid content (TFC) was measured by a colorimetric method (Singleton et al. 1999; Singleton & Rossi, 1965) with sight modifications. The absorbance was measured at 415 nm and total flavonoids were expressed as the catehin equivalents (CE) 100 g^{-1} DW of latex.

Additionally, core (CPC) and surface phenolic content (SPC) of the microencapsulated sample and encapsulating efficiency were also determined by Slinkard and Singleton (1977) method (Slinkard & Singleton 1977). Further Saenz et al. (2009) method was used for extraction (Saenz et al. 2009). For the CPC, sample (100 mg) was dispersed in ethanol, acetic acid and water in ratio of 40:6:32. Then vortexed mixture was (Tarsons, India) filtered through filter of size 0.45 µm. For the SPC, sample (100 mg) was dispersed in ethanol and methanol (1:1) mixture. The vortexed mixture was subjected to filtration as mentioned above. The results were expressed as milligram of gallic acid equivalent per hundred grams (mgGAE/ 100 g, dry weight). The encapsulating efficiency was determined by using the given Eq. (1):

$$\text{Encapsulating efficiency}\ (\%) = \frac{\text{CPC}-\text{SPC}}{\text{CPC}} \times 100 \quad (1)$$

2.6. Kinetic studies of Freeze-dried C. gigantea milk

For kinetic studies sun rays dried and freeze-dried *C. gigantea* milk samples (ADCG & FDCG) were stored in transparent container with at 40^{o}C. 20% relatively humidity (by hygrometer) and illumination (by using 36 W 4ft tube light with illumination of 2700 lumens) was maintained till 10 weeks to evaluate the effect of this storage conditions over physical properties of ADCG and FDCG. Powder degradation in the presence of above-mentioned storage conditions was expressed as coloring strength (E). FDCG and ADCG samples were periodically examined (at 2 weeks interval for powder degradation kinetic analysis) by measuring absorbance in aqueous solution (100 mg in 10ml, stirred for 10 min). The absorbance was measured with a spectrophotometer (Cary 4000, UV-Vis, Agilent Technologies UV VIS Spectrophotometer) at max = 400 nm, the maximum absorption wavelength of cardenolides. UV absorbance spectra were recorded from 200 to 400 nm. Each measurement was carried out in triplicate. Coloring strength, (CS), was determined by using following Eq. (2):

CS1% max = AV / pdC (2)

where A: absorbance at the max, V is the amount of solvent added (mL), p is the weight of the sample (g), d is the pathlength of the cell (cm) and C is a constant, (c = 100 cm^2/g). First-order reaction model was applied to determine reaction rate constant (k) and half-life period ($t_{1/2}$) (Alonso et al. 1990).

2.7. Determination of the physico-chemical properties of FDCG and AECG

To measure the effect of storage conditions over the physico-chemical properties of samples, they were stored up to 10 weeks. Sampling was done at the interval of seven days.

2.7.1. Color variation during storage

Color is an important characteristic of products especially powders. Variation in color of product at the specific storage conditions is always related with product composition due to chemical or biochemical degradation reactions. Colorimeter was used to determine color variation against same storage conditions. Samples were withdrawn at periodic level (after 2 weeks interval) to determine the color variation. Color measurements were instantly done after freeze and sun rays drying (zero time) and after 10 weeks of storage at 40 °C. Test samples (FDCG & ADCG) (0.1g) were weighted and dissolved separately in 50 mL of distilled water. Resulting solution was stirred for 1 h at 700 rpm and further centrifuged at 7000 rpm for 15 min in an Eppendorf tube. Finally, the supernatant was collected and placed in quartz cells for the measurements. Following parameters were used to calculate the cylindrical parameters (Croma, C∗) and Hue angle according to Eqs. (3 & 4). Hue angle (H°) indicates the color of the sample (0 or 360 = red, 90 = yellow, 180 = green, and 270 = blue), while Chroma (C) indicates color's purity or saturation. Color variation of FDCG & ADCG was measured by using color parameters such as: L∗ (lightness), a∗ (+ a∗ = red e − a∗ = green), and b∗ (+ b∗ = yellow e − b∗ = blue). This is done by using a spectrophotometer (Model CM-3600A, Konica Minolta, Osaka, Japan). Each measurement was carried out in triplicate. Color parameters and variation in color was examined by using following equations (3 & 4) (Cai & Corke 2000):

$C^* = [(a)^2 + (b)^2]^{1/2}$ (3)

$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ (4)

2.7.2. Moisture content determination

AOAC (2012) procedure [method No. 943.06 (Sect. 37.1.10B)] was used to determine the moisture content in test samples. This was carried out by calculating weight loss after heating the samples in hot air oven at 105°C (AOAC 2012).

2.7.3. Water activity

Electronic meter (Rotronic-HC2-Aw, Rotronic Measurement Solutions., Switzerland) was used to measure water activity (w). Direct readings were obtained once test samples were stabilized at 25°C for 10 min.

2.7.4. Solubility

Cano-Chauca et al. (2005), procedure with certain modifications was used to determine the solubility. Test samples (1g) were dissolved in 100 mL of distilled water. Resulting solution was stirred in a magnetic stirrer (MS 500, Remi, India) for 30 min and the subjected for centrifugation at 1500 rpm for 10 min at ambient temperature. Supernatant (25mL) was separated and transferred to preweighed petri dish. This is followed by the immediate drying by hot air oven at 105°C for 5 h. Solubility was determined by weight difference and expressed in percentage (%) (Cano-Chauca et al. 2005).

2.7.5. Hygroscopicity

Cai & Corke (2000) method with slight modifications was used to determine hygroscopicity. At 25°C test samples (1g) were desiccated with Nacl solution (75.3%). Hygroscopicity was determined in percentage (%) of adsorbed moisture (Cai & Corke 2000).

2.7.6 Rehydration Capacity

Rehydration capacity (RC) is the percentage weight of rehydrated sample upon dry sample. (Eq. 5). Mothibe et al. method (Mothibe et al. 2014) with slight modifications was used to perform rehydration experiments. Test samples (1g) were kept in 100 ml of water (DDW) at 35°C for 10 min. DDW treated samples were drained over mesh for 5 min. Finally, its weight was evaluated.

$$RC = \frac{mf}{m0} \quad (5)$$

2.8. Biological activity

2.8.1. Animals and treatments

Swiss albino mice (male 25–35 g weight) were obtained from the animal house of our institute. For one week before the experiment, the animals were kept in a room at 24–28°C, relative humidity 60–70% with artificial 12:12 h light: dark cycle in ventilated plastic cages (30 cm × 43 cm × 15 cm) with 6 animals per cage. Animals were fed with a standard pellet diet and sterile water was supplied *ad libitum.* The animal cages were regularly cleaned. Animals were randomized into treatment groups. Experimental procedures were approved by the Institutional Animal Ethical Committee Animal Ethics Committee (Institutional Ethics Committee-IAEC/ABMRCP/2018–2019/23). These procedures were performed in the light phase and the mice were not fasted prior to drug treatments and not reused for experiments. Mice were deprived of food but not water prior to administration of the test extracts.

2.8.2. Preparation and Dosing

FDCG and ADCG (test solutions) were freshly prepared on each day by using 0.1 % Tween 80. Test solutions were administered to mice. The dosing of animals was based on the size of the experimental animals. The volume of the vehicle used was 0.1 ml/10 g mice. Injection was administered slowly orally for the test doses, while intra-peritoneal route was used for the administration of doses.

2.8.3. Acute toxicity studies

Acute oral (p.o.) toxicity study was carried out according to the procedure of the limit dose test of Up-and-Down method. Female albino mice (25-35g) were used for acute toxicity study (Cai & Corke 2000; AOAC 2012; Cano-Chauca et al. 2005). The study was carried out as per the Organization for Economic Co-operation and Development (OECD) guidelines for the evaluation of acute oral toxicity (OECD, 2001). The animals were housed in a cross-ventilated room (12:12hr) and at constant temperature (22 ± 2.5°C) conditions. Earlier reports suggested that ADCG does not cause subacute toxicity up to the level of 3000 mg/kg body wt./day for 28 days. Limit test was performed at 3000 mg/kg p.o. as single dose and albino mice were kept without food for 3–4 h prior to dosing but had access to water *ad libitum.* The dose was administered to albino mice according to body weight. The animals were closely observed for first 30 min, then for 4 h. Food was provided after 1–2 h of dosing (Jacks et al. 2004; Nwafor et al. 2004; Lorke 1983).

2.8.4. In vivo studies

For *in vivo* studies appropriate doses for antagonists were selected from literature (Hans 2002) as well as pilot experiments, and doses that do not modify immobility were used. Effect of freshly freeze-drying *C. gigantea* (FDCGA), 10 weeks stored freeze-drying *C. gigantea* (FDCGA), freshly sun-drying *C. gigantea* (ADCGA) and 10

weeks stored sun-drying *C. gigantea* drug sample were evaluated against different experimental models including apomorphine-induced climbing behavior, l5HTP induced head twitches and MK 801 induced hyperlocomotion in mice.

2.8.4.1. Apomorphine-induced Climbing Behavior in Mice

Cage used in this study is made up of wire mesh cages with dimensions 10(L) × 10(W) × 20(H) cm of 0.4 mm thick wire and 4 mm mesh size. Mice (90) were randomly divided in to fifteen groups (n = 6). Groups I received the vehicle (0.1% Tween 80 solution; 10 ml kg^{-1}, p.o.), Group II received haloperidol (1 mg/kg), Groups III received Chitosan (2%), Groups IV-VI received FDCGA (50, 100 and 150 mg kg^{-1}), Groups VII-IX received FDCGB (50, 100 and 150 mg kg^{-1}), Groups X-XII received ADCGA (50, 100 and 150 mg kg^{-1}) and Groups XIII-XV received ADCGB (50, 100 and 150 mg kg^{-1}). All the animals were treated with apomorphine (30 mg/kg, s.c.). Immediately after, each mouse was placed at the bottom of the cage for one hour prior to the experiment. Climbing behavior was observed at 5-minute intervals till 20 minutes, starting 10 minutes post apomorphine administration. To measure climbing index following scoring system was used: 0- four paws on the floor (no paws on the vertical bars), 1-two paws on the cage, 2-four paws on the cage i.e., four feet holding the vertical bars. Study was carried out till 8 days (Jeong et al. 2007).

2.8.4.2. l5HTP induced head twitches in mice

*l*5HTP induced head twitches (*l*-5-HTP-induced behavioral stimulation) were performed to assess the antiserotonergic activity of test samples. Animals were placed in perspex cage for half an hour of habituation period and syndrome was measured. Mice were pretreated with pargyline (75 mg/kg i.p.) which is a monoamineoxidase inhibitor to prevent rapid degradation of *l*-5-HTP. Thirty minutes later, vehicle (saline 10 ml/kg i.p.), methysergide (MSG) (10 mg/kg i.p.) FDCGA (50, 100 and 150 mg kg^{-1}), FDCGB (50, 100 and 150 mg kg^{-1}), ADCGA (50, 100 and 150 mg kg^{-1}) and ADCGB (50, 100 and 150 mg kg^{-1}) were administered as it was done in above mentioned assay. After 30 minutes of interval MSG and saline treated mice administered with *l*5HTP (50 mg/kg i.p.) whereas FDCG and ADCG treated animals administered with *l*5HTP (50 mg/kg i.p., 1 h later). Fifteen minutes later mice were transferred to the cage and serotonin syndrome was assessed by monitoring behavioral parameters, such as tremors, hind limb extension, forepaw treading, head weaving and mainly head twitches till 50 min. Following score system was used to assess behavioral changes at each 10 min interval: 0- absent, 1- moderate, 2- marked (Hans 2002).

2.8.4.3 MK 801 Induced Hyperlocomotion in mice

Actophotometer (Medicraf, Inco Model 6006D) was used to measure hyperlocomotor activity in mice. Mice were divided into fifteen groups (n = 6). Groups I received the vehicle (0.1% Tween 80 solution; 10 ml kg^{-1}, p.o.), Group II received haloperidol (1 mg/kg), Groups III received Chitosan (2%), Groups IV-VI received FDCGA (50, 100 and 150 mg kg^{-1}), Groups VII-IX received FDCGB (50, 100 and 150 mg kg^{-1}), Groups X-XII received ADCGA (50, 100 and 150 mg kg^{-1}) and Groups XIII-XV received ADCGB (50, 100 and 150 mg kg^{-1}). Mice were allowed to acclimatize for 5 min. To assess basal locomotor activity mice were placed in the Actophotometer for 30min. After determining basal locomotor activity score, mice were administered respective drugs as per groups. One hour later after the administration of drugs, animals were treated with MK-801 (0.5 mg/kg, i.p.). For intraperitoneal (i.p.) administration MK-801 was dissolved in saline. López-Gil et al. 2007 report was

referred for the dose selection of MK-801 (López-Gil et al. 2007). Then mice were immediately placed again in the actophotometer for measuring the locomotor activity score at every 20, 40, 60, 80, 100 and 120 min for 10 min. Total photobeam interruption counts at 20 min interval was monitored and expressed as mean change in the locomotor activity (Chung et al. 2002; O□Neil & Shaw 1999).

2.9. Statistical Analysis

Data was presented as mean ± S.E.M and analyzed by one-way analysis of variance (ANOVA) followed by Dunnets test. $P < 0.05$ is considered significant. Statistical analysis was done with Graph Pad Prism 5 software

Results And Discussion

3.1. Phytochemical profile

C. gigantea latex is an important source of phytochemicals. *C. gigantea* latex derived freeze dried and microencapsulated ADCG and FDCG test samples were prepared by using CS. Molecular weight of CS 39,000 da was reduced, with 95% deacetylation to control properties like particle size and. size distribution. ADCG and FDCG test were qualitatively evaluated to detect alkaloids, saponins, triterpenes, flavonoids, glycosides, steroids, tannins, and phenols. Other fractions (acetone, ethanol, aqueous, n-hexane) were also considered for phytochemical investigation to check the presence of secondary metabolites and compare it with dried latex preparations (FDCG and ADCG). Steroids were detected in all the fractions whereas phenolic content was only absent in aqueous fraction. Flavonoids and glycosides were absent in aqueous and n-hexane fractions. The phytochemical investigation of *C. gigantea* revealed the presence of alkaloids, cardiac glycosides, tannins, flavonoids, sterols and/or triterpenes in prepared samples (FDCG and ADCG) (Table 1).

Table 1
Preliminary phytochemical screening of fractions of *C. gigantea* latex

Fractions	Yield (%)	Secondary metabolites						
		Alkaloids	Triterpens	Flavonoids	Steroids	Tannins	Phenols	Glycosides
Ethanol	2.1	+	+	+	+	+	+	+
Aqueous	32.1	-	-	-	+	-	-	-
Acetone	49.7	+	+	+	+	+	+	+
n-Hexane	35.2	-	-	-	+	-	+	-
FDCG*	-	+	+	+	+	+	+	+
ADCG*	-	+	+	+	+	+	+	+

+ = present, – = absent; *FDCG and ADCG samples were treated with methanol (95%)

3.2. TPC and TFC

TPC and TFC were determined in FDCG and ADCG samples depending upon treatment as shown in Table 2. Results of multivariate dispersion analyses showed that both type of treatment and samples were considerably

affected TPC and TFC ($p < 0.05$). The highest content of phenolic and flavonoid compounds was determined in FDCG samples (Table 2).

Table 2
Total phenolic and flavonoid content in samples depending upon treatment

Samples	TPC, mg GAE 100 g^{-1} DW	TFC, mg CE 100 g^{-1} DW
Fresh	23.31	11.51
FDCG	25.31	14.51
ADCG	7.21	2.32
Frozen	13.7	6.5

3.3. Encapsulating efficiency, CPC and SPC

CPC, SPC and encapsulating efficiency of FDCG were found to be 68.30 mgGAE/100 g, 10.17 mgGAE/100 g and 85.10%. Chitosan (2%) encapsulated freeze dried FDCG sample under optimized conditions offered high CPC values than SPC with high encapsulating efficiency.

3.4. Storage stability evaluation

3.4.1 Kinetic studies

First-order reaction kinetics was used to determine the relationship between coloring strength (E) vs time (in weeks). A linear relationship was observed. Figure 1 represents plot between ln(E) vs time, implying first-order reaction kinetics for cardenolides coloring strength degradation (Fig. 1). Table 3 represents rate constant (k) and half-life period ($t_{1/2}$) of the microencapsulated powders. AECG powdered samples have showed higher reaction rate constant and, consequently, shorter half-life [k ($week^{-1}$): 0.187; $t_{1/2}$ (weeks): 21.27]. Overall, FDCG samples showed the greatest protection against stability accompanied also by high half-life period ($t_{1/2}$) with values of 54.13 for cardenolides.

Table 3
Regression analysis of coloring strength in freeze dried microencapsulated samples (ADCG and FEDCG) prepared with different agents during storage at 40 ∘C for 10 weeks.

Samples	k ($week^{-1}$)	$t^{1/2}$ (weeks)	R^2
AECG	0.187	21.27	0.967
FDCG	0.067	54.13	0.991

3.4.2. Physico-chemical properties of FDCG and ADCG

Physico-chemical properties mainly moisture content, water activity (aw), and hygroscopicity are an important characteristic of powder. These properties mainly affect reconstitution of the powder.

3.4.2.1. Color stability of ADCG and FDCG

Color of ADCG and FDCG was examined to study the effect of storage conditions on the color of ADCG and FDCG. The color parameters (a*, b*, L*, C* and E*) of the microencapsulated ADCG and FDCG immediately after production and after 10 weeks of storage at 40 ◦C are presented in Fig. 2. Based on the initial values of a* and b* parameters, in ADCG case, presented a brown color in comparison with FDCG which showed pale yellow color. AECG which present higher moisture uptake, get dark (browning) as a function of the storage time (Fig. 2). The moisture content could affect the mobility of the molecular system which is directly related to the velocity of the degradation reactions. Color variation among the samples is due to the different procedure of drying were followed. In addition, FDCG sample is microencapsulated whereas ADCG sample is not encapsulated with chitosan. Due to these reasons ADCG sample showed more degradation (browning, had a high value of color parameter a*). This was observed after 10 weeks of storage, the parameter a* showed an increase in AECG presenting the t ($P < 0.05$) increase of 39.21%. A significant reduction in lightness, expressed by L* parameter, was also observed in all ADCG samples. After 10 weeks of storage FDCG samples showed lesser variation (6.5%) in chroma. The H° values varied from 57.78 to 78.60 and this confirmed the tendency of tonality for the samples to the red and yellow hue. C was high for the latex dried powder (AECG), which means that this sample have higher saturation or color purity, which is a desirable characteristic. Figure 2 shows that the drying process resulted in powders different from control ($\Delta E > 1.5$). ADCG samples showed highest ΔE value. A high value of ΔE means more color changes during treatment or storage (Maskan 2006). Due to variation in ΔE which is related to the color variation (initial and final time), AECG samples suffered significant changes during storage.

3.4.2.2. Moisture content

In the case of ADCG sample, moisture content varied from 3.12% (first day of storage) to 7.05% (last day of storage), however there was no significant difference ($p \leq 0.05$) between percentage of moisture content of FDCG samples (4.3–4.5%) for 10 weeks (Fig. 3A).

3.4.2.3. Water activity

aw values of the chitosan treated sample (FDCG) ranged from 0.07 to 0.26 (Fig. 3B). FDCG samples are within the normal range and within the recommended limit to ensure powder stability (< 0.30). However, aw values of the ADCG were not in normal range (0.33–0.62) (Fig. 3B).

3.4.2.4. Solubility

In current investigation drying procedure had no considerable ($p > 0.05$) effect on the solubility of the powders (99.00 to 99.10%) (Fig. 3C).

3.4.2.5. Hygroscopicity

In current study hygroscopicity values varied from 9.24–12.46% (Fig. 3D). This represents low hygroscopicity of powders which allows effective preservation of sample and its bioactive compounds.

3.5. Acute toxicity studies

Single oral treatment of mice with 3000 mg/kg body weight of FDCG and ADCG produced no death within the short- and long-term outcome of the limit dose test of Up-and-Down procedure. Observed behavioral manifestations include dyspnea, restlessness/agitation, generalized body tremor, feed and water refusal within 24 h post-treatment p.o. These manifestations gradually subsided after 24 h. The LD_{50} was estimated to be greater than 3000 mg/kg body weight/oral route.

3.6. Apomorphine induced climbing behavior

Current study demonstrated that antimigraine potential of FDCG (A and B) and ADCG (A and B) samples of *C. gigantea* in models of positive, negative, and cognitive symptoms of migraine. Test samples showed a positive effect in all tests performed. Classical test i.e. apomorphine mouse climbing test was performed to evaluate antipsychotic effect of samples. Apomorphine (3 mg kg^{-1}) was used to induce characteristic climbing response in mice. Apomorphine induced marked climbing behavior was inhibited by test samples and the reference drug, haloperidol. Apomorphine is a non-selective dopamine agonist which activates both D2 and D1 receptors to induce stereotype behavior such as locomotor hyperactivity, climbing, grooming, licking, and gnawing. This apomorphine-induced stereotypic reaction to a stressful stimulus is also common in migraine patients. The assay is mainly based on the dopamine theory of migraine suggesting dopaminergic activation is a primary pathophysiological component in migraine.

FDCGA (50–150 mg/kg), FDCGB (50–150 mg/kg), ADCGA (50–150 mg/kg), ADCGB (50–150 mg/kg), and haloperidol (positive control) significantly inhibited apomorphine induced climbing behavior in mice ($P < 0.01$) on day 1 and day 8 when compared to control and chitosan (2%). FDCGB 150 mg/kg samples showed significant reduction in average time spent in climbing then all ADCGA samples. FDCGB samples showed improved pharmacological activity despite of its storage for 10 weeks whereas ADCGB samples lost its therapeutic potential during same storage time (Fig. 4). ADCGB samples produced non-significant results at any of the test doses on day 8 (Fig. 4). FDCGA and FDCGB samples significantly inhibited climbing in a dose dependent pattern with a maximum inhibition by 150mg/kg (Fig. 4).

3.7. l-5-HTP induced Serotonin Syndrome

When compared to control and chitosan (2%), methysergide (positive control), FDCGA (50, 100 and 150 mg kg^{-1}), FDCGB (50, 100 and 150 mg kg^{-1}), ADCGA (50, 100 and 150 mg kg^{-1}), ADCGB (50, 100 and 150 mg kg^{-1}) showed decrease in the number of head twitches induced by *l*5HTP ($P < 0.05$) on day 1 and 8 (Fig. 5). On day 8, FDCGB 150 mg/kg samples showed significant ($p < 0.001$) decrease in the number of head twitches on day 1 and 8 when compared to control, chitosan (2%) and other test groups. The *l*-5-HTP induced behavioral syndromes except for hind limb extension were very significantly reduced by all three doses of FDCG (50, 100 and 150 mg kg^{-1}).

3.8. MK 801 Induced Hyperactivity

MK-801 administration resulted in a significant ($P < 0.001$) increase in immobility duration from day 1 to day 8 in comparison to normal control. FDCGA (50, 100 and 150 mg kg^{-1}), FDCGB (50, 100 and 150 mg kg^{-1}) and methylsergide (positive control 1 mg/kg i.p.) significantly inhibited MK 801 induced hyperactivity ($P < 0.01$) compared to positive control animals. However, administration of ADCGA (50, 100 and 150 mg kg^{-1}) and ADCGB (50, 100 and 150 mg kg^{-1}) caused no significant effect on the inhibition of hyperactivity induced by

MK 801 which corroborate that deep freeze encapsulation procedure might have protective effect on phytochemicals present in test samples which can prevent its degradation. The inhibition by FDCGB followed a dose dependent pattern with a maximum inhibition by FDCG 150 mg kg^{-1} (Fig. 6).

NMDA receptor present in nerve cells is a site to control the development of various psychological disorders. NMDA receptor antagonist, MK801 stimulates locomotor activity by increasing dopamine and serotonin metabolism. Thus, this may further increase neurotransmission of dopamine and serotonin in brain. Increase in release of these transmitters causes hyperactivity or increase in locomotory activity. Thus MK-801 stimulates dopamine and serotonin release in brain which further stimulates hyperactivity. This parameter was utilized to assess possible the serotonin–dopamine antagonistic (or atypical or second-generation antipsychotics) action of dried latex samples to further antagonize MK801 induced hyperactivity (Yan et al. 1997). Jackson et al. 2004 suggested that lower doses of MK-801 are sufficient to induce stereotypies and increases in pyramidal cell firing (Jackson et al. 2004). However, such dose failed to induce changes in extracellular glutamate (López-Gil et al. 2007). FDCGA and FDCGB samples significantly inhibited MK-801 induced hyperactivity, which corroborates their role as atypical or second-generation antipsychotic drug. This type of work is not reported thus earlier reports on antipsychotic action of FDCG and ADCG samples are not cited. However, reports on its effect on decreasing the marker neurochemical enzyme activity in scopolamine and ECS-induced amnesia model suggested the role of *C. procera* dry latex in cognition enhancement (Malabade & Taranalli 2015).

The histological observation of hippocampus region of brain showed the pathophysiological impact of various treatment including haloperidol and drug treatment with various concentrations (Fig. 7). The stained sections showed a clear and dense membrane of neurocytes in normal control and chitosan treated animals. However, the administration of haloperidol significantly disrupts hippocampal region as shown in Fig. 7. The photomicrograph of hippocampus of FDCG-A/B, ADCG-A/B (50, 100 and 150mg/kg) treated mice showed increased thickness of pyramidal cell layer in the CA3 region in dose dependent manner against haloperidol induce toxicity at 8th day.

Discussion

4.1. Phytochemical profile

Results obtained from phytochemical investigation were found to be similar in comparison to other reports on the chloroform fraction obtained from latex of *C. gigantea* in which all secondary metabolites were present except saponins and tannins (Ishnava et al. 2012). Phytochemical screening reported by Radhakrishnan et al. 2017 revealed the absence of alkaloids in methanolic fraction of latex obtained from *C. gigantean* which is contradictory to present work (Radhakrishnan et al. 2015).

4.2. TPC and TFC

Drying or processing of plant-based products is one of the oldest methods for extending their shelf life to further extend their availability throughout the year (Ahrne et al. 2007). Several drying or processing procedures can result in the leaching of bioactive compounds which may result in significant loss of phenolic content in all studied materials. One of the major causes of degradation is environmental stress that further causes the

release of active enzyme. These enzymes could cause enzymatic degradation and further affect bioactive compound content. Thus, enzymes are usually inactivated during procedures mainly due to decreased water activity (Lin et al. 2012). Hossain et al. (2010) study showed that drying process makes the plant tissue more brittle, which leads to rapid cell wall breakdown during the extraction procedure (Hossain et al. 2010). Several reports revealed that drying procedures reduced TPC and TFC content of plant product however process should be optimized in low temperatures treatment to reduce this degradation up to minimum extent (Chan et al. 2013; Ahmad-Qasem et al. 2013).

Post treatments of latex such as lyophilization, sun drying, and cold considerably affect phenolic and flavonoid content as mentioned in Table 2. These procedures significantly affect the leaching of bioactive compounds which can ultimately affect the total bioactive content in the samples. FDCGB sample (collected after 10th week of storage period) underwent extreme freezing which may allow significant retention of phenolic and flavonoid compounds in the samples. Due to this FDCG showed more content of phenols and flavonoids in comparison to fresh samples. Ibrahim et al. 2013 report on *Streblus asper* leaves revealed that ethanol extract of the freeze-dried samples exhibited higher phenolic and flavonoid content than the aqueous extract (Ibrahim et al. 2013). Gomes et al 2018 report suggested that freeze-drying is more suitable than spray drying to produce papaya powders, since these techniques retain nutrients of fresh papaya, making them viable options for pulp processing (Gomes et al. 2018). Similarly, Mphahlele et al., 2016 study suggested that freeze dried sample of pomegranate peel significantly retained bioactive compounds such as phenolic, tannin and flavonoid (Mphahlele et al. 2016). There are certain reports which also support our present work in which study on sweet potatoes (Yang et al. 2010) and onion (Arslan et al. 2010) fresh samples revealed less TPC, however freeze-dried samples showed the highest TPC. However freezing could not be mere sufficient for the retention of these bioactive compounds as frozen sample showed less phenolic and flavanoid content then freeze dried microencapsulated samples. Microencapsulation by biopolymers is further required to prevent the degradation and improve the bioavailability of the dried samples (Ahmadian et al. 2019; Rezende et al. 2018; Rosa et al. 2019; Yousefi et al. 2019; Mangiring et al. 2018). Our present findings revealed that chitosan encapsulated freeze-dried sample (FDCG) showed more TFC (14.51 mg CE 100 g $^{-1}$ DW) and TPC (25.31 mg GAE 100 g $^{-1}$ DW) content then fresh and sun rays dried samples (ADCD). Results showed that developed optimized freeze-dried procedure resulted in increase in TPC and TFC content. It was observed that phenolic and flavonoid variation in the investigated samples strongly influence color of same samples which supports Al-Farsi et al. 2018 study (Al-Farsi et al. 2018).

As discussed in the previous report's phenolic compounds from the plant material are not solely responsible for the color variation (Singleton & Rossi, 1965; Slinkard & Singleton 1977). Other secondary metabolites present in the plant material can also be responsible for this which could be triggered due to variation in temperature, pH, or by the interaction among different components of the material mainly after harvesting of latex (Cheung & Mehta 2015; Duarte-Almeida et al. 2007).

4.3. Encapsulating efficiency, CPC and SPC

Factors such as type, concentration, physical or chemical properties of polymer used in microencapsulation usually determine CPC, SPC and encapsulating efficiency. Additionally, core to coating ratio, method of encapsulation and drying (spray drying or freeze drying) also affects CPC, SPC and encapsulating efficiency of the powder. As freeze-dried samples are devoid of atomization and any sort of heat exposure. It was also

reported that SPC decrease with decrease in encapsulating agent (i.e. polymer). Thus, high core and coating materials ratio resulted in higher encapsulating efficiency. It was observed by Laine, Kylli, Heinonen, and Jouppila (2008) that freeze dried microencapsulated samples were stable during storage for a longer period (Robert et al. 2010; Ersus, Yurdagel, 2007; Changchub & Maisuthisakul 2011; Deladino et al. 2008).

4.4. Storage stability evaluation

4.4.1 Kinetic studies

As discussed above biopolymers-based microencapsulation is an active process by which bioactive compounds degradation can be prevented by extending shelf life of the final product. Furthermore, reports also supports that the bioavailability of the microencapsulated bioactive compounds is also improved which can results in various therapeutic benefits (Ahmadian et al. 2019; Rezende et al. 2018; Rosa et al. 2019; Yousefı et al. 2019; Mangiring et al. 2018). Previous reports have shown that microencapsulation of saffron petal phenolic extract resulted in the increase of polyphenolic content and antioxidant activity (Ahmadian et al. 2019). On the other hand, nature of encapsulating agent (chitosan) strongly influences stability of coloring strength. Current work proves that addition of chitosan offers significant protection ($P < 0.05$) to latex against storage conditions. The stabilizing effect offered by chitosan on bioactive compounds against different storage conditions is well documented. Chitosan offers physical protection by preventing penetration of oxygen (the most deteriorative agent) and reducing the effect of light (photodegradation), heat (thermal degradation) and moisture (decrease in moisture content enhances the viscosity of the encapsulating agent which helps in maintaining the glassy state) on the encapsulated material. Here in the present study coloring strength of chitosan encapsulated samples (FDCG and AECG) were investigated against sun rays dried samples (ADCG) for 10 weeks at 40°C. Samples were collected at 2 weeks intervals. Samples collected after 10 weeks of study were labeled as ADCGB and FDCGB whereas samples before 10 weeks of storage were labeled as ADCGA and FDCGA (Fig. 1).

Recent finding revealed that spray and freeze-drying procedures followed by the microencapsulation of saffron petal significantly prevent the destruction of antioxidant compounds by environmental factors and increased their bioavailability. Moreover, the release of microencapsulated powder in the simulated system of the digestive system improved the shelf life of the final product (Ahmadian et al. 2019). Rezende et al. reported that microencapsulated extracts from acerola resulted in increase in antioxidant activity (Rezende et al. 2018). Rosa et al. reported that microencapsulation of anthocyanin compounds extracted from blueberry (Vaccinium spp.) by spray drying prevented loss of anthocyanin and its degradation upto great extent. Report also showed that this process resulted in increase in protection and delivery of bioactive compounds (Rosa et al. 2019). Yousefı et al. 2019 study showed that freeze-dried extract-loaded microcapsules were stable during 150 days of storage (Yousefı et al. 2019). Getta et al. reported that maltodextrin affects the water content and solubility of microencapsulated propolis powders. The lowest moisture content and the highest solubility were found in this study (Mangiring et al. 2018).

4.4.2. Physico-chemical properties of FDCG and ADCG

4.4.2.1. Color stability of ADCG and FDCG

As mentioned above color is an important characteristic which can be considered as stability indicator during storage of any product (Cai & Corke 2000). Natural products are made up of different compounds which are vulnerable to oxidation and hydrolysis reactions. This can be examined by color changes. Such product when stored in an open container, the moisture adsorption can change the color of samples. For an instance sample which is stored in open environment absorb more moisture uptake which can result into color change (brown). This feature can be considered as a function of the storage time (Fig. 2) (Maskan 2006).

4.4.2.2. Moisture content

Usually freeze-dried powder contain highest moisture content but here in this case FDCG sample initially present high moisture content due to the encapsulation by chitosan (Fig. 3A) however later on no significant difference has been observed.

4.4.2.3. Water activity

aw values (water activity) i.e., unbound water which is not bound to food molecules can support the growth of microorganisms. aw values of FDCG and ADCG samples are illustrated in Fig. 3B.

3.4.2.4. Solubility

Freeze drying affects the composition and particle size of powder which can ultimately decides its solubility. In addition, selection of the encapsulating agent is very important as it may confer the crystalline state to the dried powder which may ultimately impact solubility (Cortês-Rojas et al. 2015). High solubility of the encapsulating agent increased the solubility of the encapsulated material by offering smaller particle size which can ultimately offers more surface area for hydration (Fig. 3C).

4.4.2.5. Hygroscopicity

FDCG showed the lowest hygroscopicity values, despite having higher moisture contents. This behavior was also observed by Saikia et al. (2015) (Saikia et al. 2015). The lower hygroscopicity values found for the freeze-dried powders can be related to the larger particle size when compared to the sun-dried powder (ADCG) as larger the particle size of powder, confer low surface area and thus present lower water absorption (Tonon et al. 2009) (Fig. 3D).

4.5. Acute toxicity studies

According to the OCDE guideline, any pharmaceutical drug or compound with the oral LD_{50} higher than 2000 mg/kg could be considered safe or low toxic (OECD). Result obtained from previous study suggested that *C. procera* (leaves and rootbarks) aqueous extracts showed LD_{50} higher than 3000 mg/kg (b.w.). It corroborates to the previous studies reported that *C. procera* aqueous extract is non-lethal by oral administration up to the dose of 2000 mg/kg (b.w.) for root-barks extract (Herrera-Ruiz et al. 2007), and the dose of 5000 mg/kg (b.w.) for the leaves extract (Ouedraogo et al. 2013). However, it disagrees with Mbako's group data which obtained a LD_{50} of 940 mg/kg (b.w.) for aqueous extract of the fresh leave of *C. procera* by oral route (Mohammed 2012).

4.6. Apomorphine induced climbing behavior

Apomorphine-induced stereotypic cage climbing in mice is an experimental model for studying changes in dopamine receptor sensitivity (Wilcox et al. 1980). This climbing behavior is reported to due to activation of

both dopamine D1 and D2 receptors (Moore & Axton 1990) and hence D1 and D2 antagonists are effective in this model (Vasse et al. 1998). Apomorphine induced climbing behavior and climbing time was significantly inhibited by FDCG samples in a dose dependent manner (Fig. 4). This finding supports that FDCG samples can effectively works as dopamine receptor antagonist and possibly abort a significant number of migraine attacks in a dose-dependent fashion. Inhibition of apomorphine induced climbing behavior by FDCG samples suggested that FDCG can cross the blood–brain barrier in significant concentrations. Thus, it's concluded that FDCG possessed a similar action as like D1 and D2 antagonists. This potent antidopaminergic effect of FDCG, when compared with ADCG samples, could be due to enrichment and retention of phytochemicals present in the FDCG sample. Taken together, these findings suggest that latex of *C. gigantea* may contain certain therapeutically active substances which are vulnerable to different degradation, thus freeze drying significantly prevent the degradation of phytochemicals present in latex. These compounds can actively interfere with dopamine release in the premonitory phase of a migraine attack and migraine patients may be more sensitive to its effects. However dopaminergic pathways are the only one component of a complex neurochemical cascades, thus the effect of test samples over other signaling pathways has to be performed to establish the clear mechanism of action.

4.7. l-5-HTP induced serotonin syndrome

Activity of FDCG samples can be correlated with the prophylactic anti-migraine drug methysergide, a serotonin antagonist having a critical role for serotonin in the inhibition of an acute migraine attack (Yamamato & Ueki 1981). Action of selective agonists on 5-hydroxytryptamine (5HT) receptors has clearly established a critical role of serotonin in the inhibition of an acute migraine attack. 5-HTP (a precursor of 5-HT), administration in mice induces head twitches that occur spontaneously and irregularly via central action of 5-HT. A simple method of head-twitch response induced by 5-HTP in mice, help in determining the action of potentiators and antagonists for 5-HT in the central nervous system. FDCG and methylsergide potentiated 5-HTP-induced head twitch response in mice. This potentiation of head twitch response may be due to the FDCG and methylsergide mediated inhibition of the 5-HT reuptake and resulting increase of the content of 5-HT in synapses. This finding is consistent with the fact that pargyline pretreatment attenuated the anti-immobility activity of FDCG in this test. In contrast, antimigraine drug methysergide, significantly decreased the number of 5-HTP-induced head-twitch responses. Based on therapeutic and triggered migraine studies serotonin receptors are the specific chemical mediators of migraine. It was found that MK-801 administration modifies expression of few proteins in the hypothalamus. It has been observed that modification of c-Fos protein expression in the PC/RS cortex of mice was most significant. Also, MK-801 treatment elevates presynaptic dopaminergic neuron action and in an indirect way stimulates dopamine release in the brain (Marcus et al. 2001).

4.8. MK 801 Induced Hyperactivity

Serotonin and dopamine receptors system plays a central role in regulating serotonergic and dopaminergic neurotransmission to induce behavioral and physiological changes. Based on one hypothesis serotonergic and dopaminergic system plays an important role in determining various brain disorders including migraine. This means there is a direct involvement of serotonergic and dopaminergic system in migraine. Based on vascular hypothesis, increased neurotransmission of serotonin causes initial trigger of migraine. Serotonin is a vasoconstrictor, thus sudden increase in vasoconstriction can cause localized ischemia. Elevated vasoconstriction limits blood supply and maintains cerebral perfusion which leads to the increased intracranial

pressure (Barbanti et al 2013). This resulted in pulsatile headache and vasodilation which in turn causes depletion of serotonin in the later stages. Migraine pain is often preceded, accompanied, and followed by dopaminergic symptoms (premonitory yawning and somnolence, accompanying nausea and vomiting, postdromal somnolence, euphoria and polyuria) (Barbanti et al 2013). Due to this dopaminergic antagonist are considered as an effective therapeutic agents in migraine (Peroutka 1997). In current work FDCG samples inhibited behavioral effects induced by dopamine and serotonin agonists in all the mentioned models. Thus, this action of FDCG may be through the dopamine and serotonin receptors. Thus, FDCG can be effectively used for the treatment of migraine.

Conclusion

Owing to the high level of phenolic as well as flavonoid content FDCG samples represented good physico-chemical properties. Additionally, FDCG considerably attenuated the apomorphine -induced climbing behavior, *l*-5-HTP -induced syndrome and MK 801 -induced hyperactivity in a dose dependent manner through dopaminergic and serotonergic receptors interaction. Conclusively procedure developed for shelf-life improvement of latex offered maximum protection over a period of 10 weeks with retaining its natural biological potential, thus it can be effectively utilized in the treatment or management of migraine.

Declarations

Ethics approval and consent to participate

Studies were conducted by using animal models after prior permission obtained from Institutional Animal Ethics Committee (IAEC/ABMRCP/2018-2019/23).

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests

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Authors' contribution

Saurabh Bhatia: conceptualization, experimental work, writing and editing, supervision, methodology, formal analysis; Ahmed Al-Harrasi: conceptualization and review; Arun Kumar: Histopathology; Tapan Behl: writing-original draft; Aayush Sehgal: experimental work and methodology; Sukhbir Singh: experimental work and

methodology; Neelam Sharma: experimental work and methodology; Khalid Anwer: writing-original draft; Deepak Kaushik: writing-original draft; Vineet Mittal: writing-original draft; Sridevi Chigurupati: writing-original draft; Pritam Babu Sharma: conceptualization and review; Celia Vargas-de-la-Cruz: writing-review and editing; Md. Tanvir Kabir: writing-original draft

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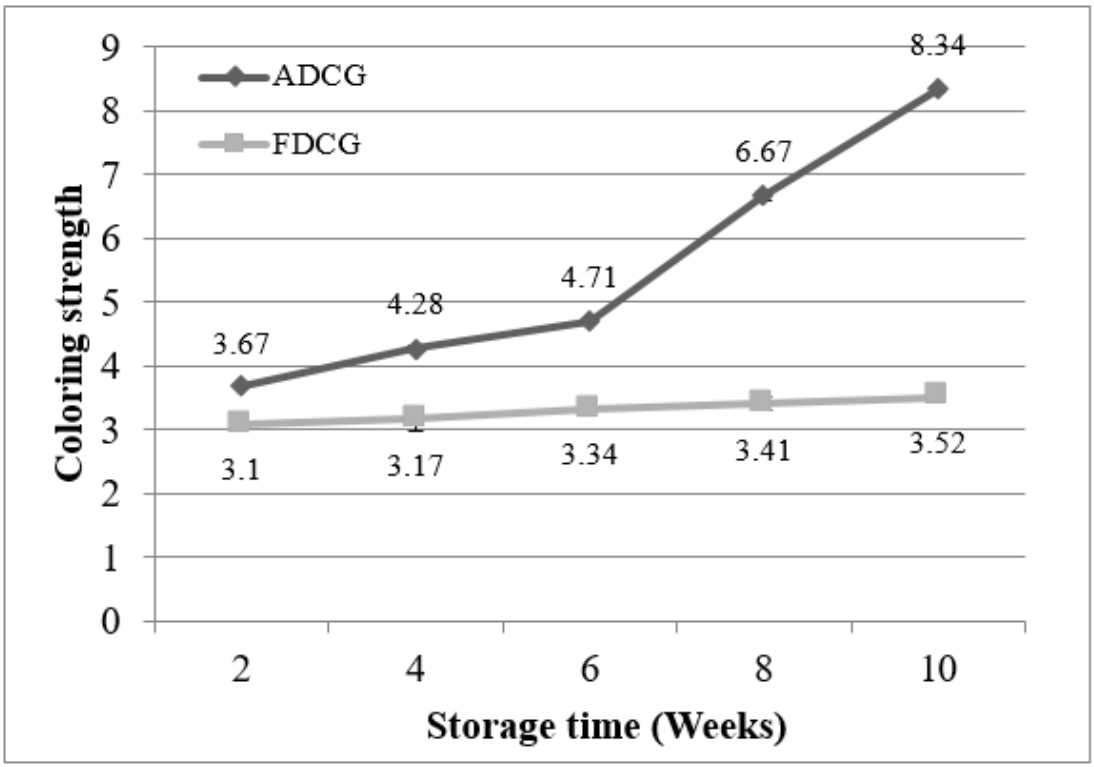
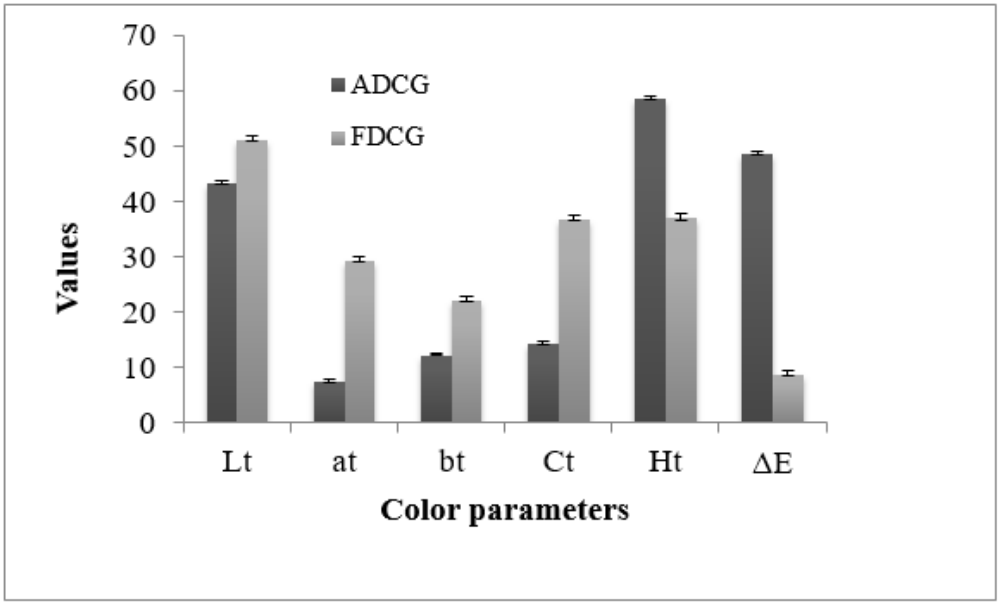
Figures



	Storage time in weeks				
Samples	2	4	6	8	10
ADCG	3.67±0.012	4.28±0.034	4.71±0.017	6.67±0.071	8.34±0.032
FDCG	3.1±0.041	3.17±0.174	3.34±0.024	3.41±0.112	3.52±0.067

Figure 1

First-order degradation plots of coloring strength (ln E vs time) for freeze dried microencapsulated sample FDCG and ADCG extracts prepared with different agents during storage at 40 ◦C for 10 weeks.



	Lightness (L*)		Redness value		Blue		Chroma		Hue=tan-1(b/a)		
Samples	L_0	L^t	a_0	a_t	b0	bt	C_0	C_t	Ht	Ht	**Delta E**
ADCG	68.7	43.2	41.2	7.5	36.1	12.2	54.77819	14.32096	41.2	58.49	48.55049
FDCG	57.8	51.2	33.9	29.4	25.2	22.1	42.24038	36.78002	36.6	36.93	8.568547

Figure 2

Color changes of ADCG and FDCG at different storage conditions determined by the chroma and Hue angle. The color related factors (a*, b*, L*, C* and E*) of ADCG and FDCG samples directly after their preparation as well as after storing them for 10 weeks at 40 ◦C

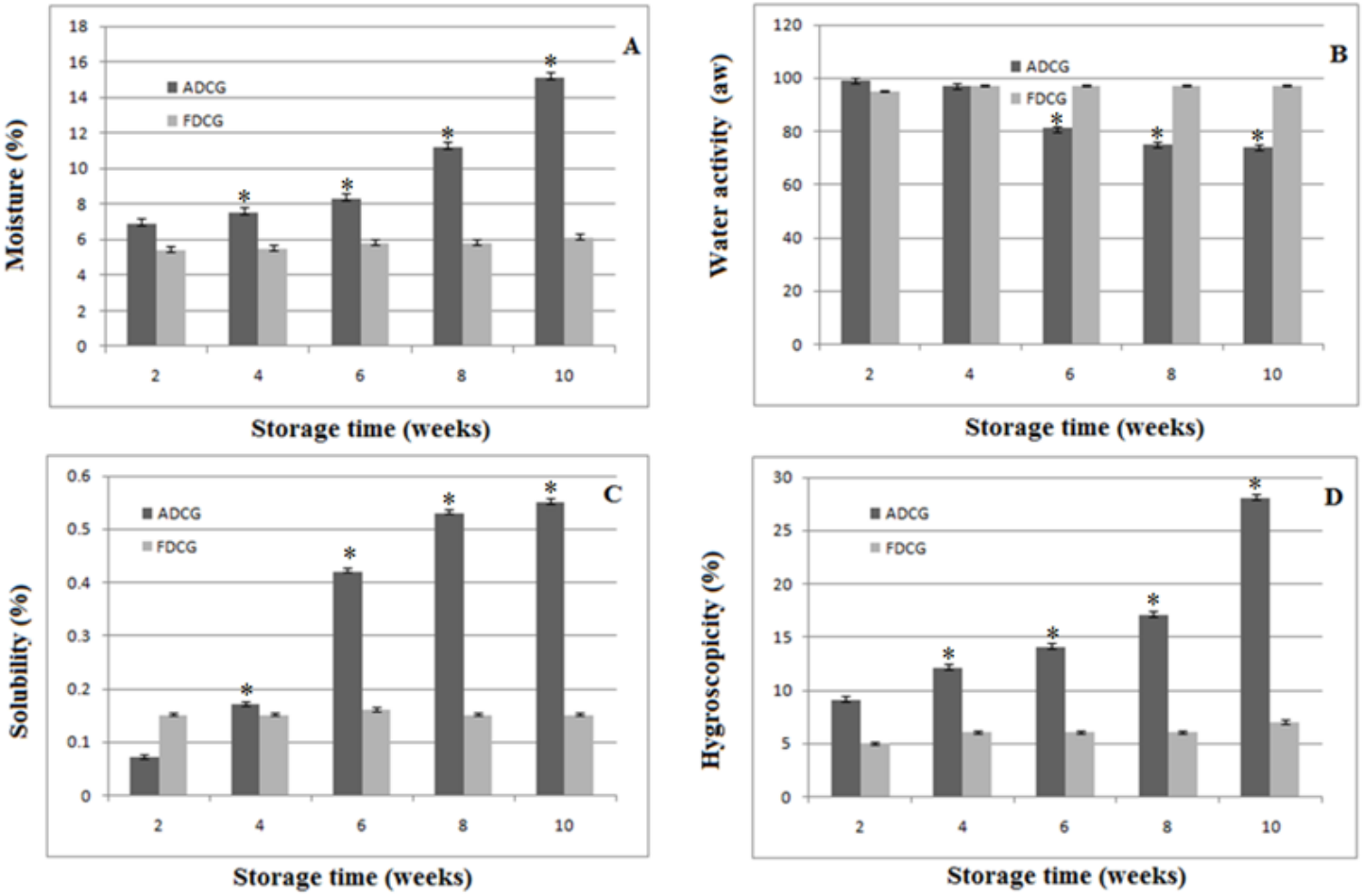


Figure 3

Physico-chemical properties of ADCG and FDCG samples. Physico-chemical properties of powder and its effect on the reconstitution of the powder.

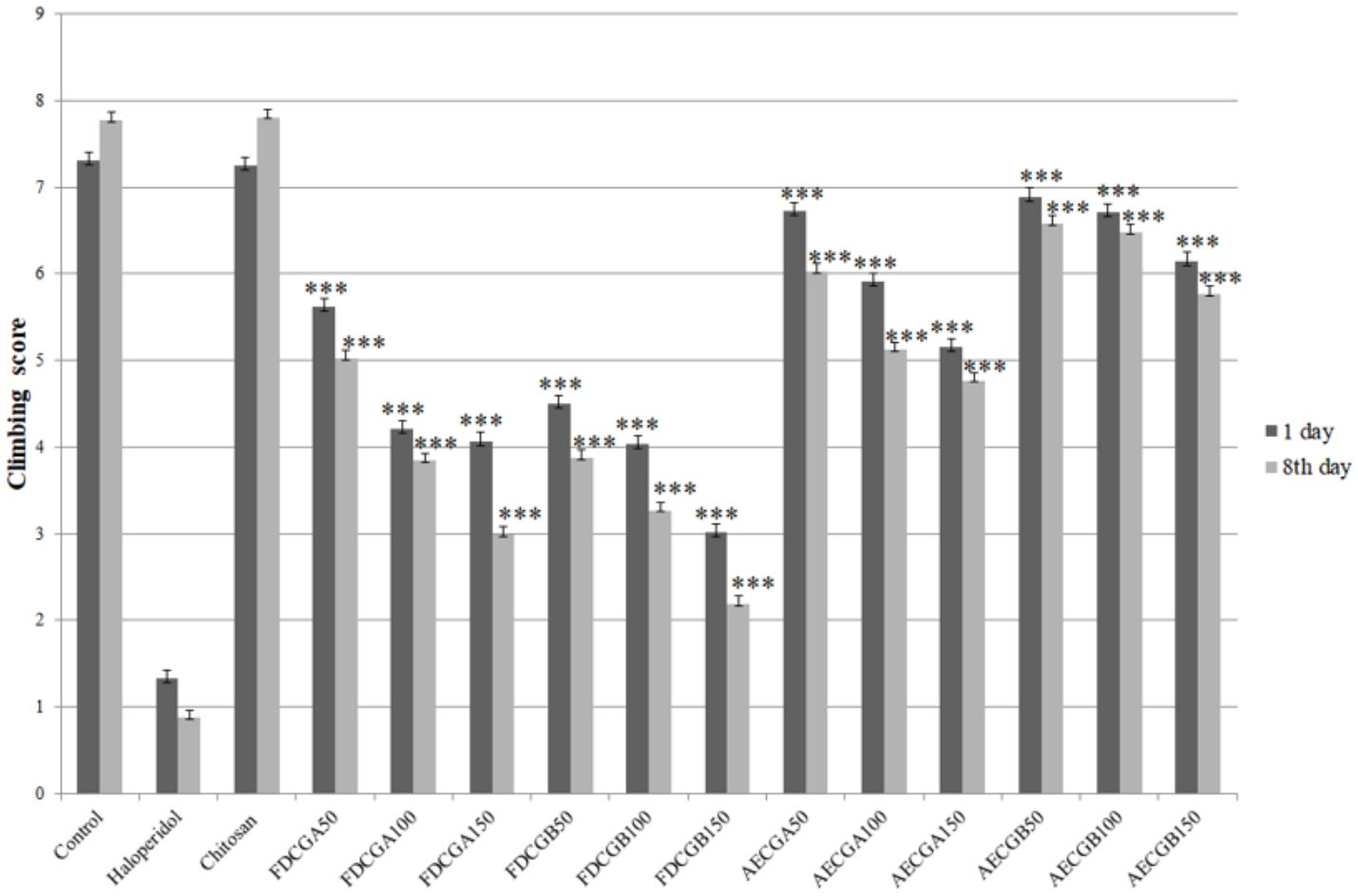


Figure 4

Inhibition of apomorphine induced climbing in mice. Effect of different Haloperidol, FDCGA (50-100mg/kg), FDCGB (50-100mg/kg), ADCGA (50-100mg/kg), ADCGB (50-100mg/kg) doses in apomorphine induced climbing behavior in mice. Each column represents mean ± SEM of total climbing score (n=6). At *p<0.05, ***p<0.001 when compared to control and chitosan (2%)

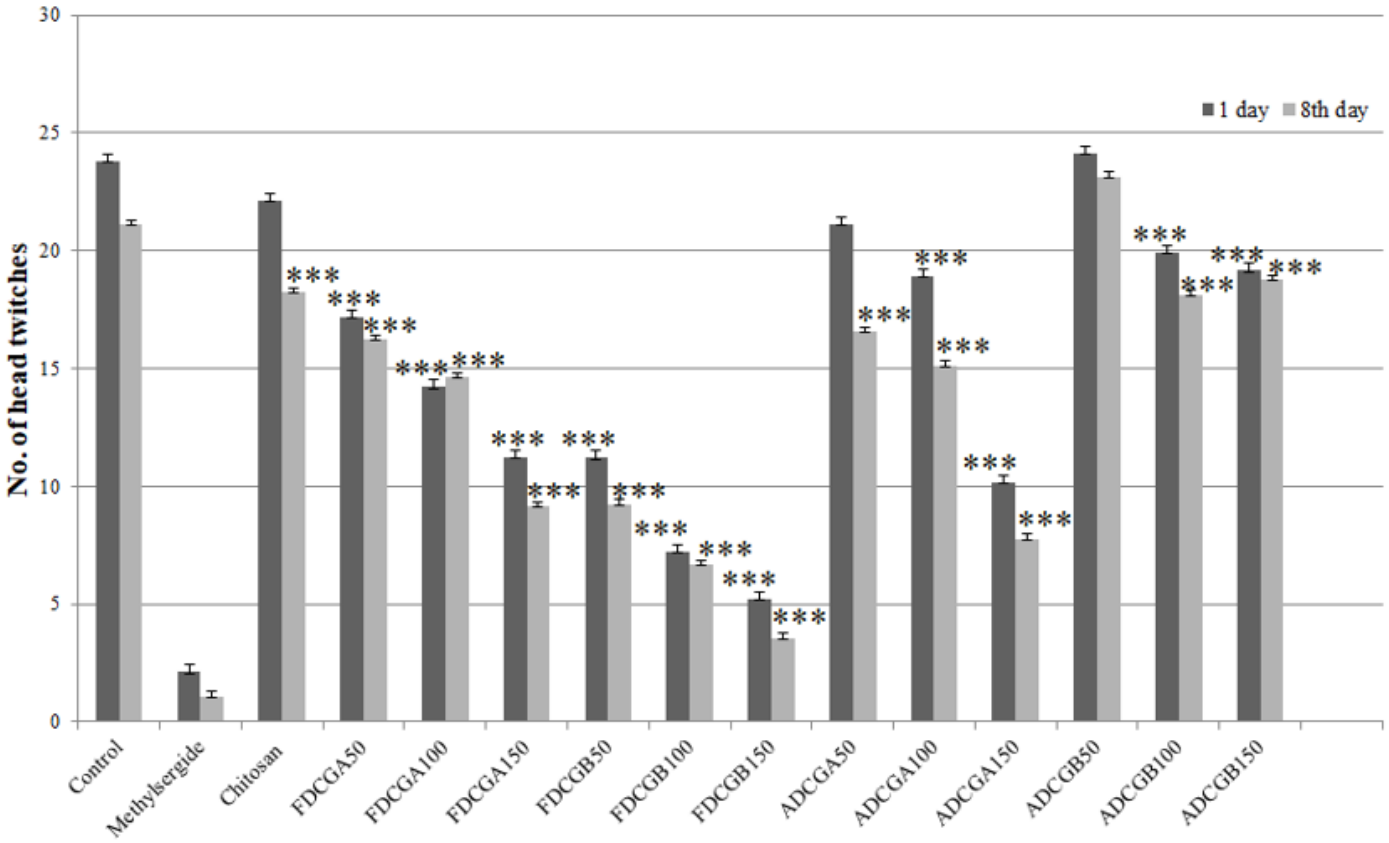


Figure 5

Inhibition of 5-HTP induced head twitches in mice. Effects of methylsergide, FDCGA (50, 100, 150mg/kg), FDCGB (50, 100, 150 mg/kg), ADCGA (50, 100, 150 mg/kg) and ADCGB (50, 100, 150 mg/kg) in 5-HTP induced head twitches in mice. Each column represents mean ± SEM of number of head twitches (n=6). At ***P<0.001 when compared to control and chitosan (2%)

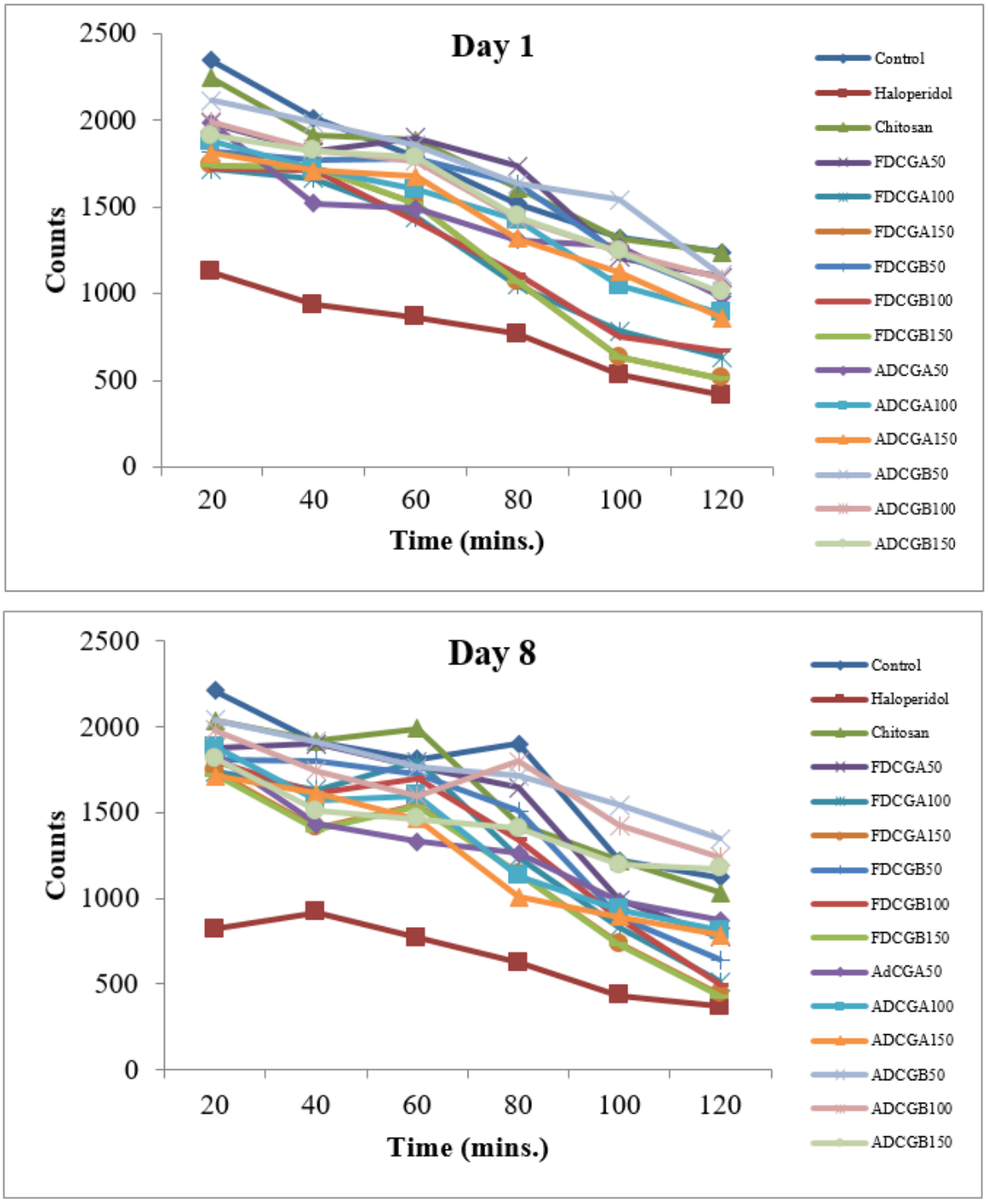


Figure 6

Effect of haloperidol, FDCGA (50, 100, 150 mg/kg), FDCGB (50-100mg/kg), ADCGA (50-100mg/kg) and ADCGB (50-100mg/kg) in MK-801 induced hyperlocomotion in mice. Each point represents the mean ± SEM of number of light beam interruption (n=6). At ***p<0.001, **p<0.01 when compared with control and chitosan (2%)

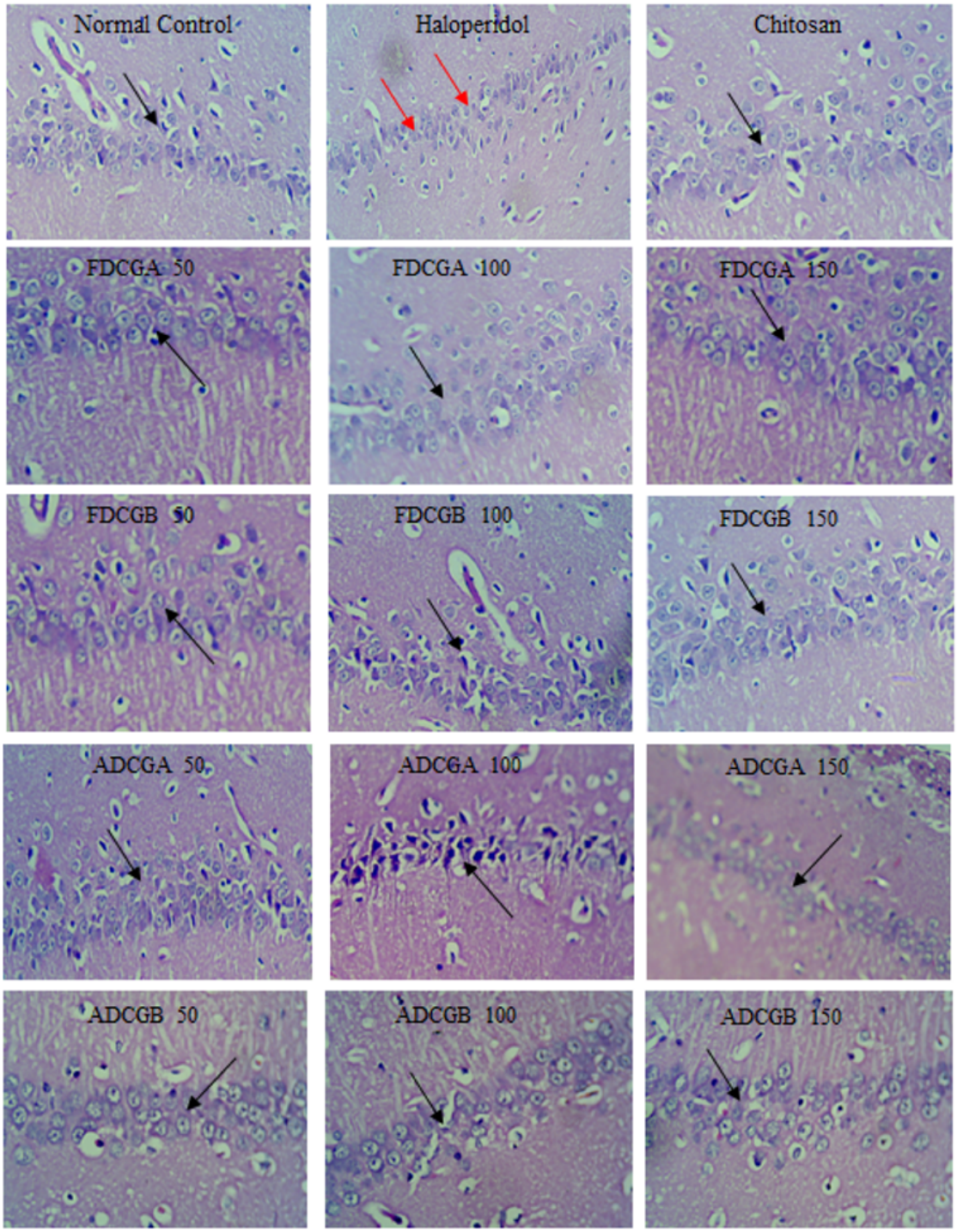


Figure 7

Effect of haloperidol, FDCGA (50, 100, 150 mg/kg), FDCGB (50-100mg/kg), ADCGA (50-100mg/kg) and ADCGB (50-100mg/kg) in MK-801 induced structural changes in hippocampus region of mice brain.

Supplementary Files

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