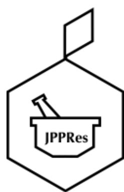

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Original Article | Artículo Original

Nanoparticulated formulations of St. John's wort (*Hypericum perforatum* L.) as smart drug delivery system combating depression incited in mice models

[Formulas nanoparticuladas de hierba de San Juan (*Hypericum perforatum* L.) como sistema inteligente de administración de fármacos para combatir la depresión inducida en modelos de ratones]

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Abstract

Context: *Hypericum perforatum* L., commonly known as St. John's wort, is practised as an alternative medicine against depression. Conversely, its remedial efficacy is indulged by various adverse effects that are recuperated in formulating nanoscaled commercial capsules encased by the biopolymer chitosan. A potential application of nanoencapsulation with regards to polymer enhances a slow controlled release of the targeted drug to achieve the desired delay until the right stimulus is obtained.

Aims: To value synthesizing biopolymeric nanocomposites encapsulating St. John's wort commercial capsules substantiating it with a study of animal model of depression to endorse the effect of nanocapsulated drug as an effective brain drug.

Methods The nanoparticulated suspension was prepared by ionic gelation technique and characterized to attest its antidepressant activity by *in vivo* studies.

Results: The drug binding efficiency was endorsed by FT-IR studies and the nanoparticles were characterized by an average particle size of 211.4 nm with a positive zeta potential of 45.9 mV. The animal despair studies on depression induced mice models displayed a significant difference in the immobility time during force swimming and tail suspension test. The commercial capsules were administed orally (p.o., 50 and 100 mg/kg). The animal despair studies were substantiated with affirmative biochemical assessments like SOD, CAT, GPx, GSH and LPO and compared with control groups.

Conclusions: The outcomes of this work manifest the calibre of St. John's wort nanocomposites in a lower dosage that can alleviate depression and reduce side effects.

Keywords: biopolymer; chitosan; depression; *Hypericum perforatum;* nanoparticles.

Resumen

Contexto: *Hypericum perforatum* L., comúnmente conocida como hierba de San Juan, es usada como una medicina alternativa contra la depresión. Por el contrario, su eficacia terapéutica se acompaña de diversos efectos adversos que se aminoran con la formulación de nanocápsulas con el biopolímero quitosano. Una aplicación potencial de la nanoencapsulación con respecto al polímero mejora una lenta liberación controlada del fármaco dirigido para conseguir el retardo deseado hasta que se obtenga el estímulo correcto.

Objetivos: Evaluar nanocompuestos biopoliméricos sintéticos que encapsulan las cápsulas comerciales de hierba de San Juan que los justifican con un estudio en modelos animales de depresión para endosar el efecto del medicamento nanocapsulado como fármaco eficaz para el cerebro.

Métodos: La suspensión nanoparticulada se preparó mediante una técnica de gelificación iónica y caracterizada por atestiguar su actividad antidepresiva mediante estudios *in vivo*.

Resultados: La eficacia de unión a fármacos fue respaldada por estudios FT-IR y las nanopartículas se caracterizaron por un tamaño medio de partícula de 211,4 nm, con un potencial zeta positivo de 45,9 mV. Los estudios de desesperación animal en modelos de depresión en ratones mostraron una diferencia significativa en el tiempo de inmovilidad durante la natación forzada y la prueba de suspensión de cola. Las cápsulas comerciales se administraron por vía oral (p.o., 50 y 100 mg/kg). Los estudios de desesperación animal se confirmaron con valoraciones bioquímicas positivas como SOD, CAT, GPx, GSH y LPO y se compararon con los grupos de controles.

Conclusiones: Los resultados de este trabajo manifiestan el calibre de los nanocompuestos de la hierba de San Juan, en una dosis más baja, que puede aliviar la depresión y reducir los efectos secundarios.

Palabras Clave: biopolímero; depresión; *Hypericum perforatum*; nanopartículas; quitosano.

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INTRODUCTION

Affective disorders are considered as a menace in the contemporary subsistence where, depression is a severe mental ailment and the world health organization (WHO, 2015) has considered it as a fret globally. *Hypericum perforatum* L. (Hypericaceae) known as St. John's wort is considered to be an effective alternative to other therapeutic agents in the treatment of psychogenic disturbances, mild to moderate depression and anxiety states (Fournier et al., 2010). Hypericin and hyperforin are the major psychoactive component of the plant that are accounted to have a potent affinity towards adenosine, serotonin 5HT-benzodiazepine, and aminobutyric acid (GABA) receptors and to weakly inhibit monoamine oxidase (Butterweck, 2003). Alike any other pharmacologically active substance the commercial formulation of St. John's wort also renders certain adverse effects such as gastrointestinal irritation, allergic reactions, fatigue and restlessness (British Herbal Pharmacopoeia, 1996; Crampsey et al., 2007).

The blemish of this commercial herbal extract can be reinstated by the prominent nanoparticulate drug delivery systems using biodegradable and biocompatible polymers. Nanodrugs are distinct and idyllic for they have specific control, sustained and targeted release characteristics. Patient compliance can be improved by using theses formulations that reduce the dosing frequency of the drugs. Therefore, the probability of developing side effects and cytotoxicity is negligibly low. Chitosan a prominent cationic polyelectrolyte biopolymer that has the potential of serving as an absorption enhancer across intestinal epithelial for its mucoadhesive and permeability enhancing property adhering to the mucosal surface and transiently opening the tight junction between epithelial cells (Wang et al., 2012). Development of therapeutics for brain disorders is one of the more difficult challenges to be overcome by the scientific community due to the inability of most molecules to cross the blood-brain barrier (BBB).

Drugs targeting central nervous system are being challenged by the complicated mechanism of the BBB, which is considered as a hurdle and results in the inability of some small and large therapeutic compounds to the targeted site. Hence the polymeric nanocomposites can consent drug delivery with low brain concentrations thus greatly enhancing the therapeutic effect on brain diseases. The study is devised in confronting depression under nanoscale by preparing nanocomposites, which are a matrix of biopolymer chitosan that encapsulates the antidepressant drugs. The encapsulation of polymers facilitates the drug delivery in a regulated manner targeting the therapeutic action, and the dosage in nanoscale lessens the adverse effects in the course of treatment. The significance of nanodrugs is reflected with the histopathological studies of cerebral cortex and study can promote this strategy as a navigator in drug development study amongst the field of neuroscience.

MATERIAL AND METHODS

Drugs and reagents

Chitosan with the deacetylation degree (DD) of 95% and the molecular weight of 360 kDa was purchased from Panacea Biotech (Punjab, India). St. John's wort capsules were bought from Sigma-Aldrich Corporation (St. Louis, USA), sodium tripolyphosphate anions and methyl isobutyl ketone from HiMedia Laboratories (Mumbai, India) and the standard antidepressant drug was obtained from Cadila Pharmaceuticals (Ahmedabad, India). All other chemicals and solvents were of analytical reagent grade.

Preparation of nanocomposites by ionic gelation method

Drug (St. John's wort capsules) loaded chitosan nanoparticles (CS/NPs) were prepared based on ionic gelation (Calvo et al., 1997) of chitosan with sodium tripolyphosphate anions (TPP). Chitosan solution (1% w/v) was prepared at various concentrations (1.0, 2.0, 3.0, 4.0 and 5.0 mg/mL) by dissolving 1 g of the content in 100 mL (1% v/v) acetic acid solution with a mild and constant stirring (magnetic stirrer for 24 h under room temperature) until the suspension became clear and transparent. Subsequently, the TPP prepared at a concentration of 0.25% w/v, was added dropwise to the chitosan

solution (0.1% w/v) and was stirred consistently. The drug loaded CS/NPs was prepared by dissolving 10 mg of St. John's wort in 5 mL of 2% w/v Tween 80 solutions and was added to the prepared chitosan solution. The suspension was incubated for 1 h at room temperature and was centrifuged at 20,000 rpm for 30 min at 10°C. The settled NPs were resuspended in distilled water for further formulation development and analysis.

Characterization of the nanocomposite formulations

Preliminary characterization was performed using Fourier transform infrared spectroscopy (FT-IR) to validate the incorporation of the drug with the biopolymer. Chitosan nanoparticles separated from suspension were dried by a freeze dryer, and their spectroscopic profiles were taken with potassium bromide (KBr) pellets on Bio-Rad FTIS - 40 model USA. Particle morphology was examined by scanning electron microscopy (Hitachi, S-4500) and, the samples were immobilized on copper grids. They were dried at room temperature and examined without being stained. The particle size range of the nanoparticles along with its polydispersity was determined using a particle size analyzer (90 Plus Particle Size Analyzer, Brookhaven Instruments Corporation). Particle size was arrived based on measuring the time-dependent fluctuation of scattering of laser light by the nanoparticles undergoing Brownian motion. The zeta potential of nanoparticles was measured on a zeta potential analyzer (Brookhaven, USA). For zeta potential measurements, samples were diluted with 0.1 mM KCl and measured in the automatic mode. All measurements were performed in triplicate.

In vitro drug release study

In vitro release of St. John's wort capsules from CS/TPP nanoparticles was studied by following the methodology implemented by Hu et al. (2002). The separated nanoparticles (10 mg) was re-dispersed in 2.5 mL freshly prepared phosphate buffer of 7.4 pH, and dispensed into a dialysis membrane bag with a molecular weight of 5 kDa. The dialysis bag (HiMedia Laboratories, Mumbai, India) was placed in 50 mL of phosphate buffer of pH 7.4. The entire system was kept under magnetic stirring. Four mL of the release medium was removed and was replaced by fresh buffer solution at regular time intervals. The amount of drug in the released medium was evaluated from the absorbance measured at 274 nm. All the release studies were conducted in triplicate and mean values were taken.

In vivo pharmacodynamics studies

Grouping of animals

The animals were accommodated under standard experimental conditions according to the rules and regulations of institutional Ethical Committee for the Purpose of Control and Suspension of Experiments on Animals (CPCSEA) under Ministry of Animal Welfare Division, Government of India, New Delhi (REF NO. BDU/IAEC/2014/NE/39). Female albino mice (16 Nos) that weighed within 22-25 g were selected and experimented (temperature of 24 ± 3°C, humidity 40-60% with 12 hour light and dark cycles) with free access to food and water *ad libitum*. The animals were assemblage into four experimental groups (four animals/group) and were acclimatized for seven days before the study. The randomly divided groups were divided as positive control (depressive without treatment), negative control (normal), standard drug - St. John's wort capsules, and nanocomposites. The details of drug dosage administrated are listed in Table 1. The animals in positive control grouped as 1 were administered with saline as vehicle (1 mL of 0.9%).

Induction of depression and treatment schedule

The animals were induced to a depressed state by injecting methyl isobutyl ketone (100 mg/kg body weight, i.p.) regularly for two weeks. The dosage of *Hypericum perforatum* is standardized by FDA and is 300 mg/kg (FDA, 2001). Further, the induction was also carried out (two weeks) with the animal despair studies during the final week and the test sessions (one week) were performed during the following week (1st, 3rd, 5th and 7th days) to access their behavioral patterns. The treatment phase was pursued for another one week immediately after the test session, and the final despair studies were conducted on the last day (14th day) after which the animals were sacrificed for biochemical studies.

Animal model of depression

Forced swim test

In order to assess the antidepressant activity of the plant extract, forced swim test (Porsolt et al., 1977) was conducted in three trials. The first trial was experimented with the depressed mice, which have not undergone the treatment, the second on non-depressed normal mice and the third on the treated mice with the commercial capsule extract and test nanocomposite. Each animal was placed individually in a 5 L glass beakers, filled with water up to a height of 15 cm and were observed for duration of 6 minutes. During the test session, the immobility time, swimming and climbing periods was observed. The mice will try to escape from this stress induced and will try to climb. It would remain immobile and renounce its attempts. This is motionless floating of the animal with its head faced above the water level is calculated as the period of depressive-like state. The water was changed periodically after each session of experiments.

Tail suspension test

Tail suspension test is another animal despair study adopted (Cryan et al., 2005) to access the anti-depressant activity. Vertically the animals were suspended down towards gravitational force and affixed with an adhesion tape. The animals tend to climb up and attempt to get rid of the sudden stress after a serious of efforts it will tend to be immobile, which is considered as a depressive behavior. This duration of immobility is observed in the animal despair study for 6 minutes.

Preparation of brain homogenate

After 24 h of behavioral observations, mice were decapitated, and the cerebral cortex region was dissected and homogenized in ice with phosphate buffer (pH 7.4) to ensure the viability of proteins using a tissue grinder (Tempstar, Kay Pee Udyog, Haryana, India). The tissue homogenates (10% w/v) were centrifuged (UV-VIS 3000+, Lab India, Maharashtra, India) at 16,000×*g*, at 4°C for 20 minutes. The supernatants obtained were used for the determination of biochemical activities such as superoxide dismutase, catalase, lipid peroxidation, reduced glutathione, and glutathione peroxidase. The protein content was quantified according to the method described by (Lowry et al., 1951), using bovine serum albumin as a standard. The experiments were conducted in triplets to avoid counterfeit predictions.

Table 1. Animal protocol design represents groups of animals and their test drug dosages administered to mice.

Groups	Treatment	Dose	Dose Volume (mL)	Routes of Administration
I	Disease Control (depression induced)(saline)	1 mL	1	Oral
II	Normal (water)	1 mL	1	Oral
III	Commercial herbal capsules*	50 mg/kg	1	Oral
		100 mg/kg	1	Oral
IV	Test nanocomposite	10 mg/kg	1	Oral

Female Swiss albino mice of 16 Nos were grouped (n=3 in each group).

*Group III animals were tested with two different dosages (n=7).

Saline was administered as vehicle for the animals in group I and considered as negative control whereas group II as positive control.

Activity of antioxidant enzymes

Superoxide dismutase

Superoxide dismutase (SOD) was assessed spectrophotometrically (Maier and Chan, 2002) based on the development of a red colored compound when diazonium compound reacts with naphthyl-amino group whose absorbance was measured at 543 nm in a spectrophotometer (UV-VIS 3000+, Lab India, Maharashtra, India). The reaction mixture consists of 1.11 mL of 50 mM phosphate buffer of pH 7.4, 0.075 mL of 20 mM L-methionine, 0.04 mL of 1% (v/v) Triton X-100, 0.075 mL of 10 mM hydroxylamine hydrochloride and 0.1 mL of 50 mM EDTA. A series of aliquots (1.2 mL) was taken and added to 100 µL of the test samples belonging to all four brain homogenates from all the four experimental groups. Riboflavin (80 µL of 50 µM) was taken and exposed the fluorescent lamps. After 10 mins Greiss reagent (mixture of equal volume of 1% sulphanilamide in 5% phosphoric acid) was added, and the absorbance of the color formed was measured at 543 nm. The samples were incubated at 30°C for 5 minutes, and SOD enzymatic activity was expressed as units (U)/mg protein.

Catalase

The activity of catalase (CAT) was analyzed by the method of Sinha (1972). The reaction mixture con-sists of 1 mL of 0.01 M phosphate buffer (pH 7.0), 0.5 mL of 0.2 M H_2O_2 and 0.4 mL H_2O. The brain homogenate samples (0.5 mL) was added to the reaction mixture containing and incubated for different time periods. The reaction was terminated by the addition of 2 mL of acid reagent (dichromate/acetic acid mixture), which was prepared by mixing 5% potassium dichromate with glacial acetic acid (1:3 by volume). To the control, the enzyme was added after the addition of acid reagent. All the tubes were heated for 10 minutes, and the absorbance was read at 610 nm. CAT activity was expressed in terms of µmoles of H_2O_2 consumed/min/mg protein.

Glutathione peroxidase

The glutathione peroxidase (GPx) level in the brain was estimated by the method (Ellman, 1959). Brain homogenate was allowed to react with H_2O_2 in the presence of GSH for a specific period, then the remaining GSH was allowed to react with DTNB [5,5'-dithiobis (2-nitrobenzoic acid)], and the mixture was incubated for 10 min. The development of yellow-colored measured at the absorbance of 412 nm. The activity of GPx was expressed as nmol NADPH oxidized/min/mg protein.

All experiments were performed in triplets.

Non-enzymic antioxidants

Assessment of glutathione content

Total reduced glutathione (GSH) was determined by the method (Moron et al., 1979) where the 0.5 mL brain homogenates from all the 4 aminal groups was mixed with 10% w/v trichloroacetic acid in ratio of 1:1 (w/v) and centrifuged at 4°C for 10 min at 5000 rpm. The supernatant obtained was mixed with 2 mL of 0.3 M phosphate buffer (pH 8.4) and 0.4 mL of distilled water, and then 0.25 mL of 0.001 M freshly equipped DTNB [5,5'-dithiobis (2-nitrobenzoic acid)] and dissolved in 1% w/v sodium citrate was added. This reaction mixture for 10 min was incubated and the absorbance at 412 nm of yellow-colored complex was noted thrice. The values are expressed as nmol/mg protein.

Determination of thiobarbituric acid reactive substance levels

The levels of thiobarbituric acid reactive substances (TBARS) in tissue were measured using the method (Ohkawa et al., 1979) in which malondialdehyde (MDA), an end-product of lipid peroxidation, reacts with thiobarbituric acid to form a colored complex. The amount of MDA, a measure of lipid peroxidation was assayed in the form of TBARS. An aliquot of 0.5 mL of brain homogenate samples and 0.5 mL Tris-HCl were incubated at 37°C for 2 h. After incubation, 1 mL of 10% trichloroacetic acid was added and centrifuged at 10 000 *xg* for 10 min. One mL of 0.67% thiobarbituric acid was added and the tubes were kept in boiling water for 10 min. After cooling, 1 mL of double distilled water was added and absorbance was measured at 532 nm along with standard MDA thrice. The values are expressed as nmol MDA/g of wet tissue.

Statistical analysis

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 17.0 (New York, USA). Data obtained were expressed as mean ± SEM. One-way analysis of variance (ANOVA) followed by multiple range test (Tukey test) was used to determine significant differences. Differences at $p<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Depression causes a large burden of malady worldwide and is a common disorder, widely distributed in the population, which is usually associated with substantial symptom severity and role impairment. This study lay downs strategies endeavoring a novel drug delivery for progressing the therapeutic index through biopolymeric drug carriers and restore mental health by facilitating antidepressant in nanoscale. Apart from the synthetic drugs, the alternate antidepressants (herbal drug extracts) would reduce the side effects more efficiently. The futuristic effort of targeting BBB would channelize the drug and reduce its scattering and adverse effects. Consequently, a topical approach is devised in confronting depression under nanoscale by preparing nanocomposites, which are a matrix of biopolymer chitosan that encapsulates the antidepressant drugs loaded as nanocomposites with chitosan by the ionic gelation method. The outcome of the study is substantiated by (Liang et al., 2011) and (Lee et al., 2010), who furnished that nanoparticles immobilized with chitosan exhibited ideal drug delivery systems for slow drug release by diffusion, by maintaining the enclosed constituent active.

Preparation and characterization drug loaded chitosan nanoparticles

The polymeric nanocomposites were prepared with chitosan as a biopolymer encasing the test drug St. John's wort capsules by ionic gelation technique. Nanoparticles were prepared by electrostatic interaction when oppositely charged macro-molecules are mixed together. The triumph of this concept relies on the intermolecular linkages created between the negatively charged groups of TPP with that of positively charged amino groups of CS (Calvo, 1997). This principle augments the encapsulation of St. John's wort commercial capsules along with the polymer. Nanocomposites were synthesized in five different concentration (1.0, 2.0, 3.0, 4.0 and 5.0 mg/mL) of chitosan where the third concentration (3 mg/mL) was optimized as standard.

Physicochemical characterization studies were made to analyze the drug-polymer interaction and the morphological features. The nature of the interaction between the drug and polymers was established through FT-IR spectrometry. The spectrum of the nanocomposite was compared with the standard spectra of chitosan, TPP, and St. John's wort capsules (Fig. 1). There are two characterization peaks of chitosan (Table 2) at 3435 cm^{-1} of ν(OH) and 2922 cm^{-1} of ν_{as}(C-H). The spectrum of chitosan-TPP (blank) nanoparticles constitutes of ν_{as}(C-H) at 2922 cm^{-1}, and there is a vibration shift of ν(OH) to 3405 cm^{-1} with a new sharp peak at 1100 cm^{-1}, which is influenced by the P=O group of TPP. The amalgamation of peaks infers the linkage of tripolyphosphoric groups with ammonium groups of chitosan in nanoparticles and a similar observation was reported earlier by Knaul et al. (1999). The spectrum of St. John's wort loaded nanoparticles illustrated an absorption peak of 2923 cm^{-1}, which depicts the interaction of St. John's wort peak of 2970 cm^{-1} of ν_{as}(C-H) with 2922 cm^{-1} of chitosan. Further the appearance of a new peak at 1100 cm^{-1} associated with the ν_s(C-O-C) of St. John's wort capsules and the phosphate group of TPP. The existence of a new peak at 1600-1629 cm^{-1} is accredited to the linkages between phosphate groups of tripolyphosphate with ammonium groups of chitosan in nanoparticles, and a similar observation was reported earlier by Calvo et al. (1997) and Knaul et al. (1999).

The plant extract of *Hypericum perforatum* L. consists of a large variety of volatile secondary metabolites such as terpenes, terpenoids, phenolic and aliphatic derivatives (Bertoli et al., 2011). The FT-IR spectra of the nanocomposite revealed the presence of alkyl halides, aliphatic amines, alkanes, amines and absence of phenol compounds derivatives, C-H (aldehydes), including cis-double bonds. Peaks in the range between 3360-3549 cm^{-1} corresponded to stretching vibrations of OH groups (from water, alcohols, phenols, carbohydrates, peroxides) as well

as from amides and studied to be significant in contributing the medical attributes of the plant (Mudasir et al., 2012). This result indicates an establishment of a network that integrates the drug together with the polymer.

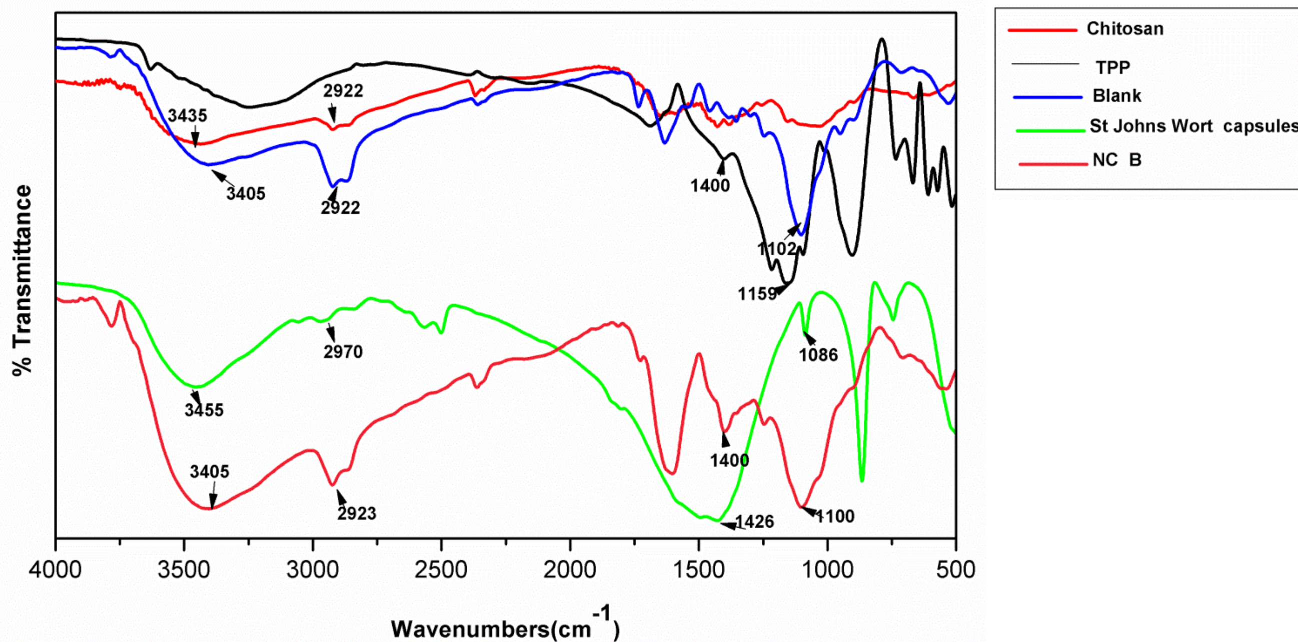

Figure 1. FT-IR spectra of chitosan, sodium tripolyphosphate anions, St. John's wort and nanocomposite.

TPP: sodium tripolyphosphate anions; Blank: chitosan, sodium tripolyphosphate; NC B: (Nanocomposite) chitosan, sodium tripolyphosphate, St. John's wort

Table 2. FT-IR analysis of biopolymer with the test drug St. John's wort and its respective nanocomposite.

Vibration mode/Group	Nanocomposite	St. John's wort capsule	Standard chitosan	TPP	Blank
ν(O-H)	3405	3455	3435		3405
νas(C-H)	2923	2970	2922		2922
C–C stretch (in–ring) aromatic 15	1400	1426		1400	
νs(C-O-C)	1100	1086			1102
P = O	1100			1159	

The shift of peak ensures the entrapment of both the polymer and drug. The nanocomposite consists of all the significant functional groups thereby illustrating its ionic interaction. TPP: sodium tripolyphosphate anions; Blank: chitosan, sodium tripolyphosphate

Fig. 2 shows the morphological characteristic of nanoparticles by the scanning electron micrograph at different resolutions. The shape and surface morphology of the nanocomposites was evaluated, which were, irregular with a size that ranges from 90.68 - 187.74 nm with an average diameter of 137.83 ± 9.0 nm. The structural features of nanocomposites exhibited microfibrillar crystalline structure with large portion of the surface structure, which was on par with the studies made by Zhang et al. (2012). This type of morphology was supported by Islam et al. (2011) and El-Hefian et al. (2010) who reported that chitosan has a non-homogenous and non-smooth surface with straps and shrinkage. The average size of the nanoparticles was measured along with the size distribution *vs.* intensity graph as shown in Fig. 3. The Dynamic Light Scattering sustains the particle size of scanning electron microscopy analysis that revealed scattering intensities corresponding to an average diameter of 211.4 nm. Presumably, the size is greater than the evaluation of scanning electron microscopy, and this may be due to the suspension of the nanoparticles in water, from that of the dry state. The zeta potential analysis of the prepared nanoparticles (Fig. 4) revealed a highly positive value of 45.9 Mv indicating a good colloidal stability. Zeta potential can affect the pharmacokinetic properties of nanosystems in the body and has a substantial influence on the stability of suspensions hence a positive zeta potential will maintain the suspension in a dispersed state Qi et al. (2005). The dynamic light scattering analysis revealed scattering intensities corresponding to an average diameter of the nanocomposites (Muller et al., 2001).

The zeta potential expresses the potential difference that exists between the dispersion medium and stationary layer of the particle (Makhlof et al., 2011). It is an important tool for understanding the state of the nanoparticle surface and predicting the long-term stability of the nanoparticles. The positive values bestowed by the nanocomposites designated a good colloidal stability. Zeta potential can affect the pharmacokinetic properties of nanosystems in the body and has a substantial influence on the stability of suspensions hence a positive zeta potential will maintain the suspension in a dispersed state (Qi et al., 2005). The study explained by Beduneau et al. (2007), illustrated the interaction of the chitosan nanocomposite with charged drugs, which reflect only a part of the amino groups that are neutralized (residual amino groups) and responsible for the positive zeta potential thereby influencing brain drug targeting. Appending this Juillerat-Jeanneret (2008) described the advantage of a positive zeta potential score on BBB, which is considered as a physiological hindrance to neuro drugs.

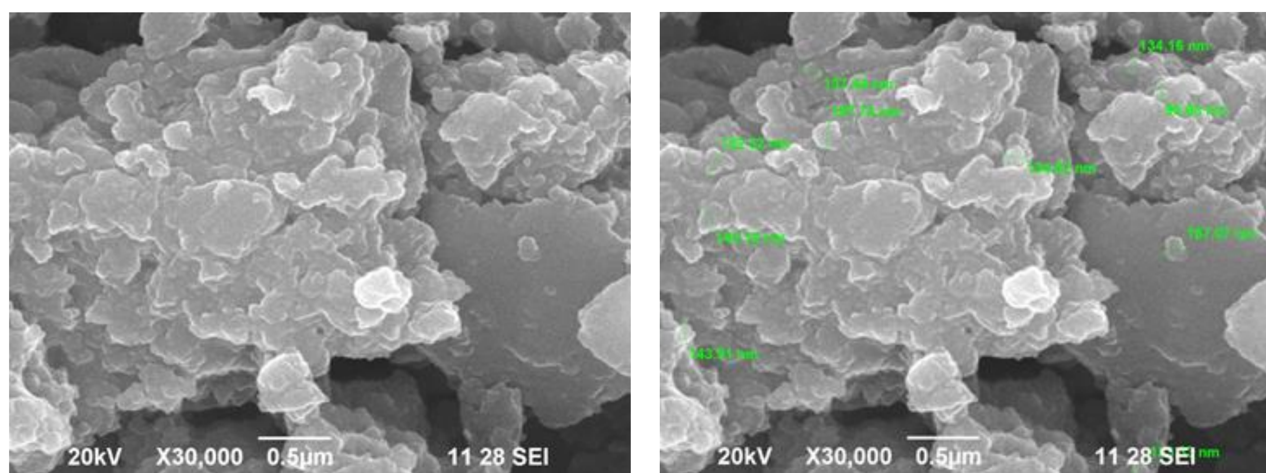

Figure 2. Scanning electron microscopy micrographs of chitosan/sodium tripolyphosphate anions nanocomposites loaded with St. John's wort capsules.

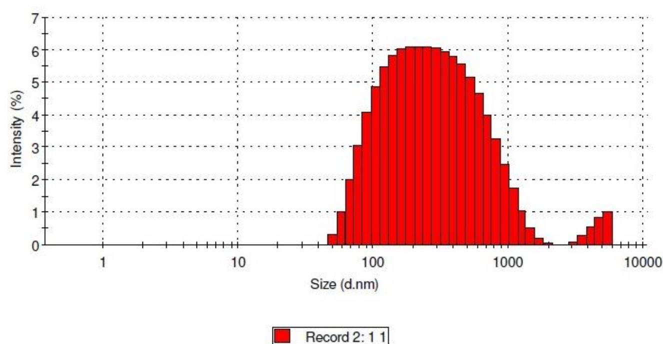

Figure 3. Dynamic light scattering measurement to determine the size distribution of nanocomposite.

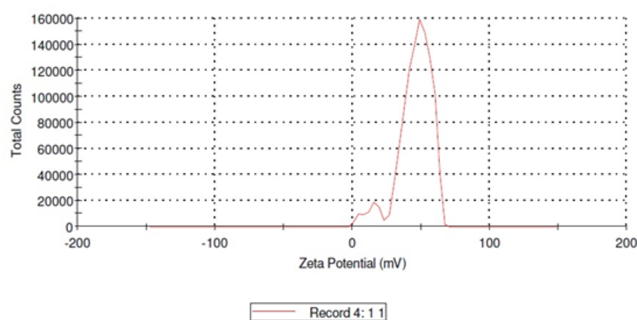

Figure 4. Zeta potential analysis to determine the stability of nanoparticles.

In vitro drug release study

In vitro drug release study is an essential parameter in developing a drug, which compiles three distinct features such as penetration (determines the dosage), dissolution and diffusion (Cairncross et al., 1978). The drug release kinetics of St. John's wort capsules was rapid where 50% of the drug was released within 6 h and a maximum release of 97.43% with a duration of 9 h (Fig. 5). The release profile of test drugs from the chitosan nanoparticles has a noticeable burst effect of rapid initial drug release within 10 h followed by a slow and sustainable release of 92.64% at the maximum duration of 24 h. The size of the drug being smaller is a significant parameter, which facilitates its transfer rapidly. An ailment generally requires an immediate action and polymeric nanocomposites restore illness instantly. The ability of the carrier to release the cargo efficiently at the desired site is an important feature of any delivery system hence the polymer-hoisted level the efficiency of the drug due to its consistent dispersion the surface. Polymeric drug carrier primarily diffuses the drug pursued by matrix degradation, and the initially hoisted level is due to the dispersion of the drug molecules on the surface. This augmented the assertion of that chitosan is a mucoadhesive, cationic polymer, which assists the drug to diffuse easily during the initial incubation period (Berscht et al., 1994; Zhou et al., 2009). This study elucidates the advantage of nanocomposites, which are dosage confined thereby restraining adverse effects.

Assessment of depression

Animal despair studies are the preferred behavioral tests that predict the intensity of depression induced. There are two significant assays called the forced swim and tail suspension test that reveals the similarity faced by a depressed human (Theirry et al., 1986; Lucki, 1997). Immobility in forced swim and tail suspension test showed significant impairment before treatment in both the standard and nanocomposite groups on the 7th day as compared to the 1st day. The post treatment with standard drugs and their relative nanocomposites shortened the immobility period in the animal despair studies performed and exhibited a dose-dependent antidepressant activity.

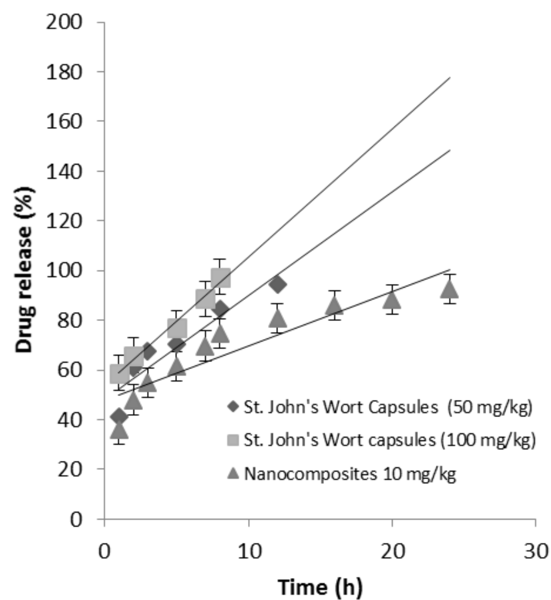

Figure 5. *In vitro* drug release of nanocomposite in accordance to St. John's wort capsules.

The results of immobility time have been presented in Tables 3 and 4. The mean value of immobility of all treatments was found to be statistically significant in comparison to control ($p<0.05$). The higher dose of commercial herbal capsules (100 mg/kg body weight) exhibited a fine control in reducing the immobility time. The treated nanocomposite relatively subsided the immobility score (95.3 ± 4.08) and excelled in accordance with the lower dosage (50 mg/kg body weight) of St. John's wort capsules. The nanocomposites established a distinct level in the dosage of 10 mg/kg body weight of the animals, which maintained them active. The nanocomposites exhibited positive results that managed the immobility period of animals. The degree of immobility was in accordance to the study of Prabhjot et al. (2014) who, experimented on tramadol HCl (centrally acting synthetic opioid) as NP-loaded in situ gel against depression.

Assessment of biochemical parameters

The development of oxidative stress in the brain was assessed and Table 5 provides the values of six different biochemical studies such as CAT, SOD, GPx, GSH and LPO. Significant differences ($p<0.05$) were observed in all the analysis among the depressed and experimental mice treated with nanocomposites. An elevated level of the key enzymes (except LPO) perceives a compatible antioxidant status thereby restoring the depressed state to normal.

Table 3. Effect of St. John's wort capsules and nanocomposites on the duration of immobility time (min) in forced swim mice models.

Group/Day	Depressed	Normal	Standard commercial herbal capsules (50 mg/kg)	Standard commercial herbal capsules (100 mg/kg)	Nanocomposites (10 mg/kg)
1	165.1 ± 4.0	-	160.0 ± 4.5	159.6 ± 5.4	162.3 ± 1.8
3	178.8 ± 6.9	-	174.5 ± 2.6	180.1 ± 3.6	180.8 ± 1.8
5	193.5 ± 4.8	-	187.1 ± 2.9	189.3 ± 5.9	186.8 ± 1.7
7	201.3 ± 8.3	-	201.5 ± 3.2	199.3 ± 7.3	199.3 ± 6.1
14	199.5 ± 7.3*	52.5 ± 1.9*	95.0 ± 2.5*	74.1 ± 4.4*	95.3 ± 4.1*

Values represented as mean ± SEM (n=3) of two independent data by one-way analysis of variance (ANOVA). Values are statistically significant ($p \leq 0.05$) between the Positive control group and the experimental groups induced with depression by means of multiple range test (Tukey test).

Table 4. Effect of St. John's wort capsules and nanocomposites on the duration of immobility time (min) in tail suspension test mice models.

Group/Day	Depressed	Normal	Standard commercial herbal capsules (50 mg/kg)	Standard commercial herbal capsules (100 mg/kg)	Nanocomposites (10 mg/kg)
1	166.3 ± 1.7	-	161.3 ± 3.0	160.5 ± 1.87	160.6 ± 4.9
3	182.1 ± 3.6	-	171.8 ± 2.5	174.6 ± 6.25	169.8 ± 5.7
5	191.0 ± 5.2	-	187.1 ± 5.4	186.1 ± 6.0	193.0 ± 5.5
7	206.3 ± 4.4	-	200.5 ± 6.4	201.1 ± 8.5	201.1 ± 3.6
14	218.5 ± 4.0*	83.1 ± 0.9	108.5 ± 1.9*	96.5 ± 3.3*	99.5 ± 9.8*

Values represented as mean ± SEM (n=3) of two independent data by one-way analysis of variance (ANOVA). Values are statistically significant ($p \leq 0.05$) between the positive control group and the experimental groups induced with depression by means of multiple range test (Tukey test).

Table 5. Effect of St John's wort loaded nanocomposites on various biochemical parameters associated with oxidative stress.

Group	Conditions	CAT (µmoles H2O2/min/mg protein)	SOD (U/mg protein)	GPx (nmol NADPH/min/ mg protein)	GSH (nmol/mg protein)	LPO (nmol MDA/g wet tissue)
I	Depressed	72.4 ± 0.4	95.4 ± 0.7	72.5 ± 0.7	19.3 ± 0.1	80.5 ± 0.3*
II	Normal	85.3 ± 0.4	119.6 ± 0.2	66.1 ± 0.1	25.7 ± 0.1	54.5 ± 1.1
III	Commercial capsules of St. John's wort	86.1 ± 0.1*	114.4 ± 0.20*	63.7 ± 0.6*	25.2 ± 0.1*	55.3 ± 0.1*
IV	Nanocomposites of St. John's wort capsules	80.7 ± 0.1*	105.3 ± 0.1*	66.5 ± 0.1*	24.5 ± 0.1*	56.8 ± 0.1*

Values represented as mean ± SEM (n=3) of two independent data by one-way analysis of variance (ANOVA). *Values are statistically significant ($p \leq 0.05$) between the negative control group and the experimental groups induced with depression by means of multiple range test (Tukey test).
CAT: catalase; SOD: superoxide dismutase; GPx: glutathione peroxidase; GSH: glutathione; LPO: lipid peroxidation.

There was a desirable decrease in all the oxidative enzymes of the depressed mice models, which were reverted after the treatment induced with the test nanocomposite. Oxidative stress asserts as a contributing factor for the psychiatric illness such as depression. The level of free radicals plays a considerable role that distinguishes a normal physiological condition from a depressed state (Bouayed et al., 2009). The biochemical assessment of the highly reactive free radicals determines the restrain intensity of the pre-pared nanocomposites against psychiatric stress and mood disorders. An elevated level of the key enzymes perceives a compatible antioxidant status thereby restoring the depressed state to normal. There were a desirable decrease in all the oxidative enzymes of the depressed mice models and was reverted after the treatment induced with the capsules of St. John's wort loaded nanocomposites. Antioxidants have the ability to defend theses free radicals where, glutathione is known as body's master antioxidant and catalase a natural endogenous antioxidant enzyme, its concentration was found to highly impair in disease conditions, which was found to be restored on treatment. An increased level of LPO was observed in depressed animals, which indicated an excessive formation of free radicals and may also result in membrane damage. The enhanced level of LPO was controlled by inducing the test drugs. Interestingly the results revealed a counteractive approach in elevating the level of the oxidative enzymes in response to the treatment of nanocomposites, which may provide an effective defense of free radicals and can offer neuroprotection.

CONCLUSIONS

This work furnishes the characterization of the prepared nanocomposites that assays the morphology and drug-polymer interaction, which further promotes to evaluate its drug parameters. The prepared nanocomposite with the biopolymer chitosan enhances drug delivery and serves as a carrier for which, the polymer–drug interaction is an essential factor. Chitosan nanoparticles had exhibited a definite ability to associate with St. John's wort capsules with a final concentration of 3 mg/mL that has the proficiency to reduce the dosage level thereby restraining adverse effects. The release profile of St. John's wort commercial capsules from nanoparticles has an initial burst effect followed by a sustained continuous release, which can improve oral absorption. The highly positive zeta potential of nanoparticles reveals better stability elevating as a prominent drug. The *in vivo* studies show a significant alteration in the biochemical parameters of oxidative enzymes after the oral administration. This study furnishes a prelude to elevate polymeric nanocomposites as efficient brain drug to restore mental illness.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Author contribution:

Contribution	Dhayabaran V	Margret A
Concepts or Ideas	X	X
Design		X
Definition of intellectual content	X	X
Literature search		X
Experimental studies	X	X
Data acquisition	X	
Data analysis		X
Statistical analysis	X	X
Manuscript preparation		X
Manuscript editing	X	
Manuscript review		X

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