Antidepressant and Cardioprotective Effects of Self-Nanoemulsifying Self-Nanosuspension Loaded with Hypericum perforatum on Post-Myocardial Infarction Depression in Rats

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Abstract
Hypericum perforatum (HP) is characterized by potent medicinal activity. However, the poor water solubility of many HP constituents limits their therapeutic effectiveness. Self-nanoemulsifying self-nanosuspension loaded with HP (HP.SNESNS) was formulated to improve the bioefficacy of HP. It was prepared using 10% triacetin, 57% Tween 20, and 33% PEG 400 and then incorporated with HP extract (100 mg/mL). HP.SNESNS demonstrated a bimodal size distribution (258.65 ± 29.35 and 9.08 ± 0.01 nm) corresponding to nanosuspension and nanoemulsion, respectively, a zeta potential of -8.03 mV, and an enhanced dissolution profile. Compared to the unformulated HP (100 mg/kg), HP.SNESNS significantly improved cardiac functions by decreasing the serum myocardial enzymes, nitric oxide (NO), and tumor necrosis factor-α (TNF-α) as well as restoring the heart tissue’s normal architecture. Furthermore, it ameliorates anxiety, depressive-like behavior, and cognitive dysfunction by decreasing brain TNF-α, elevating neurotransmitters (norepinephrine and serotonin), and brain-derived neurotrophic factor (BDNF). In addition, HP.SNESNS augmented the immunohistochemical expression of cortical and hippocampal glial fibrillary acidic protein (GFAP) levels while downregulating the cortical Bcl-2-associated X protein (Bax) expression levels. Surprisingly, these protective activities were comparable to the HP (300 mg/kg). In conclusion, HP.SNESNS (100 mg/kg) exerted antidepressant and cardioprotective activities in the post-MI depression rat model.

Keywords depression · Hypericum perforatum · isoprenaline · myocardial infarction · self-nanoemulsifying self-nanosuspension

Introduction
Myocardial infarction (MI) is the leading cause of disability and mortality worldwide [1]. It is demonstrated by a discrepancy between myocardial blood demands and coronary blood delivery [2], resulting in cardiac ischemia and cardiomyocyte degeneration. Unfortunately, this ischemia leads to irreversible cardiac damage or death. About two-thirds of MI patients demonstrated depression symptoms and, consequently, imposed the most significant burden on the country.
due to the disability and high treatment costs. Additionally, they had a higher risk of developing more progressive cardiovascular disease [3] and mortality [4] than MI patients who did not have depression.

Antidepressants, including serotonin reuptake inhibitors (SSRIs), are beneficial in treating depression by acting on the depressive symptoms associated with MI or reducing the serotonin-mediated platelet activation [5]. However, recent randomized clinical studies revealed that treating MI and depression patients with SSRIs does not cause a significant improvement in depressive symptoms and does not improve cardiovascular function [6, 7]. In addition, different treatment strategies were used to alleviate the risk of MI. Nevertheless, the outcomes were undesirable due to their side effects. Therefore there is a great demand to find new effective treatments for managing depression in MI patients.

Isoproterenol hydrochloride (ISP), a synthetic catecholamine, is adopted to induce MI in rats due to its ability to produce various functional, biochemical, and histopathological alterations in the heart tissue [8, 9]. In addition, it generates excessive free radicals that disturb the redox state resulting in necrosis of myocardial muscles [10]. Interestingly, Hu and his colleagues discussed the successful role of isopropyl adrenaline, which is considered an ISP homolog, inducing depressive-like behavior in MI rats. However, the underlying mechanisms associated with depression and the histopathological alterations of heart and brain tissue are not thoroughly investigated [11]. Therefore, using ISP to induce post-MI depression can serve as an excellent non-invasive model to investigate the antidepressant and cardioprotective properties of various synthetic or natural compounds.

Hypericum perforatum L. (HP) is a perennial herbaceous plant native to western Asia, North Africa, and Europe, commonly known as St. John’s wort or millepertuis [12]. It is used as a medicinal herb to treat a variety of diseases, including skin diseases, anxiety, and depression [12–14]. It is also characterized by potent antimicrobial, antiviral, anti-inflammatory, neuroprotective, wound healing, anti-nociceptive, and cytotoxic activities [15]. In addition, the genus Hypericum contains various classes of biologically active compounds such as flavonoids, tannins, xanthones, naphthodianthrones, phloroglucinols, procyanidins, essential oils, and amino acids [16, 17]. HP is currently marketed by many pharmaceutical companies, especially in Europe, and millions of individuals use it. Furthermore, the herb is classified as a dietary supplement by the Food and Drug Administration (FDA) and has astronomical sales estimated to exceed billions of dollars [18].

Despite the significance of HP as a medicinal plant, its therapeutic efficacy is challenging due to the poor solubility of many extract components [19], such as the highly hydrophobic hyperforin, hypericin, and flavonoids, [20] which adversely affect drug therapeutic effectiveness [21]. Consequently, it is crucial to develop a formulation incorporating hydrophilic and hydrophobic components of this herbal extract for concurrent delivery, thus enhancing their therapeutic efficacy.

Self-nano emulsifying self-nanosuspension (SNESNS) is a smart delivery system utilizing dual solubility enhancement strategies as it combines the advantages of both nanoemulsions and nanosuspensions. SNESNS has recently been developed to address the limitations of self-nano emulsifying drug delivery systems, including the difficulty of loading large drug doses, which has inadequate solubility in the self-nano emulsifying preconcentrates. SNESNS consists of an isotropic mixture of oil, surfactant, and co-surfactant, similar to a self-nano emulsifying drug delivery system. In this isotropic mixture of SNESNS, the drug is not entirely dissolved; instead, it is loaded in two forms: dissolved part and suspended microparticles which form nanoemulsion and nanosuspension spontaneously once they are diluted with aqueous fluids. SNESNS has been first reported by [22] as a simple technique to overcome the hurdle of delivering large drug doses in solubility augmenting drug delivery formulations.

Nevertheless, there are only a few reports in the literature to assess the amenability of using this technique to promote the delivery of drugs and herbal extracts. We hypothesized that the ability of SNESNS to incorporate high doses of insoluble drugs would make it a good delivery system for herbal extracts, the administration of which typically exceeds the loading capacity of self-nano emulsifying systems or nanoemulsions. Herein and for the first time, we aimed to enhance HP extract’s oral bioefficacy by incorporating it into SNESNS. In addition, we investigated the antidepressant and cardioprotective effects of HP.SNESNS by assessing the cardiac biomarker enzymes, pro-inflammatory cytokines (IL-6 and TNF-α), depressive-like behavior, brain neurotransmitters (norepinephrine and serotonin), brain-derived neurotrophic factor (BDNF), glial fibrillary acidic protein (GFAP), and Bcl-2-associated X protein (Bax), as well as heart and brain histopathological alterations.

Materials and Methods

Plant Material

The aerial parts of HP were purchased from Haraz herbal store, Cairo, Egypt, in 2019, and authenticated by Dr. Mohamed El-Gibali, the Senior Botanist at El-Orman Botanical Garden. A voucher specimen was deposited in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Cairo University (No. 2019–7–18).
Preparation of HP Ethanolic Extract

The powdered plant material (1 kg) was thoroughly extracted using 70% ethanol (5 × 1.5 L, El-Gomhouria Co., Cairo, Egypt) using an Ultra-Turrax® T25 homogenizer (Janke & Kunkel IKA-Lab., Staufen, Germany). The ethanolic extract was evaporated under reduced pressure to dryness (65 g) and then was maintained in the desiccator over anhydrous CaCl₂ for biological assay.

Metabolic Profiling Using LC/MS/MS

A 400 μl aliquot of the sample was re-suspended in methanol: water (200 μl, UPLC-grade, 1:1, v/v) and transferred to the autosampler. 2 μl was injected and separated on RP High Strength Silica (HSS)T3 C18 column (100 mm × 2.1 mm containing 1.7 μm diameter particles, Waters), using a Waters Acquity UPLC system (Manchester, UK). The mass spectrum was attained by full scan MS in positive ionization mode on an Exactive high-resolution Orbitrap-type MS (Thermo-Fisher, Bremen, Germany) [23]. Metabolites were identified by their mass spectra and compared to our in-house database and the reference literature.

Formulation and In Vitro Characterization of HP.SNESNS

Identification of Self-Nanoemulsifying Regions and Construction of the Ternary Phase Diagram

Thirty-six mixtures were prepared by varying the concentrations of triacetin as an oil, Tween 20 as a surfactant, and PEG 400 (Sigma-Aldrich, Germany) as a cosurfactant. After weighing and vortexing the mixtures, 1 gm of each formula was diluted with 250 mL of water while stirring at 25 rpm at 37°C, and the mixtures were visually examined for the formation of either clear transparent or milky turbid mixtures. A ternary phase diagram was constructed utilizing Sigma-Plot® (V.12.0) software, and the prepared formulations were highlighted to differentiate the self-nanoemulsifying region, which refers to the formulations that are capable of spontaneously forming clear transparent mixtures devoid of any visible oil globules after dilution with water [24, 25].

Preparation of HP.SNESNS

In order to determine the loading capacity of HP extract in the formulation, a solubility study was conducted in 10 mL glass vials by adding 2 mL of the selected SNEDD formulation with an excess amount of HP extract. The mixtures were kept in an isothermal shaker for 72 h and then centrifuged to separate the undissolved extract. Finally, the HP content of the supernatants was determined using a UV spectrophotometer at 260 nm. Solubility of HP extract in water and each SNEDD component, including triacetin, Tween 20, and PEG400, has also been determined. A self-nanoemulsifying formulation was selected from the ternary phase diagram to be used to prepare HP.SNESNS. The formula with the composition of 10% triacetin, 57% Tween 20, and 33% PEG 400 (w/w) was prepared as follows: for each 150 gm of the formulation: 15 g of triacetin, 85.5 g Tween 20, and 49.5 g PEG 400 were mixed, and then 100 mL of the prepared mixture was incorporated with 10 g of HP extract (to yield a concentration of 100 mg/mL) with the aid of homogenization for 5 min so that part of the extract is dissolved while the remaining part exists as undissolved microparticles. The formula was prepared and subjected to further characterization.

Transmission Electron Microscopy (TEM)

After dilution with deionized water, the morphology of the prepared SNESNS was examined using TEM (Joel, JEM-1230, Tokyo, Japan). A drop of the diluted sample was placed on a copper grid and stained with a 2% phosphotungstic acid solution. The excess staining agent was removed with the aid of a filter paper, and then the grid was left to completely dry at ambient temperature.

In Vitro Dissolution Study

To assess the viability of the SNESNS formulation to reliably enhance the dissolution of HP extract, the dissolution study was conducted in non-sink conditions at which water was utilized as a discriminative dissolution medium. Amounts of SNESNS equivalent to 200 mg extract and the unformulated extract powder were filled into colorless hard gelatin capsules, and then the capsules were introduced into the USP II dissolution apparatus (Hanson Research, SR8PLUS, USA) filled with 900 mL water, maintained at 37 ± 0.5°C, and agitated with a paddle rotating at a speed of 75 rpm. Five-milliliter samples were withdrawn periodically at predetermined time intervals of 2, 5, 10, 15, 30, and 60 min and immediately replaced by equal amounts of water to maintain the initial volume. The dissolved amounts of the extract at each interval were determined by measuring the UV absorbance of the withdrawn
samples compared with the extract solution at 260 nm using a UV spectrophotometer and utilizing water as a blank (Schi-
madzu, 1600, Japan).

Animals and Experimental Design

Male Wistar rats (150–180 g) were purchased from a pri-
ivate breeding facility, Giza, Egypt. They were maintained
in a 12:12 h light/dark cycle with a constant temperature
(22°C ± 2°C) and humidity (50% ± 10%). Rats were allowed
free access to a commercial diet and tap water ad libitum.
Rats were randomly assigned into five groups, with seven
rats in each group. Group I: saline/normal control rats was
gavaged orally with normal saline once per day (2 mL/kg)
for 21 days. Group II: isoproterenol/ISP control rats were
administered orally with normal saline once per day (2 mL/
kg) for 19 days. Group III: ISP + HP 100 rats were treated
with HP (100 mg/kg) once per day for 19 days. Group IV:
ISP + HP 300, rats were treated with HP (300 mg/kg) once
per day for for 19 days. Group V: ISP + HP.SNESNS, rats
were treated with SNESNS loaded with HP (100 mg/kg)
once per day for 19 days. MI was induced with ISP (85 mg/
kg, s.c.) on the first and second day in all groups except
Group I and treated with the corresponding treatment for
19 days. Twenty-four hours after the last dose, all groups
were subjected to behavioral assessment at 10 a.m and con-
tinued for 7 days. The ISP and HP doses were determined
based on previous studies [15, 26, 27].

Behavioral Assessment

Open Field Test (OF)

The locomotor activity and rats’ anxiety-like behavior
were investigated using the open field test. The procedures
were conducted in accordance with [28, 29]. Briefly, rats
were gently placed into one side of a square wooden box
(70×70×35 cm) and kept for 3 min. The measuring param-
eters were the number of squares crossed by the rat using all
paws and the frequency of rearing activity.

Elevated Plus Maze (EPM)

The rats were tested in the EPM as described by [30, 31] for
5 min. The measured parameters were the number of open
and closed arms entries and the time the animal spent in the
open and closed arms.

Forced Swim Test

The forced swim test is used as a screening tool to assess
depressive-like behavior in rodents [32]. The procedures
were conducted in two sessions, 24 h apart. The first session
is the training session where the rat was positioned on a cir-
cular arena filled with tap water (22 ± 2°C) for 15 min. The
rat was then subjected to a 5-min testing session. After both
training and testing sessions, the rats were allowed to dry in
a plastic cage. The recorded parameters were the mobility
duration, including swimming and climbing, and immobility
duration, including small movements to maintain the rat
head above the water. The rat was tested for 5 min, with the
first minute discarded due to the animal’s struggle [33].

Y-Maze Test

The Y-maze was used to test the spatial working memory
of rodents [34]. The procedures were conducted for 5 min
as described previously [29, 35]. The measured parameters
were the number of arm entries and the spontaneous alterna-
tion percentage calculated as follows: [number of alterna-
tions / (number of total arm entries-2) × 100%].

Blood and Tissue Harvesting

Blood was collected from the rat’s retro-orbital plexus 24 h
after the last behavioral test and centrifuged at 4000 r.p.m
for 10 min to collect sera. Then, the collected sera were stored
at − 80°C for biochemical analysis of myocardial injury
markers (creatine kinase (CKMB) and lactate dehydrogenase
(LDH)). The rats were then euthanized by cervical disloca-
tion, and heart and brain tissues were carefully removed,
blotted between filter paper folds, and weighed in an analyti-
cal balance. A polytron homogenizer was used to prepare
10% tissue homogenate in 0.05 M phosphate buffer (pH
7) at 4°C. The homogenate was centrifuged at 10,000 rpm
for 20 min to remove the cell debris, intact cells, nuclei,
erthrocytes, and mitochondria. The supernatant (cytoplas-
mic extract) was used to measure NO and TNF-α in cardiac
homogenate and IL-6, TNF-α, norepinephrine, and seroto-
in, BDNF in brain homogenate.

Determination of Heart Weight Index (HWI)

After dissecting the hearts of rats, the heart weight index was
calculated using the following formula (heart weight / total
final body weight × 100).

Biochemical Analysis

Determination of Serum Myocardial Injury Enzymes Using
ELISA

The serum levels of CKMB and LDH were determined using
an enzyme-linked immunosorbent assay (ELISA) kit (Cata-
log No: DEIA-FN285 for CKMB and LS-F5026 for LDH)
according to the manufacture instructions.
Determination of Cardiac NO and TNF-α Using ELISA

According to the manual instructions, nitric oxide, and tumor necrosis, factor-alpha levels in the heart were measured using ELISA kits (Catalog No: STA-802 for NO and SEA133Hu for TNF-α).

Determination of Brain Inflammatory Cytokines and Neurotransmitters Using ELISA

The brain levels of IL-6, TNF-α, norepinephrine, and serotonin were estimated using ELISA kits following the manufacturer’s instructions (Catalog No: SEA079Hu for IL-6, SEA133Hu for TNF-α, MBS269993 for norepinephrine, and LS-F27987 for serotonin).

Western Blotting Analysis of Brain-Derived Neurotrophic Factor (BDNF)

The protein concentration in the brain homogenate was first determined using a BCA protein assay kit, and 20 μg of tissue samples were boiled for 5 min, loaded on 10% polyacrylamide gel (SDS-PAGE), and then transferred onto PVDF membranes. The membranes were blocked in tris-buffered saline with Tween 20 (TBST) buffer and 3% bovine serum albumin at room temperature for 1 h. The membranes were incubated with primary antibodies against BDNF (dilution 1:500, rabbit monoclonal to BDNF, clone ID: EPR1292, Epitomics Co, USA) overnight at 4°C, rinsed with TBST (3–5 times) for 5 min, and then incubated with HRP-conjugated secondary antibody (dilution as 1:1000) for 1 h at room temperature and rinsed (3–5 times) with TBST for 5 min. The blots were detected by enhanced chemiluminescence (Millipore). The densitometry of protein bands was identified against beta-actin using ChemiDoc MP Image analysis software.

Histopathological Examination of Myocardial and Brain Tissues

Hearts were cut into transverse sections, while brains were cut into sagittal sections. Then, they were fixed in neutral buffer formalin (10%) for 24 h, embedded in paraffin, and sectioned at 4–5-μm thickness for hematoxylin and eosin staining [36]. Slides were examined using a light microscope (Olympus, BX43). The histological scores of heart and brain tissues were evaluated according to [37, 38], with some modifications as described in (Table I).

Immunohistochemical Examination for Glial Fibrillary Acidic Protein (GFAP) and Bcl-2-Associated X Protein (Bax)

For immunohistochemical (IHC) assays, brain sections were deparaffinized and rehydrated with graded descending concentrations of alcohol and then treated with peroxidase solution to avoid nonspecific staining for 15 min at room temperature. Subsequently, sections were washed three times using phosphate-buffered saline (PBS) and incubated with a polyclonal anti-GFAP antibody (1:100, SAB4501162, Sigma-Aldrich, St. Louis, MO) for astrocyte identification and a monoclonal anti-Bax antibody (1:100, HPA027878, Sigma-Aldrich, St. Louis, MO) for apoptosis detection (each was incubated for 30 min). Afterward, slides were rinsed three times in PBS and incubated with secondary antibodies for 30 min. Sections were treated with DAB-chromogen-substrate (2 mL) for 15 min to enhance color reaction and then counterstained with hematoxylin. Positive reactions were assessed as area % using ImageJ software.

Statistical Analysis

The data were expressed as means ± standard error (SEM), and the statistical analysis was conducted using SPSS (Version 24; SPSS Inc., Armonk, NY, USA). One-way analysis of variance (ANOVA) followed by Tukey post hoc test was used, and partial eta squared ($\eta_p^2$) was reported as an effect size index. GraphPad Prism (Version 6, San Diego, CA, USA) was used to draw histograms. The data were expressed as median ± standard deviation (SD) using Kruskal–Wallis test, followed by Dunn’s multiple comparison test for histopathological scoring. The level of statistical significance was set at $P \leq 0.05$.

Results

Metabolic Profiling of HP

Using UPLC/HRMS, more than 20 compounds were tentatively identified and belonging to various metabolite classes, including flavonoids, phenolic acids, and phloroglucinols. Metabolite assignments were made by comparing retention time and MS data (accurate mass, isotopic distribution, and fragmentation pattern in positive ionization modes) and subsequent confirmation with the literature data. The chromatographic and spectroscopic data are summarized in Table II and Fig. 1.
Formulation and In Vitro Characterization of HP.
SNESNS

The viability of the formulations to self-emulsify upon dilution along with gentle agitation was judged. Various formulations were prepared with the compositions illustrated in Table III; ternary phase diagram is shown in Fig. 2A. As observed from the results, only 8 formulations could form clear transparent nanoemulsions with a common percent of oil composition of 10%. Increasing oil concentration by more than 10% led to the loss of the self-nanoemulsifying ability of the system. Additionally, it is noticeable that Tween 20 concentrations should be ≥ 29.15 to maintain self-nanoemulsification properties. Hence, the formula with a composition of 10% triacetin, 57% Tween 20, and 33% PEG 400 was selected to load the drug as it contained a compromised surfactant/cosurfactant ratio and demonstrated good viscosity since it was crucial to adjust the Tween 20 concentration to be high enough to ensure self-emulsification of triacetin yet low to maintain the flow-ability of the system. The solubility of HP extract in water, triacetin, Tween 20 and PEG 400 was 8.62 mg/mL, 0.84 ± 0.31, 3.50 ± 1.1, and 1.03 ± 0.5 mg/mL, respectively. In contrast, the solubility of HP in SNEDD was higher than that in the individual components with a value of 34.89 ± 2.1 mg/mL; consequently, upon loading one mL of the SNESNS

| Lesion | Description (heart score system) | Score |
|--------|----------------------------------|-------|
| Myocardial necrosis | Normal | 0 |
| < 25% of the field | 1 |
| 25–50% of the field | 2 |
| 50–75% of the field | 3 |
| > 75% of the field | 4 |
| Inflammatory cell infiltration | Normal | 0 |
| < 25% of the field | 1 |
| 25–50% of the field | 2 |
| 50–75% of the field | 3 |
| > 75% of the field | 4 |
| Thrombus formation | − ve | 0 |
| + ve | 1 |
| Fibrosis | Normal | 0 |
| < 25% of the field | 1 |
| 25–50% of the field | 2 |
| 50–75% of the field | 3 |
| > 75% of the field | 4 |
| Extravasation of RBCs | − ve | 0 |
| + ve | 1 |
| Edema | − ve | 0 |
| + ve | 1 |

Heart and brain damage was assessed by summation the total lesions score
### Table II: The Identified Metabolites from the Ethanolic Extract of HP

| R_t | MS^+ Error (ppm) | Identified compound | Molecular formula | MS/MS^+ fragments |
|-----|------------------|---------------------|-------------------|------------------|
| 4.26| 355.1026 0.68    | 5-O-cafeoylquinic acid (chlorogenic acid) | C_{16}H_{19}O_{9} | 163.03, 135.044 |
| 5.03| 355.1025 0.511   | O-cafeoylquinic acid isomer | C_{16}H_{19}O_{9} | 291.08, 163.03 |
| 5.29| 579.15 0.444     | Procyanidin dimer | C_{30}H_{22}O_{12} | 485.16, 289.07, 195.06, 177.05 |
| 5.76| 291.0864 0.499    | Catechin | C_{15}H_{16}O_{6} | 234.98 |
| 6.42| 481.0984 1.44     | Myricetin 3-O-glucoside | C_{21}H_{32}O_{13} | 319.04, 137.023 |
| 6.72| 611.1611 0.669     | Rutin | C_{27}H_{31}O_{16} | 303.04, 207.13 |
| 6.88| 611.1616 1.471     | Kaempferol-3-O-glucopyranosyl-galactopyranoside | C_{22}H_{27}O_{16} | 303.04, 153.01 |
| 7.08| 479.0825 0.925     | Miquelianin | C_{21}H_{22}O_{13} | 303.05 |
| 7.14| 465.1033 1.113     | Quercetin-3-O-galactoside (hyperoside) | C_{21}H_{22}O_{12} | 303.05 |
| 7.27| 451.1242 1.534     | Dihydroquercetin (astilbin) | C_{21}H_{21}O_{11} | 303.04 |
| 7.49| 435.0927 1.269     | Quercetin 3-O-arabinopyranoside | C_{20}H_{19}O_{11} | 303.04, 149.02 |
| 7.5 | 449.1085 1.519     | Quercetin-3-O-rhamnoside (isoquercetin) | C_{21}H_{22}O_{11} | 303.04, 153.01 |
| 7.61| 435.0927 1.2       | Quercetin 3-O-arabinopyranoside | C_{20}H_{19}O_{11} | 303.05 |
| 7.9 | 433.1136 1.609     | Vitexin | C_{21}H_{22}O_{10} | 271.05, 154.99 |
| 9.47| 701.15027 0.247    | Skyrin-2-O-glucopyranoside | C_{26}H_{31}O_{15} | 539.09 |
| 9.55| 303.05 0.102       | Quercetin | C_{15}H_{13}O_{7} | 229.04, 153.01 |
| 10.8| 287.0548 −0.713    | Kaempferol | C_{15}H_{11}O_{6} | 153.018 |
| 11.16| 539.0979 1.163    | Amentaflavone (Biapigenin) | C_{30}H_{18}O_{10} | 445.25, 293.13 |
| 13.34| 521.0864 −0.534   | Pseudohypericin | C_{30}H_{17}O_{9} | 471.31, 293.13 |
| 14.75| 401 −5.672        | Hyperatomanan/hypercalyxone A | C_{22}H_{15}O_{4} | 293.13 |
| 15.17| 569.3416 0.868    | Hydroperoxy furufyperforin | C_{33}H_{32}O_{6} | 493.31 |
| 16.22| 553.3897 1.624    | Furohyperforin | C_{35}H_{34}O_{4} | 485.32, 293.137 |
| 16.32| 553.3897 1.407    | Furohyperforin | C_{35}H_{34}O_{5} | 293.13 |
| 16.26| 483.3473 0.897    | Hyperpolyphyllirin | C_{31}H_{22}O_{4} | 293.13 |
| 16.66| 469.3315 0.519    | Hyperforin | C_{30}H_{22}O_{4} | 287.16 |
| 16.67| 537.3948 1.756    | Hyperforin | C_{32}H_{24}O_{4} | 469.33, 277.14 |
| 16.91| 551.4094 −0.23    | Adhyperforin | C_{36}H_{35}O_{4} | 293.13 |

**Fig. 1** Total ion chromatogram of HP L. ethanolic extract in positive ionization mode.
formulation with 100 mg HP extract, 65.11 mg remains undissolved, while the solubilized fraction is 34.89 mg.

The size distribution chart is depicted in Fig. 2b, which reveals that the diluted SNESNS demonstrated two peaks corresponding to the presence of nanoemulsion globules and the nanosized particles of the formed nanosuspension with a mean size of 9.08 ± 0.01 nm (intensity 36.45%) and 258.65 ± 29.35 nm (intensity 61.45%), respectively. TEM micrographs are depicted in Fig. 2c, and it is evident that the SNESNS formulation was able to form a double nanosystem (nanoemulsion and nanosuspension appear as white droplets and black spots, respectively). The PDI of the system is high with a value of 0.93 ± 0.03 due to simultaneous detection of both nanosuspension and nanoemulsion peaks. The results of in vitro dissolution are depicted in Fig. 2d. It is evident that the SNESNS formulation improved the dissolution profile of HP extract compared to the unformulated HP extract with statistically significant differences (P < 0.05). The SNESNS formulation released 72.3 ± 0.6% of the extract only in 5 min, while the unformulated HP extract dissolved only 14.04 ± 5%. In addition, the unformulated HP extract could not be dissolved by more than 42.07 ± 1% even after 60 min at the end of the study. In general, the rapid dissolution of the formulation is favorable to ensure the complete absorption of the extract from the GIT after administration.

### Behavioral Parameters

#### Open Field Test

There was a statistically significant difference between the means of different groups in the number of crossings [F (4, 29) = 23.4, p < 0.001, η² = 0.76, Fig. 3a] and rearing frequency [F (4, 29) = 8.83, p < 0.001, η² = 0.54, Fig. 3b] as determined by one-way ANOVA. Tukey’s multiple comparisons test revealed that ISP-exposed rats demonstrated a significant reduction in the number of crossings and rearing frequency compared to control rats. Conversely, treatment either with the unformulated extract in a dose of 300 mg/kg or with the HP.SNESNS at a dose of 100 mg/kg significantly increased the number of line crossing and the rearing frequency compared to the ISP group.

#### Elevated Plus Maze

There was a statistically significant difference between the means of different groups in the number of open arms [F (4, 29) = 9.43, p < 0.001, η² = 0.92, Fig. 4a] and closed arms entries [F (4, 29) = 3.8, p < 0.01, η² = 0.87, Fig. 4c] as well as the duration of time spent in open [F (4, 29) = 3.85, p < 0.012, η² = 0.84, Fig. 4b] and closed arms [F (4, 29) = 3.85, p < 0.01, η² = 0.94, Fig. 4d] as determined by one-way ANOVA. Tukey’s multiple comparisons test revealed that ISP-exposed rats showed a significant reduction in the time spent and the number of entries in open arms associated with a significant elevation in the time spent in closed arms compared to rats in the control group. However, treatment with HP in both doses and the HP.SNESNS at a dose of 100 mg/kg significantly increases the number of line crossing and the rearing frequency compared to the ISP group.

### Table III

| Formula | % Triacetin | % Tween 20 | % PEG 400 |
|---------|-------------|------------|-----------|
| 1       | 9.61        | 9.74       | 80.64     |
| 2       | 10.18       | 20.36      | 69.44     |
| 3       | 11.17       | 29.15      | 59.67     |
| 4       | 11.04       | 39.57      | 49.38     |
| 5       | 11.90       | 47.45      | 40.63     |
| 6       | 10.17       | 57.05      | 32.77     |
| 7       | 11.13       | 68.38      | 20.48     |
| 8       | 11.27       | 77.02      | 11.71     |
| 9       | 19.19       | 13.83      | 66.97     |
| 10      | 19.82       | 20.31      | 59.86     |
| 11      | 20.87       | 29.34      | 49.78     |
| 12      | 21.58       | 40.41      | 38.00     |
| 13      | 19.57       | 51.33      | 29.09     |
| 14      | 22.29       | 54.81      | 22.88     |
| 15      | 21.37       | 65.01      | 13.60     |
| 16      | 29.83       | 11.14      | 59.02     |
| 17      | 30.65       | 19.76      | 49.57     |
| 18      | 29.69       | 30.29      | 40.01     |
| 19      | 30.19       | 39.97      | 29.83     |
| 20      | 28.93       | 48.74      | 22.32     |
| 21      | 30.36       | 58.02      | 11.61     |
| 22      | 39.80       | 11.50      | 48.68     |
| 23      | 39.23       | 20.40      | 40.36     |
| 24      | 40.05       | 30.31      | 29.63     |
| 25      | 40.18       | 39.03      | 20.78     |
| 26      | 38.85       | 48.21      | 12.93     |
| 27      | 48.38       | 10.11      | 41.49     |
| 28      | 49.04       | 22.11      | 28.85     |
| 29      | 46.79       | 30.54      | 22.66     |
| 30      | 48.35       | 37.78      | 13.85     |
| 31      | 58.30       | 10.02      | 31.67     |
| 32      | 58.09       | 21.22      | 20.68     |
| 33      | 58.48       | 30.82      | 10.69     |
| 34      | 67.76       | 10.52      | 21.70     |
| 35      | 68.08       | 18.99      | 12.92     |
| 36      | 74.92       | 12.21      | 12.87     |
Forced Swim Test

There was a statistically significant difference between the means of different groups regarding mobility \([F (4, 29) = 19.48, p < 0.001, \eta^2_p = 0.72, \text{Fig. 5a}]\) and immobility durations \([F (4, 29) = 19.48, p < 0.001, \eta^2_p = 0.72, \text{Fig. 5b}]\), as determined by one-way ANOVA. Tukey’s multiple comparisons test revealed that ISP-exposed rats significantly elevated in the immobility period compared to the control group. However, treatment with HP in both doses and the HP.SNESNS at a dose of 100 mg/kg ameliorated the depressive-like behavior of the ISP as there was a dose-dependent reduction in the immobility period.

**Fig. 2**

(a) Ternary phase diagram depicts the prepared 36 formulations where the green area represents the self-nanoemulsifying region where the black dots reveal the composition of each self-nanoemulsifying formula, while the yellow triangle represents the formulations that were not able to self-emulsify spontaneously upon mixing with water. (b) Particle size distribution by intensity depicts two peaks corresponding to nanoemulsion globules (left) and nanosuspension particles (right). (c) TEM micrographs of HP.SNESNS formulation revealing black particles of the formed nanosuspension after dilution with water along with white nanoemulsion droplets of smaller droplet size. (d) In vitro dissolution profiles of HP.SNESNS and the unformulated HP extract.
Y-Maze

There was a statistically significant difference between means of different groups in the frequency of Y-maze arm entries \[F(4, 29) = 6.16, p < 0.001, \eta_p^2 = 0.46, \text{Fig. 5c}\] and SAP \[F(4, 29) = 4.18, p < 0.008, \eta_p^2 = 0.366, \text{Fig. 5d}\], as determined by one-way ANOVA. Tukey’s multiple comparisons test revealed that the frequency of arm entries is

Fig. 3 Effects of HP and HP.SNESNS on the locomotor behavior of rats with post-MI depression. a Number of crossings. b The number of reaings in the open field. Data are expressed as mean ± SEM, one-way ANOVA followed by post hoc Tukey test for seven rats in each group. * Significant from the control group, # significant from the ISP group, and $ significant from ISP + HP 100 group \((P < 0.05)\)

Fig. 4 Effects of HP and HP.SNESNS on the anxiety-like behavior of rats with post-MI depression in the elevated plus maze. a Open arm entry. b Open arm entry. c Close arm entry. d Close arm duration. Data are expressed as mean ± SEM, one-way ANOVA followed by post hoc Tukey test for seven rats in each group. * Significant from the control group and # significant from the ISP group \((P < 0.05)\)
significantly decreased in the ISP-exposed rats compared to rats in the control group. Nonetheless, treatment with the HP in both doses or the HP.SNESNS at a dose of 100 mg/kg significantly increased the locomotor activity of the rats in the Y-maze (Fig. 5c).

Furthermore, the ISP-exposed rats demonstrated severe impairment in spatial memory, as indicated by lower percentages of spontaneous alternation behavior than the control group. On the contrary, treatment with HP in both doses and the HP.SNESNS at a dose of 100 mg/kg caused upgrading in the spatial cognitive ability, as evidenced by a dose-dependent increase in the SAP, but the difference was not statistically significant (Fig. 5d).

**Heart Weight Index**

There was a statistically significant difference between different groups in the heart weight index means \[ F (4, 29) = 4.49, p < 0.006, \eta^2_p = 0.38, \text{Fig. 6a} \], as determined by one-way ANOVA. Tukey’s multiple comparisons test revealed that ISP-exposed rats increased the heart weight index compared to the control group, however non-significant. On the other hand, treatment with either HP in a dose of 100 mg/kg or 300 mg/kg caused a dose-dependent reduction in the heart weight index, but the difference was not statistically significant. Interestingly, the incorporation of HP in a SNESNS caused a significant increase in the heart weight index compared to the ISP group.

**Biochemical Parameters**

Effects of HP and HP.SNESNS on the Cardiac Biomarkers, NO, and TNF-α of Rats with Post-MI Depression

One-way ANOVA demonstrated a statistically significant difference between different groups regarding cardiac biomarker levels (creatine kinase \[ F (4, 29) = 611 \]...
.77, $p < 0.001$, $\eta_p^2 = 0.98$, Fig. 6b] and lactate dehydrogenase \([F (4, 29) = 131.40, p < 0.001, \eta_p^2 = 0.94, \text{Fig. 6c}]\), NO \([F (4, 29) = 295.13, p < 0.001, \eta_p^2 = 0.97, \text{Fig. 6d}]\), and TNF-α \([F (4, 29) = 301.46, p < 0.001, \eta_p^2 = 0.97, \text{Fig. 6e}]\). Tukey’s multiple comparisons test revealed that ISP-exposed rats displayed substantially increased levels of cardiac biomarkers (creatine kinase and lactate dehydrogenase), NO, and TNF-α compared to the control group. However, treatment with HP in both doses and the HP.SNESNS at a dose of 100 mg/kg significantly decreased these parameters. Remarkably, the results obtained after treatment with HP.SNESNS at a dose of 100 mg/kg were comparable to those of HP at a dose of 300 mg/kg.

**Histopathological Examination of Heart Tissue**

Heart sections from the control group revealed normal histological architecture. In contrast, the ISP-treated group demonstrated severe damage to the myocardium, including necrotic myofibers, inflammatory cell infiltration, fibroplasia, and micro-thrombosis associated with endothelial swelling. Similar changes were observed in (ISP + HP...
100) group. In addition, both HP 300 and HP.SNESNS 100 treated groups significantly improved myocardium structure (Fig. 7).

**Effects of HP and HP.SNESNS on the Brain Pro-inflammatory, Inflammatory Cytokines, and Neurotransmitters of Rats with Post-MI Depression**

One-way ANOVA demonstrated a statistically significant difference between different groups in means of the pro-inflammatory and inflammatory cytokines (IL-6 [$F(4, 29) = 189.55, p < 0.001, \eta_p^2 = 0.96$, Fig. 8a] and TNF-α [$F(4, 29) = 165.31, p < 0.001, \eta_p^2 = 0.95$, Fig. 8b]), neurotransmitters (norepinephrine [$F(4, 29) = 129.95, p < 0.001, \eta_p^2 = 0.94$, Fig. 8c], and serotonin [$F(4, 29) = 542.25, p < 0.001, \eta_p^2 = 0.98$, Fig. 8d]). Tukey's multiple comparisons test revealed that ISP-exposed rats demonstrated a substantial increase in the pro-inflammatory and inflammatory cytokines (IL-6 and TNF-α) associated with a decline in brain neurotransmitters (norepinephrine and serotonin).

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**Fig. 7**  
(a) Photomicrograph of heart tissues stained with (H&E). Remarkable marks are revealed on the figure as follows: inflammatory cell infiltration (arrowhead), coagulative necrosis (curved blue arrows), fibroplasia (black arrows), micro-thrombosis associated with swelling of the endothelium (curved black arrows).  
(b) Heart damage score. Data expressed as median±SD using Kruskal–Wallis test, followed by Dunn’s multiple comparison Test. * Significant from the control group ($P < 0.05$)
compared to rats in the control group. However, treatment with the HP in both doses or the HP.SNESNS at a dose of 100 mg/kg caused a significant elevation in the pro-inflammatory and inflammatory cytokines accompanied by increased brain neurotransmitters. Interestingly, treatment with HP.SNESNS at a dose of 100 mg/kg was comparable to the HP at a dose of 300 mg/kg.

**Histopathological Investigations of Brain Tissues**

Brain sections stained with H&E from the control group showed normal neurons and neuroglia of the cerebral cortex as well as intact structure of the hippocampus, which is classified into three main parts: hippocampus proper, dentate gyrus, and subiculum. The hippocampus proper is divided into four regions based on the morphology and size of pyramidal cells (CA1-CA4). In the ISP group, the cerebral cortex revealed neurophagia of necrotic neurons and faintly stained shades of granule cells, which lost their nucleoli. The hippocampus area revealed a decrease in the thickness of the cells at CA1, which reached into one row of cells in some fields, in addition to size reduction as well as the absence of pyramidal cells in CA3. Furthermore, marked vacuolation was observed in the molecular layer’s dentate gyrus associated with apoptotic pyramidal cells.

No significant improvement was observed in the brain histological structure of rats treated with HP at a dose of 100 mg/kg. Additionally, necrotic neurons in the cerebral cortex decrease the thickness of hippocampal pyramidal cells, and vacuolation of the dentate gyrus was observed. Treatment with either HP at a dose of 300 mg/kg or HP.SNESNS at a dose of 100 mg/kg preserved cerebral cortex nerves’ structure and enhanced the thickness of granular and pyramidal cells in the hippocampal region; only dark-stained neurons were observed (Fig. 9).

**Effects of HP and HP.SNESNS on the Brain-Derived Neurotrophic Factor (BDNF) of Rats with Post-MI Depression**

One-way ANOVA demonstrated a statistically significant difference between different groups in means of BDNF protein expression \[ F(4, 29) = 14.40, p < 0.001, \eta^2_p = 0.66 \], Fig. 10. Tukey’s multiple comparisons test revealed that ISP-exposed rats showed substantially decreased BDNF...
protein expression compared to rats in the control group. However, treatment with the HP in both doses significantly decreased the BDNF protein expression dose-dependently. Remarkably, favorable results obtained after treatment with HP.SNESNS at 100 mg/kg doses were more significant than those of HP at 300 mg/kg (Fig. 10).

Immunohistochemical Investigation

Compared to the control group, the cerebral cortex and hippocampus of the ISP and ISP + HP 100 groups showed moderate GFAP expression ($P < 0.001$, $P < 0.01$, respectively). In addition, astrocytes in these groups have processes that are scattered, disbanded, and damaged processes with indistinct cell bodies. In the (ISP + HP 300) and (ISP + HP.SNESNS 100) groups, the expression of GFAP was enhanced, and the astrocytes regained their typical structure and revealed distinct, abundant process and opaque cell bodies (Fig. 11a, c).

On the contrary, Bax positive expressed neurons featured with brown-yellow cytoplasm under the light microscope. The cerebral cortex neurons showed significant Bax expression in both the ISP group and ISP + HP100 group compared to the control group ($P < 0.001$), which signified apoptosis. The expression was more evident in neurons than in astrocytes. Both (ISP + HP 300) and (ISP + HP.SNESNS) groups demonstrated insignificant Bax gene expression, with no significant differences between both groups. Surprisingly, we found no Bax expression in the hippocampus and amygdala (Fig. 11b, d).

Discussion

Post-MI depression is considered a significant health issue that leads to recurrent cardiac events and high mortality rates among a significant number of patients. Despite the advancement in myocardial infarction treatment, the results are not satisfactory. Therefore, there is a great need to find more therapeutics with fewer side effects. SNESNS is a newly developed drug delivery system that has evolved as a modification of self-nanoemulsifying drug delivery systems to make them suitable to accommodate large drug doses and to enhance the solubility and efficacy of drugs depending on the synergism of two solubilization techniques which are
self-nanoemulsification and in situ formation of nanosuspension [22, 39, 40]. The favorable advantages of SNESNS present it as a delivery system of choice for herbal extracts, which contain multiple components, including hydrophobic and hydrophilic materials, as the formed W/O nanoemulsion can load both materials.

In addition, the components whose concentrations exceed their saturated solubility in the system can be precipitated as fine particles of a large surface area, thus enhancing the overall solubility. The particle size of the developed HP.SNESNS exhibited that fine nanosuspension and nanoemulsion have spontaneously formed upon dilution with water. The diluted SNESNS dispersion demonstrated a negative value of zeta potential, indicating the stability of the colloidal system through the electrostatic repulsive forces, and this may be attributed to the extracted content of acids such as 5-O-caffeoylquinic acid (chlorogenic acid) and O-caffeoylquinic acid isomer, which are suggested to be intercalated in the aqueous phase of the formed nanoemulsion due to their hydrophilicity.

Furthermore, the TEM micrographs confirmed the formation of nanoemulsion along with the presence of nanosuspension of the HP extract components. Remarkably, the formulated SNESNS could significantly ameliorate the in vitro dissolution profile of the HP extract by more than five-fold within only 5 min, even though the dissolution study has been conducted in non-sink conditions. It was hypothesized that the rapid and augmented dissolution profile of the extract from the formulated SNESNS might contribute to enhancing the extract absorption from GIT. In general, these favorable results of in vitro characterization of HP.SNESNS suggested the conductance of in vivo further evaluation to assure the enhancement of the therapeutic efficacy of the HP extract.

In our in vivo study, MI was induced by S/C injection of ISP at a dose of 85 mg/kg for two consecutive days. Then, the treatment strategies begin after the ISP challenge and continue for 19 days, according to our previous study [41]. MI and depressive-like behavior of the ISP intoxicated group were investigated at the end of the experiment. MI was indicated in rats by the elevation in the myocardial injury enzymes like CK-MB and LDH, and the elevation of NO and TNF-α is associated with myocardial necrosis evident in the histopathological picture of the heart. Additionally, depression was confirmed after MI by assessing the depressive-like behavior, inflammatory cytokines, neurotransmitters, and BDNF in brain tissue. Furthermore, histopathological alterations of the brain were detected, and the immunohistochemical expression of GFAP and Bax were performed.

ISP administration resulted in the myocardium enzymes (CK-MB and LDH) leaking into circulation. These enzymes were elevated after myocardial necrosis, a significant mark in MI, which aligns with the findings of [11]. However, administration of HP at a dose of 300 mg/kg and the HP.SNESNS at a dose of 100 mg/kg resulted in restoring the normal levels of these enzymes. Moreover, nitric oxide (NO) acts as a regulatory mediator in the cardiovascular system and is produced via the activation of inducible nitric oxide synthase (iNOS). Despite its anti-inflammatory and anti-thrombotic properties, excessive NO is the primary cause of myocyte toxicity and tissue damage [42].

In the current research, ISP intoxicated rats displayed elevated cardiac levels of NO compared to control. Conversely, HP administration (100 mg/kg and 300 mg/kg) resulted in a dose-dependent decrease in the NO levels. Interestingly, the cardiac levels of NO in the HP.SNESNS-treated group were comparable to normal levels.

In addition, many pro-inflammatory cytokines like NF-κB, IL-6, and TNF-α are elevated in the MI group. They are responsible for the increased expression of iNOS by stimulating the cells in the blood vessel wall, therapy leading to excessive NO production. In our study, the cardiac tissue of ISP-treated rats exhibited elevated levels of TNF-α compared to control rats. On the contrary, administration of HP (100 mg/kg and 300 mg/kg) results in a dose-dependent decrease in the TNF-α levels. Interestingly, the cardiac levels of TNF-α in the HP.SNESNS-treated group...
were comparable to normal levels, consistent with our histopathological investigations of cardiac tissue results. Cardiac tissue from ISP-exposed rats revealed coagulative necrosis associated with inflammatory cell infiltration and fibroplasia. ISP-induced oxidative damage leads to free radical production that induces myocardial membrane injury through the peroxidation of phospholipids [43]. Consequently, cardiomyocyte demise in the infarcted area by either apoptosis or necrosis [44]. The infarcted area enters the inflammatory phase, characterized by marked infiltration of neutrophils and macrophages that trigger the dead tissue. Afterward, macrophages engulf the dead neutrophils and release growth factors that promote fibroblast activation [45]. However, administration of HP (100 mg/kg) and HP.SNESNS at a dose of 100 mg/kg improves the histological structure of heart tissue, which could be linked to its anti-inflammatory capacity via inhibiting pro-inflammatory gene expression and reduction of prostaglandin E2 (PGE2) production [46]. Moreover, hyperforin (flavonoid active principle) enhances the blood flow in the blood vessels [18]. Therefore, it prevents thrombus formation in the infarcted heart tissue and maintains oxygen delivery to heart tissue.

After confirming the MI from the data above, we demonstrate the occurrence of post-MI depression. Elevation of pro-inflammatory cytokines (IL-6 and TNF-α) is strongly linked with depression pathogenesis [47, 48]. In the current research, the brains of ISP-exposed rats demonstrated elevated IL-6 and TNF-α levels. In contrast, administration of HP (100 mg/kg and 300 mg/kg) caused a dose-dependent reduction in these levels. Notably, HP at a 300 mg/kg dose in treated rats was comparable to the HP.SNESNS-treated rats.

Accumulating evidence suggested the effect of cytokines on the neurotransmitters system. It can influence either the release or the synthesis or the reuptake of monoamine transmitters [49], leading to neurotransmitters system disturbance, which can affect the behavior as reported in previous research [50]. In the current study, ISP-exposed rats demonstrated a reduction in the brain levels of monamines (norepinephrine and serotonin) compared to control rats. These findings are in agreement with Liu et al. study [51] though they induced MI
by ligating the left anterior descending coronary artery. On the contrary, administration of HP (100 mg/kg and 300 mg/kg) caused a dose-dependent increase in these levels. Surprisingly, HP at a 300 mg/kg dose in treated rats was comparable to the HP.SNESNS-treated rats, and the two doses were superior to the 100 mg/kg HP treated rats.

As previously mentioned, neurotransmitter disturbance is responsible for the severity of depressive symptoms [50]. In our study, we assessed the depressive-like behavior in MI rats using an open field test, elevated plus maze, and forced swim test. The cognition dysfunction was also associated with the depressive episodes and was assessed using the y maze. The forced swim test is a measure of behavioral despondency [52]. In the current study, the ISP-exposed rats displayed a reduction in the mobility duration associated with an elevation in the immobility time compared to control rats, indicating the depressive state of the MI rats.

On the contrary, administration of HP (100 mg/kg, 300 mg/kg, and 100 mg/kg HP.SNESNS) increased the mobility time in the FST. The antidepressant effect of HP was previously reported [53], and these findings are consistent with those reported by Liu and colleagues [51]. Open field tests, and the elevated plus maze is conducted to measure the anxiety-like behavior associated with MI. Our data revealed that ISP intoxicated rats demonstrated a significant increase in their anxious state expressed by a decrease in their general motor activity in the open field test as well as the decrease in the open arm entry and duration associated with an increase in the time spent in closed arms of EPM. Conversely, HP (100 mg/kg, 300 mg/kg, and 100 mg/kg HP.SNESNS) improved the emotional state of the MI rats. The anxiety-like behavior associated with MI rats was previously reported [54].

In addition, depression is accompanied by a cognitive dysfunction [52], as evidenced by a decrease in the working spatial memory of ISP-exposed rats and a decrease in the spontaneous alternation percentage, a measure of the natural tendency of rats to alternate between three different arms. In contrast, administration of HP (100 mg/kg, 300 mg/kg, and 100 mg/kg HP.SNESNS) increased the cognitive abilities of the MI rats.

BDNF is an essential neurotrophic family member responsible for neuronal plasticity [55]. It contributed to the pathogenesis of depression as low levels of hippocampal BDNF were associated with depression [56]. In the present study, ISP-exposed rats displayed low levels of brain BDNF. However, administration of HP (100 mg/kg and 300 mg/kg) increased the BDNF levels in a dose-dependent manner. In contrast, rats treated with 100 mg/kg of HP.SNESNS restored almost their normal levels. Furthermore, astrocytes play a crucial role in the pathophysiology of depression due to their interaction with synapses and stimulating the reuptake of glutamate through the excitatory amino acid transporters (EAAT) 1 and 2 present in its body and processes [57]. The the ISP-exposed group demonstrated low expression levels of the GFAP-immunoreactive astrocytes.

Conversely, administration of HP (100 mg/kg and 300 mg/kg) increased the cortical and hippocampal GFAP levels in a dose-dependent manner. In contrast, the 100 mg/kg HP.SNESNS group was similar to HP 300 mg/kg group. An earlier study revealed that the cortical and hippocampal GFAP levels are attributed to decreased brain BDNF [58].

Finally, ISP could enhance the expression of Bax protein in the cerebral cortex. No Bax expression was detected in the hippocampal area, regardless of apoptosis and necrosis, which were confirmed in hippocampal neurons by H&E stain. This result could be linked to the inactivity of Bax in this area, and the necro-apoptotic changes could be attributed to caspase-3 activity. Furthermore, administration of HP (300 mg/kg) and 100 mg/kg HP.SNESNS demonstrated anti-apoptotic activity by diminishing the levels of Bax expression in neurons of the cerebral cortex. In general, the use of HP extract could mitigate the depressive illness in the MI depressed rat model.

Interestingly, loading of HP extract in a SNESNS could significantly augment the efficacy of the HP extract, as evidenced by the comparability of the results of the rats that received 300 mg/kg body weight from the HP extract and the rats received only 100 mg/kg from the HP loaded in a SNESNS. Consequently, there was a promising amelioration of HP extract’s oral delivery and bioefficacy from SNESNS, which is interpreted as an augmented therapeutic activity, including antidepressant and cardioprotective effects against the post-MI depression in the developed rat model. These findings can be attributed to the ability of the formulation to simultaneously form a nanoemulsion and nanosuspension of the extract, resulting in enhanced and rapid dissolution of the HP extract in the gastrointestinal fluids and consequently improved its absorption. Subsequently, our developed SNESNS can decrease the HP extract’s regular doses, thus significantly decreasing the treatment costs and minimizing the side effects in patients.

It should be noted that polyphenolic compounds, the main class in hypericum, the predominant class in hypericum, are characterized by heart-protective, anti-inflammatory, and antioxidant properties. Flavonoids and anthocyanins have been found to have cardioprotective effects via different pathways, including decreasing vascular inflammatory responses, oxidative injury, and plasma lipids [59]. Therefore, the cardioprotective properties demonstrated in our plant extract could be due to the presence of chlorogenic acid [60]; catechins [61]; rutin [62]; Kaempferol- O-glucopyranosyl-galactopyranoside and Kaempferol [63]; hyperoside [64]; quercetin [65–67]; vitexin [68]; hypercin [69]; and hyperforin [70]. Furthermore, the flavonoids detected in the plant were reported before for their antidepressant effects;
rutin, vitexin, amentoflavone, quercetins, and astilbin [71], in addition to the two majors anthocyanins; hypericin [72] and hyperforin [59].

**Conclusion**

In conclusion, a self-nanoemulsifying self-nanosuspension formulation was developed to enhance the oral delivery of HP extract and potentiate its therapeutic effects as an antidepressant and cardioprotective agent. The formulation was created using 10% triacetin as an oil, 57% Tween 20 as a surfactant, and 33% PEG 400 as a co-surfactant and loaded with the extract at a concentration of 100 mg/mL. The formulation exhibited nanoemulsion and nanosuspension upon dilution, thus increasing the surface area available for dissolution of the extract. Consequently, the therapeutic effects of the extract could be significantly amplified. The in vivo study revealed a significantly improved cardiac function and decreased depression. The in vivo evaluation of the formulation revealed a decline in the myocardial enzyme markers, NO, and TNF-α with restoring the normal architecture of the heart tissue. In addition, it decreases anxiety, depressive-like behavior, and cognitive dysfunction, along with decreased brain TNF-α, elevating neurotransmitters, and BDNF. Interestingly, HP.SNESNS augmented the immunohistochemical expression of cortical and hippocampal GFAP levels and downregulated the cortical Bax expression. Finally, SNESNS represents a suitable delivery system for HP extract with the potential to enhance its activity and decrease treatment costs. Pharmacokinetics/pharmacometabolomics-pharmacodynamics (PK/PM-PD) studies will be conducted in the future for the investigation of the synergism between the bioavailable compounds within the HP extract.

**Author Contribution** Heba M. A. Khalil, Dina B. Mahmoud, Riham A. El-Shiekh, Alaa F. Bakr, Amira A. Boseila, Sally Mehanna, Reham A. Naggar, Hesham A. Eliwa: Conceptualization, in vivo study, formal analysis, writing—original draft. Dina B. Mahmoud and Amira A. Boseila: preparation and characterization of the formula, writing—original draft. Riham A. El-Shiekh: Conceptualization, plant extraction, formal analysis, writing—original draft. Heba M. A. Khalil: Editing the final draft and supervision.

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**Data Availability** Data available on request from the authors.

**Declarations**

**Ethics Approval** All the experimental procedures followed the Veterinary Institutional Animal Care and Use Committee (Vet-IACUC) (Approval Number: VetCU03252019030), Faculty of Veterinary Medicine, Cairo University.

**Consent for Publication** Not applicable.

**Competing Interests** The authors declare no competing interests.

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