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Original Article

Is *Hypericum perforatum* extract comparable to Milnacipran, Agomelatine in rats with Subclinical Hypothyroidism?

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ABSTRACT

Subclinical hypothyroidism is becoming a global health problem due to its increasing prevalence and potentially deleterious effects on cognitive, mood memory, and other higher brain functions. Recently, an important concern is the inefficiency of levothyroxine for depressive symptoms in patients with SCH. The aim of this work was to compare the possible effects of three different antidepressant drugs; Milnacipran as a standard antidepressant, Agomelatine, another antidepressant acting via melatonin receptors, and Hypericum perforatum, the proven antidepressant, on the depression-like behavior performances, the serum level of cortisol, serum level of TNFα, the level of monoamines and Brain-Derived Neurotrophic Factor (BDNF) in the hippocampus in an experimentally–induced model of SCH in rats. 60 adult male rats were chosen and divided into 6 groups. The rats with SCH were induced by hemi-thyroid electrocauterization in 4 groups: model group, Milnacipran-treated group (10 mg/kg/day), Agomelatine-treated-group (40 mg/Kg/day), and H. Perforatum treated-group (200 mg/kg/day). The treatment duration was 2 weeks. The depressive-like behavior was assessed by OFT and FST. The biochemical assessment involved the hippocampal monoamines and BDNF, TNF alpha, and cortisol levels in serum at the end of the study. Milnacipran, Agomelatine, and *H. Perforatum* treatment alleviated the behavioral performance changes and decreased the elevated serum cortisol and TNF alpha. Also, the drugs used increased the Hippocampal monoamines and BDNF levels in the SCH rats. Comparing the three tested drugs, the results of the *H. Perforatum* treatment were comparable to that of Milnacipran and superior to Agomelatine results in most of the tested parameters. Treatment with *H. Perforatum* extract resulted in marked improvement of behavioral and biochemical changes in the SCH model group, which was in a comparable manner to the standard antidepressant drug Milnacipran. Also, an improvement was observed with Agomelatine but it was lesser than that of Milnacipran and *H. Perforatum* extract.

Keywords: Depression, *Hypericum Perforatum*, Milnacipran, Agomelatine, SCH, OFT, FST

Introduction

Subclinical hypothyroidism (SCH) is a health problem worldwide due to its potentially deleterious effects and increasing prevalence. [1-4] In adults, the SCH prevalence ranges from 4% to 20% of the population in different regions [5].

SCH is characterized by raised thyrotropin (TSH) in combination with a normal-range of free circulating thyroid hormones. [6-8]

SCH has drawn intensive interest due to its detrimental effect on cognitive, mood memory, and other higher brain functions [9]. It has been reported that depression is more prevalent in SCH patients than those with overt hypothyroidism and that SCH increases the risk of depression more than 4 times [10].

Levothyroxine might be ineffective for depressive symptoms in SCH patients [11], and the possibility of overtreatment is an adverse effect of levothyroxine treatment [12]. The present study

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aimed at comparing the possible effects of three different antidepressant drugs; Milnacipran as a standard antidepressant, Agomelatine, another antidepressant acting via melatonin receptors and Hypericum perforatum, the proven antidepressant, on the depression-like behavior performances, the serum level of cortisol, serum level of TNFα, the level of monoamines and BDNF in the hippocampus in an experimentally–induced model of SCH in rats.

Materials and Methods

60 healthy mature male albino rats, matched for age and weight (150-200g) were bred in the animal house of Research Institute of Ophthalmology. They were harbored on a 12-h light/dark cycle in a fully ventilated room at fixed room temperature (21±2°C) each rat in a separate cage for at least 1 week before the start of the experiment, fed with a standard laboratory diet composed of 62% corn, 10% soybeans, 10% cottonseed oil, 5% fish powder, 2% bone powder, 2% sodium chloride, and 1% vitamins, and allowed to access water ad libitum. All experimental procedures were performed following the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, which comply with the international laws for use and care of laboratory animals [13]. All experiments were performed under aseptic conditions in the Laboratory of Medical Biochemistry Department, Faculty of Medicine, Cairo University. The protocol was approved by the Cairo University Institutional Animal Care and Use Committee **(IACUC).** Approval number CU/III/F/18/19.

II- Drugs

1- **Savella ® Milnacipran HCl** (Allergan USA, Inc.) was obtained from a local pharmacy in the form of 50-mg tablets, and crushed and suspended in distilled water, freshly prepared, administered by gastric gavage, at a dose of 10 mg/kg/day orally for 2weeks starting from day 15 [14].

2- **Agomelatine,** Doxanero® (Liptis, Egypt) was obtained from a local pharmacy in the form of 25-mg tablets and crushed and suspended in distilled water, freshly prepared, administered by gastric gavage, at a dose of 40 mg/Kg/day orally for 2 weeks starting from day 15 [15].

3- **Extract of Hypericum perforatum L. capsules** (0.3% Hypericin) was obtained from Life Extension Products, USA. (Catalog Number: 01396). The extract was crushed, freshly dissolved in 2%Tween-80 dropwise till clear solution [16]. Then it was administered by gastric gavage, at a dose of 200 mg/kg/day for 2 weeks starting from day 15 [17].

4- **Ketamine (Ketamar):** (Amoun company-Egypt) was supplied as 10-ml ampoule; each 1 ml contained 50 mg ketamine.

5- **Ethyl carbamate (Urethane):**(El-Gomhorya-Egypt) was supplied as a white crystalline powder and dissolved in distilled water.

III - Kits

1- Rat fT3/Free Triiodothyronine ELISA Kit (Competitive EIA) Catalog No. LS-F25839, Lifespan Bioscience, USA.

2- Rat T4 / Thyroxine ELISA Kit (Competitive EIA) Catalog No. LS-F10013, Lifespan Bioscience, USA.

3- Rat Thyroid Stimulating Hormone (TSH) ELISA Kit Catalog Number. CSB- E05115r, Cusabio, USA.

4- Rat cortisol ELISA Kit, Catalog Number. CSB-E05112r, Cusabio, USA.

5- Rat Norepinephrine (NE) ELISA Kit. Catalog No. MBS269993, My Bioscience, USA.

6- Rat Dopamine (DA) ELISA Kit Catalog No: MBS026032, My Biosource, USA.

7- Rat Serotonin (ST) ELISA Kit Catalog No: MBS9362408, My Bioscource, USA.

8- Rat BDNF ELISA Kit, RayBio® inc. Catalog #: ELR-BDNF, GA.

9- Rat TNF-alpha ELISA Kit RayBio® inc. Catalog #: ELR-TNF-α, GA.

Methods of Experimental Design

- **Experimental Procedure**

Thyroid gland Hemi-electrocauterization [18]: 60 Male 2-months-old Wistar rats matched for age and weight (180-200 g) were used in the experiment. They were randomly divided into sham, SCH, and normal control groups.

The SCH group is furtherly divided into 4 groups (diseased and the 3-treated groups). All rats were subjected to training to the behavioral tests for 4 days. Then from day 15, the animals started to receive the medications until day 30.

The tests were repeated on days 1, 15, and 30. On day 15, after anesthesia, blood samples (about 1 cm^3) were obtained from the retro-orbital sinus of the animals to confirm the establishment of the SCH model. The blood sample collection was repeated at the end of the experiment to assess the TNF-α and Cortisol.

Then, the rats were sacrificed, each rat was decapitated and the hippocampus was dissected. Hippocampal tissue was homogenized in phosphate buffer saline (PBS), then centrifuged at 8000rpm for 10 minutes and the supernatant was kept frozen at -80°C till analysis for assessment of BDNF, serotonin, noradrenaline, and dopamine.

SCH rat model: At the beginning of the experiment, rats of groups II, III, IV, V, and VI were anesthetized by intramuscular injection of 46 mg/kg ketamine [19], fixed on the sterile control

table in the supine position. After local shaving and routine sterilization, the skin of the neck was cut longitudinally, and the trachea was exposed. The thyroid tissue of the right thyroid lobe and isthmus were damaged by 24W high- frequency power coagulation. The sham operation Group exposed the thyroid without electrocoagulation damage [12]. Intramuscular injection of ceftriaxone and diclofenac was carried out every 12 hours postoperatively for 3 days following the operative procedure. Local antibiotic (Fusidic acid ointment) was applied to the wound daily for the first 3 days.

The animals were fed with a normal diet and drinking water, returned to normal state 2 days after operation, considering the pain and locomotion within their cages.

• Study Design

60 rats were divided into the following groups: [n =10] **Group I (Control group):** rats orally received **1 ml** saline for the whole study duration. **Group II (Sham-operated group):** Thyroid glands were surgically exposed without electrocautery and closure of skin incision. Rats received 1 ml saline orally for the rest of the study duration. For the rest 4 groups, Hemi-electrocauterization of the thyroid gland of the rats were performed and the SCH model was evaluated 2 weeks later by measuring the **plasma fT3, T4, and TSH.** Then, the **successful SCH rats** were randomly divided into 4 groups each with 10 rats: **Group III (Model Group):** The rats orally received 1 ml of saline/day for 2 weeks **Group IV: (Milnacipran treated model group):** Rats orally received Milnacipran in a dose of 10 mg/kg/day for 2 weeks [14]. **Group V**: (**Agomelatine treated model group)** Rats orally received Agomelatine at a dose of 40 mg/Kg/day for 2 weeks [15].

Group VI: (***Hypericum perforatum*** **(HP) treated model group)** Rats received HP extract in at dose of 200 mg/kg/day for 2 weeks [17].

Behavioral Tests

1- Open Field test:

• Principle: [20]; modified by [21]

Open field test (OFT) behavioral test is one of the most known primary behavioral tests to evaluate animal behaviors including anxiety, exploration, and locomotor activity, elicited by placing the subject in a novel open space with a surrounding wall to prevent escape. It is used in the assessment of animal behaviors in various models of neuropsychological disorders including depression and anxiety, the animal behaviors were recorded manually.

Apparatus: [22]

The open field apparatus was done using a Plexiglas apparatus 72x72 cm with 36-cm walls; the floor of the box was divided into sixteen 18x18 cm squares by a red marker; the bottom of the OF arena was mildly rough to enable animals to move without sliding; the open field arena was equipped with a camera (20-megapixel, Cannon).

• **Procedure:** The box was put in the corner of a quiet room and lit by a lamp and avoid unintentional illumination and light reflections, which influences the behavior and possibly the tracking procedure too. The rats were subjected to OFT, from 10:00 am to 12:00 am on day 1. During the first hour, they were transported to the test room in their home cages, and at all times, they were handled with their tail bases to adapt to the environment (data not shown). Then, each rat was placed into one of the four corners of the open field and allowed to explore the apparatus for 5 minutes and the following behavioral patterns were monitored and observed for the following:

- **Latency time,** time in seconds elapsed until the rat started to move**.**
- **Ambulation Frequency,** number of squares crossed by rats with all 4 paws
- **Rearing Frequency,** the count, the rat stood on its hind legs in the field
- **Grooming frequency,** the count, the rat licked or scrated itself while stationary [23].

After the experimental session, the rats were carefully removed from the OF and returned to their cages and the open field arena was cleaned with 70% ethyl alcohol and allowed to dry between the subjects' tests to avoid internal olfactory cues, which may change the exploratory pattern of the next animal [24].

2- Forced Stress swim test

Also known as the behavioral despair test, [25]. The rodents were subjected to a brief and acute period of stress and the time during which they respond in an active versus a passive way was recorded.

Principle:

The Forced-swimm test (FST) or Porsolt swim test (PST), is a common assay used to study depressive-like behaviour in the animal subjected to the forced swimming in a cylindrical container of water from which it cannot escape, instead, it will first make efforts to escape by active swimming but will eventually show immobility, which may reflect the level of behavioral despair.

• Apparatus and Procedure:

The Forced Swim-Test was done using a transparent cylindrical plastic containers measuring 60 cm height × 20 cm diameter, were filled with water at room temperature (23 ± 1 °C), and a camera was fixed in front of the containers and all activities were manually video-taped. The test was performed in 2 sessions, 24 hours apart, the first session was the pre-test stage (15 min) and the second session was the test stage (5 min) [26].

Each rat was transported in its home cage into the waiting room at least 30min before behavioral testing in order to adapt to the testing environment. Then each rat was placed in the water-filled cylinder container for 5min. After that, the rat was removed from the container, dried by a pad, and placed in the transient drying cage with a heating pad under it and a heat lamp above it. Water was changed after every session to avoid any influence on the next rat. The rats' behavior was analyzed using a time sampling technique to rate the predominant behavior over a 5-min interval, including:

Struggling Time: The time in seconds during which the animal attempts to jump out of the tank, climb the walls, and dive into the tank [27].

Immobility Time: The time in seconds in which the animal remains floating passively in the water in an upright position [28].

Biochemical Assessment

A) Measurement of Serum Cortisol:

2 weeks after the operation, about 1ml of blood samples were collected to evaluate whether the SCH rat model was successfully established. 24h after the last behavioral test, the animals were anesthetized with chloral hydrate, and blood was drawn from the abdominal aorta. Serum cortisol was measured using ELISA kits according to the manufacturer's instructions.

B) Estimation of serotonin, norepinephrine, and dopamine contents of the Hippocampus:

At the end of the experiment, serotonins, as well as catecholamines (norepinephrine and dopamine), were determined in rats' hippocampus according to the method of [29] using rat ELISA kits.

Tissue Homogenates

–preparation of the brain Hippocampus: Hippocampal tissues were rinsed with PBS* to remove excess blood and weighed before homogenization. The tissues were finely minced and homogenized in 5-10 mL PBS* with a glass homogenizer on ice. The cells were lysed by freezing (-20°C)/thaw (room temperature) for 3 times.

* 1x PBS (0.02mol/L, pH 7.0-7.2)

The homogenates were centrifuged at 5000×g for 5 minutes. The supernatants were collected for assay.

-**Serotonin:** Principle of the assay: 5-HT ELISA kit applies the competitive enzyme immunoassay technique utilizing a polyclonal anti- 5-HT antibody and a 5-HT-HRP conjugate. The assay buffer and sample were incubated with 5-HT-HRP conjugate in a pre-coated plate for 1h. Then, the wells were decanted and washed 5 times. Next, the wells were incubated with the HRP enzyme substrate. The product of the reaction formed a blue complex. Finally, a stop solution was added to stop the reaction, which then turned the solution yellow. The color intensity was measured at 450 nm using a spectrophotometer. Since 5-HT-HRP conjugate and 5-HT from samples compete for the anti-5-HT antibody binding site, the color intensity is inversely proportional to the 5-HT concentration. Because the number of sites is limited, as more sites are occupied by 5-HT from the sample, fewer sites remain for 5-HT-HRP conjugate binding. A standard curve was plotted relating the color intensity (OD) to the concentration of standards. The 5-HT concentration in each sample was interpolated from this standard curve Rat Serotonin (ST) ELISA Kit ***(My Biosource, Catalogue no MBS9362408).***

-Noradrenaline: Double-sandwich ELISA technique was used according to the Manual***: (My Biosource Cat. No. MBS269993).*** The detecting antibody was a biotin-labeled polyclonal antibody and the pre-coated antibody was Rat NE monoclonal antibody. The biotin-labeling antibody and the samples were added into the wells of the ELISA plate and washed with PBS. Then Avidin-peroxidase conjugates were added into ELISA wells. The color intensity and the testing factors in the samples had a positive correlation.

-**Dopamine:** The quantitative sandwich ELISA technique was used in this assay. The antibody specific for D2D was pre-coated onto a microplate. Samples and standards were added into the wells. D2D was bound by the immobilized antibody, and the unbound substances were removed. Then, a biotin-conjugated antibody specific for D2D was added to each well. After washing, avidin conjugated Horseradish Peroxidase (HRP) was added into the wells. After that, the wells were washed to remove any unbound avidin-enzyme reagents and the substrate solution was added into the wells, and color developed in proportion to the amount of D2D bound in the initial step. The color development was stopped and the color intensity was measured ***(My Biosource, Cat. No: MBS026032).***

C) Serum Tumor necrosis factor-α (TNF-α): At the end of the test, TNF-α was quantitatively measured in rat serum as an in-vitro ELISA using the specific kit [30]. For this, an antibody specific for Rat TNF-α was coated on each well. Samples and standards were added into the wells and TNF-α present in the samples bound to the wells through the immobilized antibody. The wells were then washed and biotinylated anti-rat TNF-α antibody was added. After removing the unbound biotinylated antibodies, HRP-conjugated streptavidin was added into the wells. Then, the wells were washed, the TMB substrate was added into each well and color was developed in proportion to the amount of TNF-α bound. The stop solution changed the color from blue to yellow, and the color intensity was measured at 450nm. ***(RayBio® manual).***

D) Brain-Derived Neurotrophic Factor (BDNF):

At the end of the experiment, BDNF was quantitatively assessed using a specific ELISA kit [29]. For this, an antibody specific for BDNF was coated onto the wells. Samples and standards were added into the wells and BDNF present in a sample bound to the wells through the immobilized antibody. Then, the wells were

washed and biotinylated anti-rat BDNF antibody was added. After removing the unbound biotinylated antibody, HRP-conjugated streptavidin was added into the wells. The wells were washed again, a TMB substrate solution was added into the wells, and color was developed in proportion to the amount of bound BDNF. The stop solution changed the color from blue to yellow, and the color intensity was measured at 450nm ***(RayBio® inc. Catalog).***

Statistical Methods

Data were analyzed using SPSS v.23. Then, data were summarized using the mean and standard deviation. Comparisons between groups were done using two-way (ANOVA) with multiple comparisons using post hoc Tukey-comparison test. P-values of less than or equal to 0.05 were considered statistically significant, while P-values of more than 0.05 were considered insignificant.

Results

- Behavioural Tests

A) Open-field Test *1- The Latency time*

As shown in figure 1 & table 1, The mean latency time for the normal control group (group I) was 3.28 ± 0.35 seconds and was 3.13 ± 0.54 seconds for the sham-operated group (group II), with a non-significant statistical difference between the two groups.

Rats with SCH (Model group III) showed a statistically significant increase (P<0.000) in the mean latency time (12.06 ± 0.35 seconds). The percentage increase was 267.68% compared to group I.

The milnacipran-treated group (group IV) showed a mean latency time of 2.76±0.77 seconds. Compared to the SCH model group, it showed a statistically significant difference with a percentage reduction of 77.11 % (P<0.000).

Considering the Agomelatine-treated group (group V), the mean latency time was 5.02 ± 0.77 seconds with a statistically significant difference compared to groups III and IV (P<0.000). The mean latency time was attenuated by about 58.37% compared to group III.

The *Hypericum perforatum* extract-treated group (group VI) showed significant improvement in the mean latency time (2.91 ± 0.47 seconds), with a statistically significant difference from groups III and V (P<0.000) and a statistically non-significant difference from group IV. The percentage of reduction in the mean latency time was about 75.87% than that of the SCH model group, refer to table 1 and figure 1.

2- The ambulation frequency

Table 1 and figure 2 show that, the mean ambulation frequency (total number of crossed squares by the 4 paws) of the control normal group I and Sham group-II were 56.2 ± 4.66 and 55.5 ± 5.19 counts in the 5min-observation, respectively, showing a statistically non-significant difference between the two groups (Table 1).

Regarding the SCH (Model) group (group III) the ambulation frequency was 13.5 ± 1.58 counts in the 5min-observation, which showed a statistically significant (P<0.000) reduction by about 75.98% compared to the control group.

The Milnacipran-treated SCH group (group IV) showed 53.8 ± 4.76 counts/5min-observation with a statistically significant increase compared to the SCH model group III (P<0.00) with a percentage increase of about 298.52%.

In the agomelatine-treated SCH group (group V), the mean ambulation frequency was 50.2 ± 5.2 counts/5min-observation time with a statistically significant difference from group III (P<0.000) with a percentage increase of 58.37%. Meanwhile, a statistically non-significant decrease was observed in the number of counts compared to group IV.

In the Hypericum perforatum extract-treated SCH group (VI), the mean ambulation frequency was 51.1 ± 5.34 counts / 5min-observation time with a statistically significant increase compared to group III (P<0.000) with a percentage increase of 278.52%, which was statistically non-significant compared to groups IV and V.

3- The Rearing Frequency

As shown in figure 3 and table 1, The mean Rearing Frequency (Number of times the animal stood stretched on its hind limbs with/without forelimbs support) was found 18.6 ± 1.07 count/5min observation period for the normal control group I, while it was 18.1 ± 1.37 count/5min observation period for the sham group II with a statistically non-significant difference between the two groups.

Rats with SCH (Model group III) showed a statistically significant decrease P< 0.000 of mean rearing frequency (5.9 ± 0.74 count/5min observation period). The percentage decrease was 68.28% compared to group I.

Milnacipran-treated SCH group (group IV) showed a mean rearing frequency of 18.6 ± 1.26 counts/5 min observation period. Compared to the SCH group (model group III), it showed a statistically significant increase with a percentage increase of about 215.25% (P<0.000).

Considering the Agomelatine-treated SCH group (group V), the mean rearing frequency was 15.1 ± 1.1 counts/5min observation period with a statistically significant difference compared to groups III and IV (P<0.000). Compared to group III, the mean rearing frequency was increased by about 155.93%.

The Hypericum perforatum extract-treated SCH group (group VI) showed a significant improvement in the mean rearing frequency (18.1 ± 1.2 counts/5min observation period), with a statistically significant increase compared to groups III and V (P<0.000) and a statistically non-significant increase in comparison to group IV. Compared to group III, the mean rearing frequency of this group was increased by about 155.93%.

4- The grooming frequency

As shown in table 1 & figure 4, Regarding the mean grooming frequency (Number of times of face scratching and washing with the hind leg and licking of its genitals and fur) were 3.9 ± 0.74 and 4.4 ± 0.97 counts/5min observation period respectively for groups I and II with the statistically non-significant difference between the two groups.

Rats with SCH (Model group III) showed a statistically significant reduction of 5.9 ± 0.74 and 1.9 ± 0.74 counts/5 min observation period of mean grooming frequency. The percentage decrease was 51.28% compared to group I.

Milnacipran-treated group (group IV) showed a mean grooming frequency of 4.9 ± 0.74 counts/5min observation. Compared to the SCH model group, it showed a statistically significant difference with a percent increase of 157.89% (P<0.000).

Considering the Agomelatine-treated group (group V), the mean grooming frequency was 2.7 ± 0.67 counts/5min observation period. A non-significant difference was observed in comparison with group III, where a 42.11% increase was observed.

The Hypericum perforatum extract-treated group (group VI) showed marked improvement in the mean grooming frequency (4 ± 0.94 count/5min observation period), with a significant difference from group III and group V (P<0.000) and a non-significant difference from group IV. The percentage increase in the mean grooming frequency was about 110.53% than that of the SCH model group.

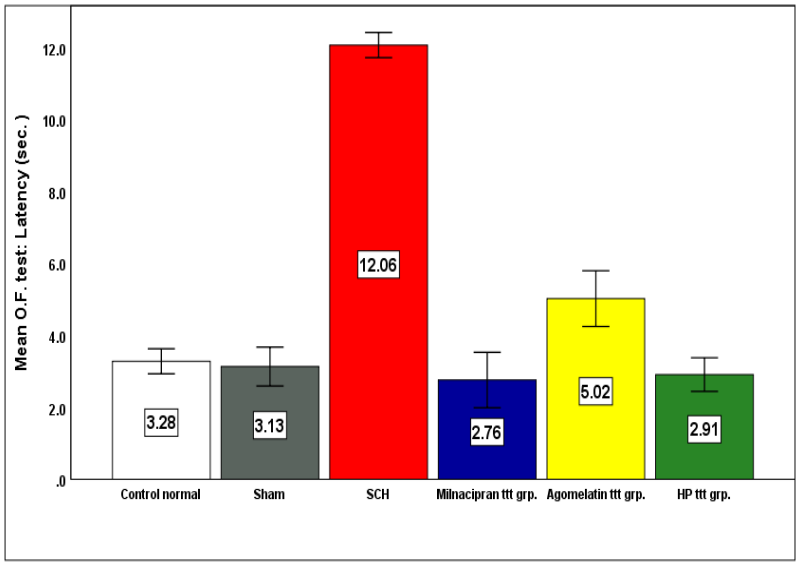

Figure 1: The mean latency time in Open-field Test in different studied groups
The bars represent the mean ± SD, (n = 10)
Statistical analysis was conducted by ANOVA with post hoc Tukey- comparison test.

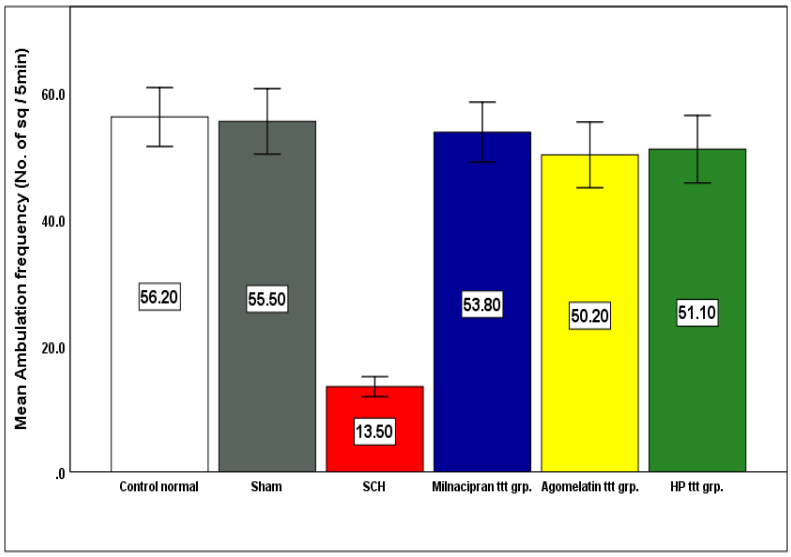

Figure 2: The mean Ambulation Frequency in the study groups
(The bars represent the mean ± SD, n = 10)
Statistical analysis was conducted by ANOVA with post hoc Tukey- comparison test.

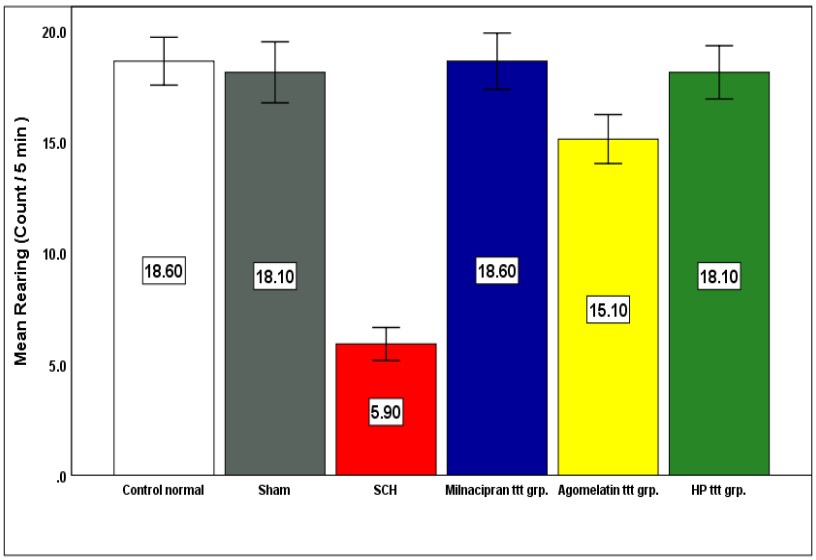

Figure 3: The mean rearing of different studied groups regarding Frequency.
(The bars represent the mean ± SD, n = 10)
Statistical analysis was done by ANOVA with post hoc
Tukey- comparison test.

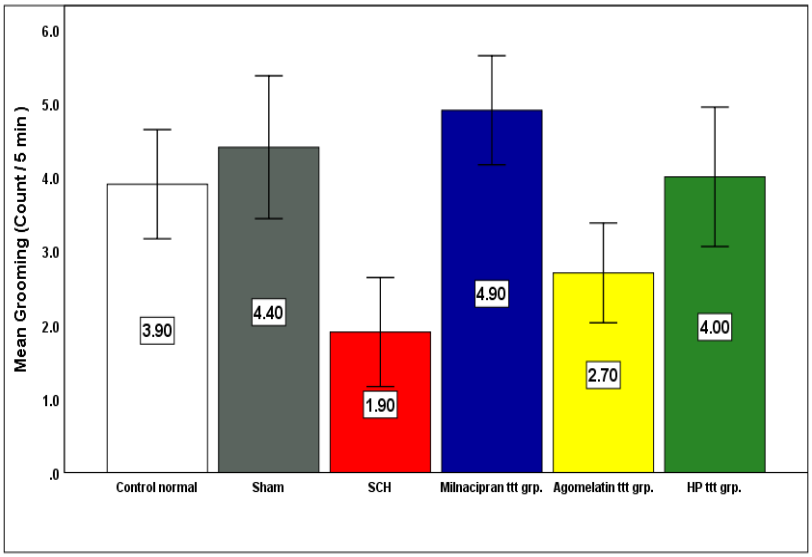

Figure 4: The mean Grooming Frequency in different studied groups.
(The bars represent the mean ± SD, n = 10).
Statistical analysis was conducted by ANOVA with post hoc Tukey- comparison test.

Table 1: Comparison between the effect of Milnacipran, Agomelatine, and H. perforatum extract on Open Field Behaviors of Rats with SCH.

Groups / Open Field behaviour	Control Group I	Sham-operated Group II	SCH Group III	Milnacipran treated Group IV	Agomelatine treated Group V	HP treated Group VI
Latency (sec.)	3.28 ± 0.35	3.13 ± 0.54	12.06 ± 0.35[a]	2.76 ± 0.77[b]	5.02 ± 0.77[bc]	2.91 ± 0.47[bd]
Ambulation Frequency	56.2 ± 4.66	55.5 ± 5.19	13.5 ± 1.58[a]	53.8 ± 4.76[b]	50.2 ± 5.2[b]	51.1 ± 5.34[b]
Rearing Frequency	18.6 ±1.07	18.1 ±1.37	5.9 ± 0.74[a]	18.6 ± 1.26[b]	15.1 ± 1.1[bc]	18.1 ± 1.2[bd]
Grooming Frequency	3.9 ± 0.74	4.4 ± 0.97	1.9± 0.74 [a]	4.9± 0.74 [b]	2.7± 0.67[c]	4± 0.94 [bd]

Number of animals: 10 rats per each group, SCH= Subclinical Hypothyroidism, HP=Hypericum Perforatum.
a: Significantly different from the normal control group.
b: Significantly different from the model control (SCH) group.
c: Significantly different from the Milnacipran-treated group.
d: Significantly different from the Agomelatine-treated group

Table 2: Comparison between the effect of Milnacipran, Agomelatine and H. perforatum extract on Forced Swim Test Behaviours of Rats with SCH

Groups / *Forced Swim Behaviour*	Control Group I	Sham-operated Group II	SCH Group III	Milnacipran treated Group IV	Agomelatine treated Group V	H.P. treated Group VI
Immobility Time (sec)	127.44 ± 4.16	129.11 ± 4.04	252.08 ± 11.56 [a]	143.65± 3.38 [b]	153.4± 5.85 [bc]	139.34 ± 4.41 [bd]
Struggling time (sec)	173.45 ± 3.93	174.06 ± 4.24	48.57 ± 3.11 [a]	147.44± 8.05 [b]	136.54± 5.29 [bc]	154.09± 4.26 [bd]

Number of animals: 10 rats per each group, SCH= Subclinical Hypothyroidism, HP=Hypericum Perforatum.
a: Significantly different from the normal control group.
b: Significantly different from the model control (SCH) group.
c: Significantly different from the Milnacipran-treated group.
d: Significantly different from the Agomelatine-treated group.

B) Forced Swim Test

a- The immobility time

As shown in table 2 & figure 5, the mean immobility time for the normal control group (group I) was 127.44 ± 4.16 seconds and was 129.11 ± 4.04 seconds for the sham-operated group (group II), with a statistically non-significant difference between the two groups.

Rats with SCH (Model group III) showed a statistically significant increase (P<0.000) of mean immobility time (252.08 ± 11.56 seconds in a 5min observation period). The percentage increase was 95.24% compared to group I.

Milnacipran-treated group (group IV) showed a mean immobility time of 143.65 ± 3.38 seconds. Compared to the SCH model group, it showed a statistically significant difference with a percentage reduction of 43.01% (P<0.000), please refer to table 2 and figure 5Considering the Agomelatine-treated group (group V), the mean immobility time was 153.4 ± 5.85 seconds with a statistically significant difference compared to groups III and IV (P<0.000). The mean immobility time was attenuated by about 39.12 % compared to group III.

The Hypericum perforatum extract-treated group (group VI) shown a significant reduction in the mean immobility time (139.34 ± 4.41 seconds), with a significant reduction compared to groups III, IV, and V (P<0.000). The percentage decrease in the mean immobility time was about a 44.72% increase than that of the SCH model group (Figure 5, Table 2).

b- The struggling time

Table 2 and figure 6 show, the mean struggling time for the normal control group (group I) was 173.45 ± 3.93 seconds and was 174.06 ± 4.24 seconds for the sham-operated group (group II), with a statistically non-significant difference between the two groups.

Rats with SCH (Model group III) showed a statistically significant reduction (P<0.000) of mean struggling time 48.57 ± 3.11 seconds. The percentage of reduction was 72.10% compared to group I.

Milnacipran-treated group (group IV) showed a mean struggling time of 147.44 ± 8.05 seconds. Compared to the SCH model group, it showed a statistically significant increase with a percentage of 203.56% % (P<0.000)

Considering the Agomelatine-treated group (group V), the mean struggling time was 136.54 ± 5.29 seconds with a statistically significant difference compared to groups III and IV (P<0.000). The mean struggling time was increased by about 181.12% compared to group III. The Hypericum perforatum extract-treated group (group VI) showed a significant improvement in the mean struggling time (154.09 ± 4.26 seconds), with a significant difference from groups III and V (P<0.000) and a non-significant difference from group IV. The percentage of increase in the mean struggling time was about 217.25% compared to that of the SCH model group (Figure 6, Table 2).

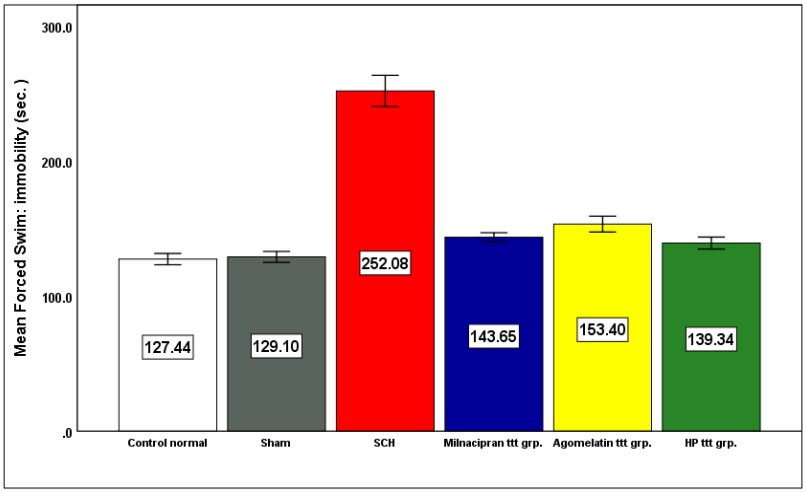

Figure 5: The Forced Swim Test Behaviour (Mean Immobility time) in different studied groups.
The bars represent the mean ± SD, n = 10.
Statistical analysis was conducted by ANOVA with post hoc Tukey- comparison test.

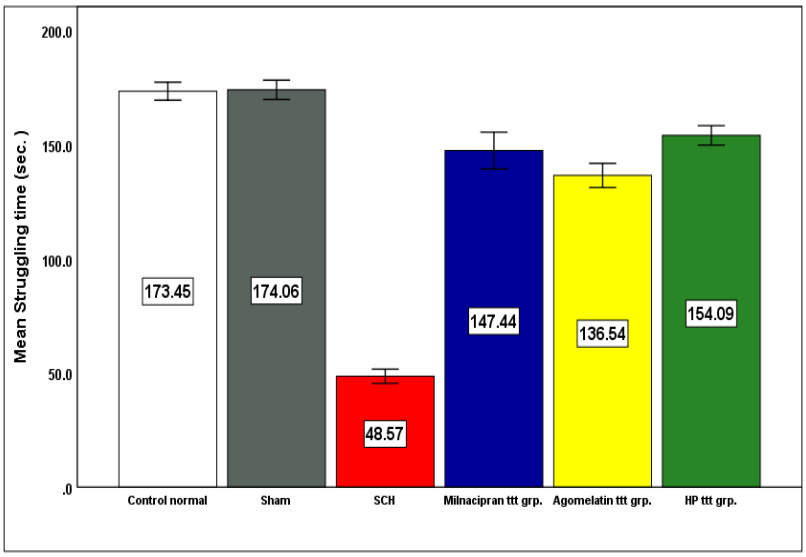

Figure 6: The Forced Swim Test Behaviour (Mean Struggling time) in different studied groups.
The bars represent the mean ± SD, n = 10.
Statistical analysis was conducted by ANOVA with post hoc Tukey- comparison test.
The Struggling Time= The time in seconds during which the animal attempts to jump out of the tank, climb the walls, and dive into the tank

II- Assessment of Biochemical changes

A) Assessment of Serum Cortisol Level

As shown in Figure 7, Regarding the mean normal control group (group I) cortisol level was 2.08 ± 0.31 ng/ml, while it was 2.17 ± 0.42 ng/ml for group II, with an insignificant difference between the two groups.

The SCH group (group III) showed a mean serum cortisol level of 8.41 ± 1.16 ng/ml with a statistically significant increase compared to both groups I and II (P<0.000).

The Milnacipran treated SCH group (group IV) revealed a significant improvement in the mean cortisol level of 4.18 ± 0.47 ng/ml. This result was statistically significant with a percentage reduction of 50.3% compared to the SCH group.

Agomelatine treated group (Group V) showed a mean level of 2.89 ± 0.77 ng/ml with a significant difference from groups III and IV (P<0.000) with a percentage reduction of 65.64% than that recorded in the SCH model group.

Figure 6 and table 2 reveal the H. Perforatum-treated group (group VI) serum cortisol level was 2.6 ± 0.71ng/ml with a statistically significant difference from groups III (with a percentage reduction of 69.08%) and IV (with a percentage reduction of 18.78%) (P<0.000). Meanwhile, an insignificant difference was observed compared to group V.

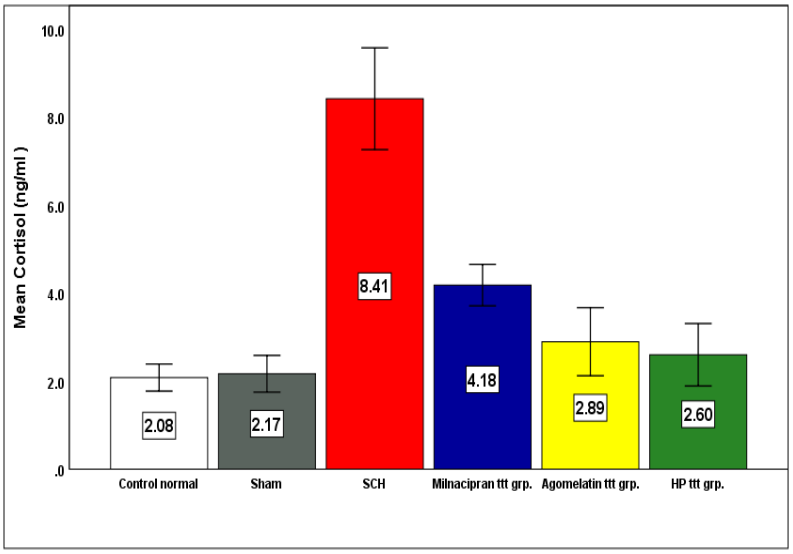

Figure 7: The Mean Serum Cortisol in different Studied groups.
The bars represent the mean ± SD, n = 10.
Statistical analysis was conducted by ANOVA with post hoc Tukey- comparison test.

B) **Assessment of Hippocampal Monoamines; Serotonin, Noradrenaline, and Dopamine**

i) Hippocampal Serotonin Level: Figure 8 and table 3 show that, the control group I Serotonin level was 91.06 ± 7.59 ng/ml, while it was 91.5 ± 4.27 ng/ml in group II, with an insignificant difference between the two groups .

Regarding the SCH untreated model group III, the mean serotonin was 23.75 ± 3.18 ng/ml with a significant difference from the normal control group (group I) where P<0.000 with a percentage reduction of about 52.90%.

The Milnacipran-treated SCH group IV revealed a mean serotonin level of 51.91 ± 4.8 ng/ml with a significant increase compared to group III, (P<0.000) and the percentage increase was about 118.57%.

Regarding the Agomelatine-treated group V, the mean hippocampal serotonin level was 45.66 ± 8.52 ng/ml with a statistically significant increase compared to group III, where P<0.000, the percentage increase was 92.25%. Meanwhile, a non-significant difference was seen compared to group IV.

The Hypericum Perforatum-treated group (group VI) showed a serotonin level of 49.81 ± 3.9 ng/ml with a statistically significant increase compared to SCH model group (group III), (P<0.000) with a percentage increase of 109.73%. An insignificant difference was seen compared to groups IV and V.

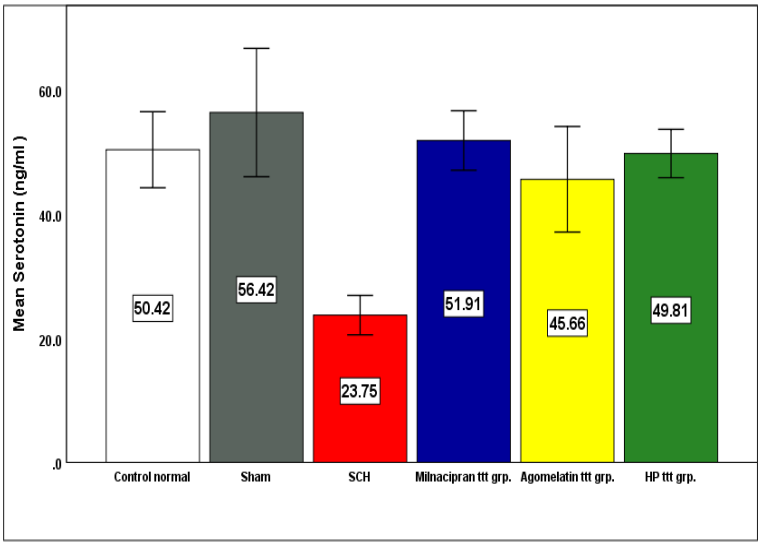

Figure 8: The mean Hippocampal Serotonin Level in different studied groups.
The bars represent the mean ± SD, n = 10.
Statistical analysis was conducted by ANOVA with post hoc Tukey- comparison test.

ii) Hippocampal Noradrenaline Level:

As revealed in table 3 and figure 9, the mean Noradrenaline level in normal control group I was 133.65 ± 8.64 pg/ml, while it was 137.93 ± 20.18 pg/ for group II, with an insignificant difference between the two groups.

Considering the Model SCH group (group III), the mean hippocampal Noradrenaline was 78.54 ± 11.24 pg/ml with a significant reduction compared to group I (P<0.000), and a percentage reduction of 41.23%.

For the Milnacipran-treated SCH group IV, the mean hippocampal Noradrenaline was 102.26 ± 7.22 pg/ml with a statistically significant increase compared to group III, (P-value = 0.001) percentage increase was 30.20%.

Regarding the Agomelatine-treated SCH group, the mean hippocampal Noradrenaline was 100.62 ± 11.29 pg/ml with a significant increase from group III with a percentage increase of 28.11%, (P<0.000) while insignificantly changed compared to group IV.

The H. Perforatum-treated group IV showed a mean hippocampal Noradrenaline level of 104.29 ± 10.65 pg/ml showing a significant difference from group III (P<0.000) with a percentage increase of 32.79%, and an insignificant difference from groups IV and V.

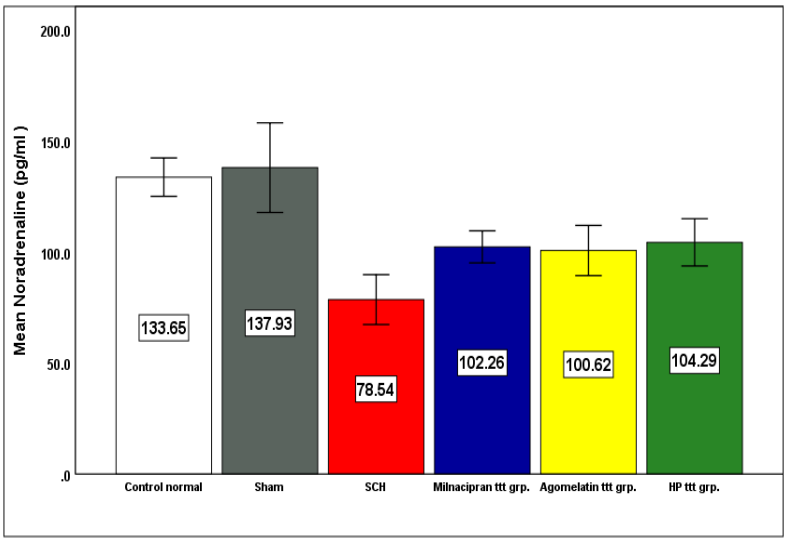

Figure 9: The mean Hippocampal Noradrenaline Level in different studied groups.
The bars represent the mean ± SD, n = 10.
Statistical analysis was conducted by ANOVA with post hoc Tukey- comparison test

iii) Hippocampal Dopamine Level

As shown in figure 10 and table 3, the mean normal control group I dopamine level was 91.06 ± 7.59 ng/ml, while it was 91.5 ± 4.27 ng/ml for group II, with an insignificant difference between the two groups.

The SCH group III showed a mean hippocampal dopamine level of 39 ± 7.25 pg/ml with a statistically significant decrease compared to group I where P<0.000 with a percentage reduction of about 57.17%.

The Milnacipran treated group (group IV) revealed a significantly improved mean hippocampal dopamine level of 71.14 ± 6.75 pg/ml with a statistically significant increase compared to group III (P<0.000) with a percentage increase of about 82.05% %.

Agomelatine treated rats (group V) showed a mean hippocampal dopamine level of 77.68 ± 2.61 pg/ml with a significant increase compared to group III (P<0.000) and a percentage increase of about 82.41%, while an insignificant difference was observed compared to group IV.

Regarding the H. Perforatum-treated group (group VI), the mean Hippocampal Dopamine level was 80.38 ± 4.83 pg/ml with a significant increase compared to groups III and IV (P<0.000) with a percentage increase of 106.10% compared to the SCH model group**.** Meanwhile, an insignificant difference was observed compared to group V.

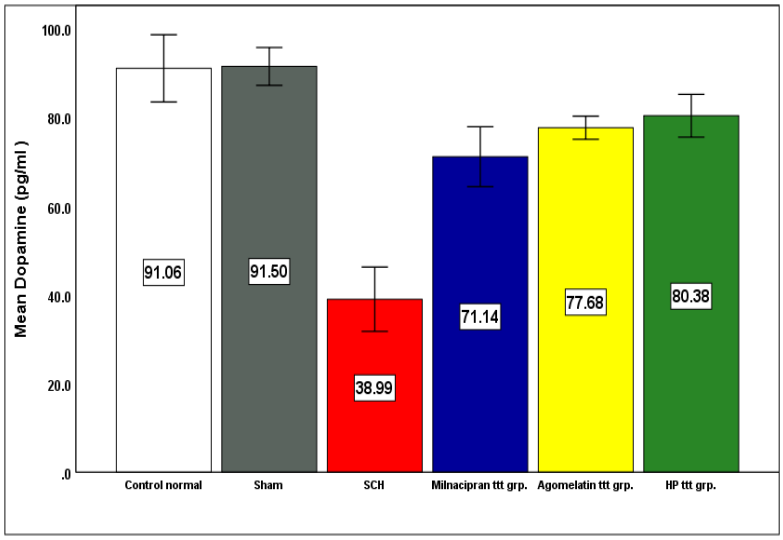

Figure 10: The mean Hippocampal Dopamine Level in different studied groups.
The bars represent the mean ± SD, n = 10.
Statistical analysis was conducted by ANOVA with post hoc Tukey- comparison test.

Table 3: Comparison between the effect of Milnacipran, Agomelatine, and H. perforatum extract on Hippocampal Serotonin, Noradrenaline, and Dopamine Level of Rats with SCH.

Groups / Mono-amines	Control Normal Group I	Sham-operated Group II	SCH Group III	Milnacipran treated Group IV	Agomelatine treated Group V	HP treated Group VI
Serotonin	50.42 ± 6.13	56.42 ± 10.34	23.75 ± 3.18 [a]	51.91± 4.8 [b]	45.66± 8.52 [b]	49.81± 3.9 [b]
Noradrenaline	133.65 ± 8.64	137.93 ± 20.18	78.54 ± 11.24 [a]	102.26± 7.22 [b]	100.62± 11.29 [b]	104.29± 10.65 [b]
Dopamine	91.06 ± 7.59	91.5 ± 4.27	39± 7.25 [a]	71.14± 6.75 [b]	77.68± 2.61 [b]	80.38± 4.83 [bc]

Number of animals: 10 rats per each group, SCH= Subclinical Hypothyroidism, HP=Hypericum Perforatum.
a: Significantly different from the normal control group.
b: Significantly different from the model control (SCH) group.
c: Significantly different from the Milnacipran- treated group.
d: Significantly different from the Agomelatine- treated group.

C) Assessment of Serum Tumour necrosis factor (TNF) α Level:

The mean normal control group I TNF α level was 14.12 ± 1.85 pg/ml as shown in figure 11, while it was 14.72 ± 1.04 pg/ml for group II, with an insignificant difference between the two groups.

Regarding the Model SCH group (group III), the mean serum TNF-α was 100.04 ± 6.79 pg/ml with a statistically significant increase compared to group I, (P<0.000), and a percentage increase of 608.50%.

For the Milnacipran-treated group IV, the mean serum TNF-α was 47.57 ± 11.1 pg/ml with a statistically significant reduction (P<0.000) compared to group III. The percentage reduction was about 52.45% as compared to group III.

Considering the Agomelatine-treated group, the mean serum TNFα was 43.16 ± 19.63 pg/ml with a significant difference compared to group III, (P<0.000), and the percentage reduction was 56.86%, which was insignificant compared to the Milnacipran group (group IV).

The H. Perforatum-treated group IV showed a mean serum level of 42.95 ± 16.47 pg/ml showing a significant decrease compared to group III (P<0.000) and the percentage reduction was 57.07% in comparison to the model group, which was insignificant compared to groups IV and V.

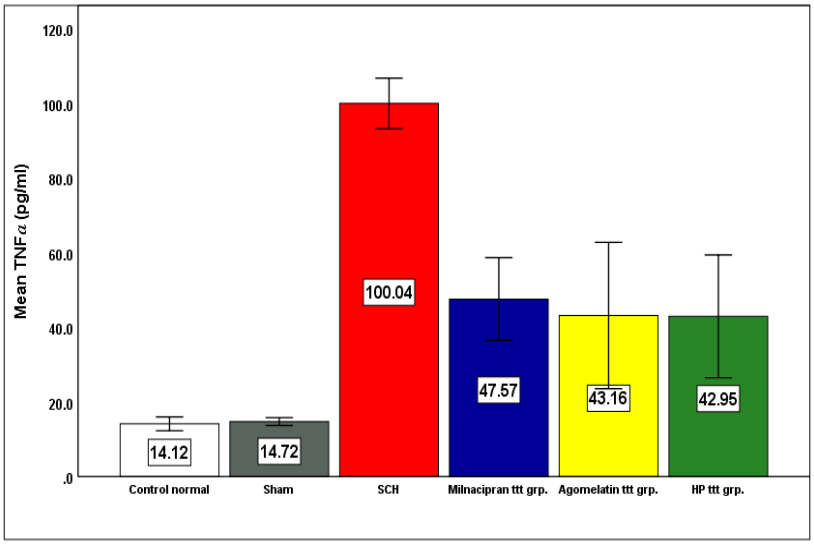

Figure 11: The mean serum TNF-α Level different studied groups.
The bars represent the mean ± SD, n = 10.
Statistical analysis was conducted by ANOVA with post hoc Tukey- comparison test.

D) Assessment of BDNF Level:

The mean BDNF level was 176.8 ± 9.02 pg/ml in normal control group I, while it was 180.2 ± 5.72 pg/ml for group II, with an insignificant difference between the two groups, as revealed in figure 12.

The Subclinical hypothyroid group III showed a mean BDNF level of 58.24 ± 5.81pg/ml with a significant reduction compared to group I (percentage reduction of 67.06%) (P<0.000).

The Milnacipran treated group IV revealed a significant improvement of the mean BDNF level (120.02 ± 14.98 pg/ml) with a significant increase in comparison to group III (P<0.000). The percentage of increase was 106.08 % as compared to the model group.

Agomelatine treated group V showed a mean level of 121.92 ± 9.87 pg/ml with a statistically significant increase compared to group III (P<0.000). About 109.34% increase was observed compared to group III, meanwhile insignificant difference from group IV.

Regarding the *H. Perforatum*-treated group VI, BDNF was 135.91 ± 10.25 pg/ml with a significant increase compared to group III (P<0.000) and the percentage of increase was 133.3%. A significant increase was also observed in comparison with group IV (P=0.008) and group V (P =0.027).

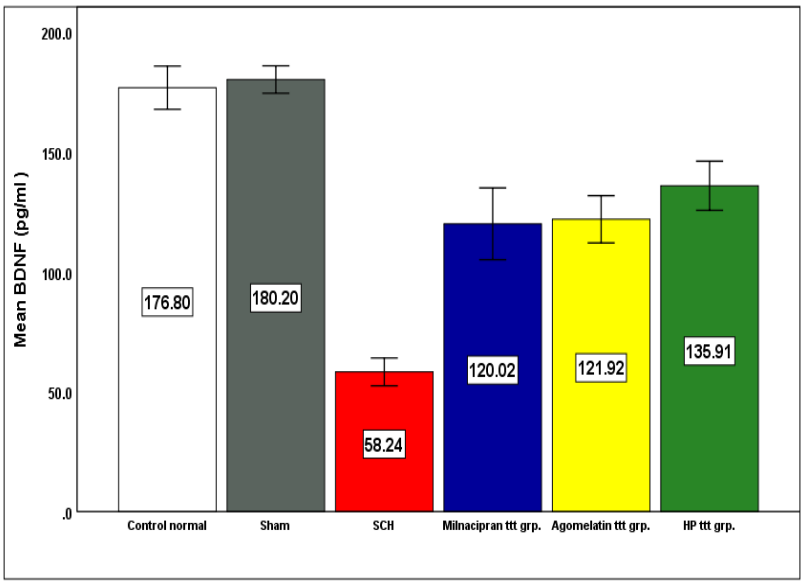

Figure 12: The mean BDNF in different studied groups
The bars represent the mean ± SD, n = 10.
Statistical analysis was conducted by ANOVA with post hoc Tukey- comparison test.

Discussion:

In the present study, the model of SCH was achieved by a new surgical technique, which is unilateral thyroid electrocoagulation. Rats were allowed to complete wound healing; the thyroid profile (elevated TSH with normal levels of serum fT_3 and T_4) was assessed two weeks later to confirm the model establishment.

The result of the procedure was similar to the study of ***Ge et al.*** where unilateral thyroid coagulation damage of one thyroid lobe in rats induced mild hyperplasia and consequently, serum TSH was elevated maintaining the level of serum fT_3 and T_4 within normal, which is the sub-clinical hypothyroidism by biochemical definition [18].

Induction of SCH in the rats of the present study resulted in changes in the behavioral tests, increased level of serum cortisol, and reduced levels of hippocampal serotonin, noradrenaline, dopamine, and BDNF. Also, increased serum TNFα was recorded.

As regards the behavioral assessment of the SCH model group in the present study, in open field test (OFT), there was a considerable prolongation of the latency time in the open field arena and also a reduction in the ambulation rearing, and grooming frequencies.

Regarding the behavioral assessment by the forced swim test (FST) in the SCH model group, in the present study, marked prolongation of the immobility time and reduction of the struggling time (including swimming, diving, and climbing) were observed. Immobility in the FST was considered as an index of despair behavior, which is one of the prominent symptoms of depression.

These results were consistent with that of [12], where the SCH rats revealed a depressive attitude in the OFT and the FST. The dopamine, noradrenaline, and serotonin in the hippocampus were significantly reduced.

In the present work, SCH rats were observed to have a profound reduction in the levels of BDNF in the hippocampus. This was in accordance with [31], in another model of depression (corticosterone-induced depression in a rat model), which showed a significantly increased immobility time in FST and hippocampal BDNF levels and reduced sucrose intake.

Data of the present work was consistent with the data previously stated by [32], in which BDNF was reduced in patients with depression and these levels were normalized following antidepressant treatment. Similarly, in another study, where a rat model of depression by maternal deprivation and young-adult corticosterone treatment was conducted. This model induced significant and unique reductions of BDNF gene expression in the hippocampus. These rats also showed marked behavioral disruption [33].

BDNF is the most abundant neurotrophin affecting the function of both peripheral and central nervous systems. Neurotrophins are a class of growth factors promoting neuronal proliferation,

differentiation, and survival. They are highly expressed in the CNS as well as other tissues [34].

The neurotrophin hypothesis has received significant attention in recent studies, and BDNF is an important neurotrophic factor that is widely measured [32]. The hypothesis suggests that the reduction of neurotrophic signaling is a key factor in depression pathophysiology, and its restoration is the basis of antidepressant treatment measures. BDNF can explain the ability of antidepressants to induce hippocampal neurogenesis or other forms of synaptic plasticity [35].

Recently, it was declared that low BDNF levels may lead to decreased dendritic numbers, neuronal atrophy, neuronal degeneration, and thus low synaptic activity and clinical symptoms of depression. Various investigations have reported depressed patients to have a low level of BDNF in the hippocampus and prefrontal cortex [36].

BDNF is believed to have a precursor (pro-BDNF) that is cleaved into mature BDNF by tPA (tissue plasminogen activator) proteolysis. Mature BDNF favors long-term potentiation and anti-apoptotic properties, whereas pro-BDNF favors long-term depression and neuronal apoptosis. Cleavage of pro-BDNF by tPA/plasmin is also crucial for long-term hippocampal plasticity. The inability of pro-BDNF to convert into mature BDNF leads to major depressive disorder (MDD) pathogenesis [37].

Therefore, since the increased levels of glucocorticoid co-occurred with MDs reduce the rate of neurogenesis and BDNF levels, and also induce the retraction of dendrites, so the survival and proliferation of new hippocampal neurons are vital for MDD patients [38].

The encountered elevated serum cortisol level is a sign of the hypothalamic-pituitary-adrenal (HPA) axis activation in the SCH model in the present study. Regarding the serum cortisol level, the results of the present study were consistent with [18], regarding the behavioral tests and the HPA axis activation, where they compared between the SCH model and overt hypothyroidism regarding the HPA axis activation (the index of adrenal cortex, serum corticosterone level, and hippocampal mRNA CRH expression) and behavioral alteration in FST and sucrose preference test in Wistar rats. The serum corticosterone level was elevated in the SCH in their work, however not to the same extent as the hypothyroid rats. FST revealed significantly increased immobility compared to the sham group and less than the hypothyroid group.

Also, [12] assessed the HPA axis activation by weighing the adrenal glands in rats and plasma corticosteroids. Similarly, a study conducted by [39] concluded that a rat model of chronic unpredictable mild stress caused alterations in the HPA axis in the form of corticosterone elevation, changes in the value of the ACTH and adrenal index.

The biological clock of the brain, i.e. the suprachiasmatic nucleus (SCN) shows lower vasopressin production and a smaller circadian amplitude in depression, which may explain the sleeping disorders in this disease and may contribute to the strong Corticotropin-releasing hormone (CRH) activation, which plays a crucial role in HPA axis regulation, i.e. the final common pathway in the stress response. [40].

In the present study, the SCH group exhibited a reduced level of monoamine obtained from the hippocampus homosylate. This result is in accordance with a previous study, where Wistar–Kyoto (WKY) and Wistar rats were exposed to acute forced-swim stress as a stressful stimulus. The neurochemical results showed distinct patterns of stress-induced and baseline monoamine turnover in the rats, including alterations to dopamine and serotonin (5-HT) turnovers in the nucleus accumbens and prefrontal cortex, 2 critical brain areas implicated in depression, anxiety, and drug reward [41].

Also, the results are in line with the study by [42] in which an unpredicted chronic mild stress model of depression in rats was used and similar results were obtained regarding the monoamines, elevated serum cortisol levels, and the prolongation of the immobility during FST.

In addition, the results of [15] after six days of intraperitoneal clonidine administration in a dose of 0.8 mg/kg clonidine induced alteration in neurotransmitters in the form of a decrease in brain serotonin (5-HT), dopamine, and norepinephrine contents, also significantly increased latency time, and decreased grooming, rearing, and ambulation in the OFT, whereas, in the FST, decreased struggling time and increased immobility were observed.

The results of the current study revealed elevated serum TNF-α levels in the SCH group. This is in accordance with [43] in a rat model of depression by Unpredictable Chronic Mild Stress Model [15], and in a clonidine-induced depression model of rats, where an increase in serum TNF-α was observed together with other mediators like IL-6 brain MDA and GSH contents. That was confirmed by behavioral tests FST, OFT, tail suspension test changes, splash test, and the resident/intruder test.

[44], in genetically manipulated with selectively carrying mutational deletions of TNF-α receptors, mice for depression and anxiety-like behaviors. They concluded that TNF-α receptor activation under normal circumstances promotes depression-like behavior in these mice by using a panel of behavioral assays. They used FST, OFT, and other behavioral tests and demonstrated decreased freezing in a fear-conditioning paradigm (scrambled foot shock) and decreased immobility in the forced swim test. They suggested that selective inhibition of all types of TNF-α receptors' signaling may be effective in treating depression symptoms.

The systemic activation of the immune system in depression may play a crucial role in the depression etiology. Stress is associated with increased secretion of several hormones (e.g. glucocorticoids), and catecholamines could provide an explanation for endocrine, behavior, and central neurochemical alterations. The innate immune system plays a key role in depression and it determines the type of adaptive immune response involving T-helper lymphocytes (Th1 or Th2). In the

Th1 response, macrophages release pro-inflammatory cytokines such as interferon-gamma (INF-γ), TNF-α, and both interleukins (IL)-1 and 2 [45].

These findings can be attributed to what previously stated by ***Wysokiński & Kłoszewska***: ''The diagnosis of SCH must be considered in every patient with depression" as recommended by the American Association of Clinical Endocrinologists. This also confirms the need to monitor TSH levels regularly in patients with all types of mood disorders [46].

In the present work, the Milnacipran-treated group showed a statistically significant improvement of the OFT and FST parameters. Also, a statistically significant reduction of serum cortisol and TNF-α levels was recorded. In addition, the hippocampal neurotransmitters and the BDNF level were significantly increased in comparison to the levels in the model group.

Xu et al., (2006) evaluated the impact of the chronic administration of venlafaxine, quetiapine, and their combination, on BDNF expression and hippocampal cell proliferation in rats subjected to chronic restraint stress over the last 2weeks of a 3-week drug administration course [47]. They deduced that CRS decreased BDNF expression and hippocampal cell proliferation. Also, chronic administration of venlafaxine or quetiapine prevented these reductions in BDNF expression and hippocampal cell proliferation caused by CRS (6 h/day for 14days); prevented BDNF reduction; increased hippocampal cell proliferation; and in stressed rats in a dose-dependent manner.

Similarly, [48], demonstrated that repeated intraperitoneally administration of 10 mg/kg venlafaxine, another SNRI, in rats increased BDNF mRNA levels in both the dentate gyrus and CA3 region of the ventral hippocampus in a chronic unpredicted stress model of rats modified forced swim test in stressed rats.

In the study of [14], lesions of the ventromedial prefrontal cortex of Wistar rats induced impulsive deficits -one of the symptoms of major depression- and repeated Milnacipran treatment ameliorated that impulsive deficit. Repeated Milnacipran remediated the mature BDNF levels and also postsynaptic density, dendritic spine density, and excitatory currents in the few surviving neurons in the ventromedial prefrontal cortex of ventromedial prefrontal cortex-lesioned rats as tested by behavioral parameters.

Chronic administration of a NET inhibitor (NRIs, NDRI, SNRIs, TCAs) overrides the down-regulation of the postsynaptic β_1-receptor, leading to increased noradrenergic neurotransmission activity in the CNS. Moreover, some actions of NET inhibitors are also mediated by postsynaptic α_1-adrenoceptors, which do not appear to be downregulated during chronic treatment with NET inhibitors. The net effect of NET inhibitors on the noradrenergic neurotransmission during chronic treatment is further complicated by regional differences in the distribution of postsynaptic α_1- and β_1-adrenergic receptors within the CNS [49].

In the present work, the Agomelatine-treated SCH group showed significant reversion of the OFT and FST parameters as well as the serotonin, noradrenaline, dopamine, and BDNF levels in the hippocampus.

These results are in accordance with that of [50], in which the acute intraperitoneally or orally administration of 4, 16, or 32 mg/kg Agomelatine to mice or rats significantly reduces the immobility duration in an FST-induced depression model in comparison with Melatonin**.**

In another study using the chronic corticosterone animal model of anxiety and depression state, the behavioral consequences of either chronic Agomelatine (10–40 mg/kg per day) or fluoxetine (18 mg/kg per day) were assessed in a number of paradigms such as the FST, OFT, novelty-suppressed feeding (NSF), and the splash test (ST). Also, the effects of Agomelatine on increasing number of proliferating cells and neurogenesis in the ventral and dorsal hippocampal regions. Both fluoxetine and Agomelatine were administered for 4weeks. The results of the forced-swimming test showed that Agomelatine at the doses of (10 and 40 mg/kg per day) and fluoxetine increased mobility duration in corticosterone and non-corticosterone-treated rats. All behavioral parameters with fluoxetine and Agomelatine were effective in reversing anxiety/depression-like phenotypes induced by excess glucocorticoids [51].

Depressive patients often experience a number of sleep disturbances, like difficulty in falling asleep, staying asleep, disturbed nocturnal sleep, early-morning awakening, etc. Several studies have suggested that depression is related to circadian rhythm disturbances; therefore, an antidepressant that resets disturbed circadian rhythms and benefits sleep quality may have a more beneficial antidepressant effect. Agomelatine is a melatonergic agonist of MT1/MT2 melatonergic receptors, with antagonism of 5-HT2C serotonergic receptors [52].

The therapeutic effect of Agomelatine in depressive disorders is related to its action on MT1 and MT2 melatonergic receptors, largely present in the hypothalamus SCN, and also to its 5-HT2C antagonism. 5-HT2C receptors are concentrated in the SCN, cortico-limbic structures, hippocampus, amygdala, and frontal cortex, and these structures are involved in the regulation of mood and cognition [53].

Results of the present study were consistent with the results achieved by the study of [15], where Agomelatine significantly improved rats depressed with clonidine for 2 weeks, which was regarding the OFT and FST parameters, as well as brain neurotransmitters, TNF-α and IL-6.

In the present study, Agomelatine significantly reversed the elevated cortisol level but to a lesser extent than Milnacipran and more effective than *H. Perforatum.*

Similarly, in a model of psychosocially stressed male tree shrews, 40 mg/kg/day of Agomelatine for 4 weeks normalized the cortisol level and the core body temperature. Agomelatine also reversed the stress-induced reductions in locomotor activity as well [54].

In a previously published study, it was shown that acute Agomelatine administration increased BDNF in the prefrontal cortex and chronic administration of Agomelatine increased BDNF in the hippocampus in rats [55].

A randomized**,** double-blind clinical trial in patients with major depressive disorder favored of Agomelatine compared to sertraline on the relative amplitude of the circadian rest-activity cycle was observed at the end of the first week. In parallel, a significant improvement of sleep efficiency and sleep latency from the 1st to 6th weeks was observed with Agomelatine as compared to sertraline. Over the six-week treatment period, anxiety and depression symptoms significantly improved with Agomelatine compared to the SSRI sertraline [56].

Sleep-enhancing effects are essentially important to Agomelatine's therapeutic effects. More specifically, improvement of sleep may target certain mechanisms implicated in depressive and anxiety pathophysiology. Impaired sleep has a adversely affect mechanisms that play pathophysiological roles in inflammation, anxiety and mood disorders, oxidative stress, and mitochondrial function and integrity [57, 58].

The results of this study revealed a reversal of the behavioral alterations in the OFT and the FST in the *H. Perforatum*-treated group, comparable to the standard drug, Milnacipran, while it was significantly more efficient than Agomelatine, regarding the latency time, ambulation frequency, rearing, grooming in OFT, and struggling time in the FST. Thus, it is obvious that it alleviated the depressive-like symptoms in the SCH rats.

However, *H. Perforatum* showed less significant prolongation of immobility time in comparison to Agomelatine.

This was in accordance with a previous study in which acute restrain stress caused neurobehavioral alterations and oxidative damage in mice for 6 hours. *H. Perforatum* was administered in two different doses, 50 and 100 mg/kg, 0.5h prior to exposing animals to acute immobilized stress. There was a significant attenuation of restraint stress-induced behavioral (antianxiety-like effect, reduced tail-flick latency, and improved locomotor activity) and oxidative damages in comparison to the control [59].

Another study was conducted to evaluate the therapeutic effect of *H. perforatum* on behavioral alterations (mirror chamber, plus maze, zero maze) and oxidative damage induced by sleep deprivation for 72 hours in LACA mice. 200 and 400 mg/kg of standardized *H Perforatum* extract (p.o), and 10 mg/kg of imipramine (i.p.) were administered for five days, starting two days before sleep deprivation. The results revealed significantly improved anti-anxiety effect, locomotor activity, body weight, as well as reduced oxidative damage in sleep-deprived animals [58].

The mechanism of action of *H. perforatum* as an antidepressant remains unclear [60]. *H. perforatum* shares some mechanisms of action with conventional antidepressant drugs. It may act via novel mechanisms of action, which needs further exploration [61].

A number of theories have been proposed. One possible mechanism is inhibition of the uptake of dopamine (DA), norepinephrine (NE), and serotonin (5HT) from the synaptic cleft of interconnecting neurons causing an increase in synaptic concentrations that might be associated with the increase in free intracellular sodium ion concentrations [62].

Zirak and colleagues suggested that hyperforin serve as potent but nonspecific inhibitors of synaptosomal 5-HT, NA, and DA suppressing serotonin uptake into brain synaptosomes with no interaction with the transporter molecule [61].

Moreover, in the rat locus coeruleus, *H. perforatum* extract increases extracellular levels of serotonin, noradrenaline, dopamine, where the cell bodies of the Noradrenergic neurons originate and spread to different parts of the CNS, including the limbic system in which the emotion, motivation, and libido are under control [63] and modulated adenosine, GABAA, GABAB, and glutamate receptors [64, 65]. Furthermore, *H. perforatum* has a long-term effect on the expression of genes involved in the HPA axis regulation, leading to decreased levels of plasma corticosterone and adrenocorticotropic hormones [66].

In the present work, H Perforatum has shown marked alleviation of the level of TNF alpha and serum cortisol at a comparable level to Milnacipran and Agomelatine.

The release of inflammatory cytokines may explain the pathophysiology of major depression. The glucocorticoid receptor is one of the main mechanisms by which cytokines may contribute to depression, inducing neuroendocrine challenge [67]. In major depression, the two most consistent biological findings are the increase in the hyperactivity of the hypothalamic-pituitary-adrenal axis and inflammation [68], but the molecular and clinical mechanisms underlying these abnormalities are still unclear [69].

H. perforatum has an antioxidant action by reactive oxygen-quenching, metal chelating, and scavenging free radical activities. Its extract is an effective antioxidant and lipid peroxidation inhibitor by altering brain malondialdehyde, glutathione peroxidase, and glutathione levels in rats [60].

In addition to inhibiting monoamine reuptake, HP activates adenosine and gamma-aminobutyric acid receptors, downregulates beta-adrenergic receptors, and upregulates serotonin receptors—and all of these mechanisms may contribute to feelings of contentment and calm [65].

The efficacy and safety of H. perforatum in the treatment of depressive disorders have been widely investigated in clinical trials with different designs (open trials or randomized, controlled trials); different populations (adolescents, adults, or the elderly); and different control groups (placebo or antidepressant) [61].

It is to be noted that, Besides its antidepressant activity, St. John's wort is known for its noticeable effect on the activity and expression of genes involved in drug metabolisms and this is responsible for its interactions with other drugs [70, 71].

H. perforatum increases the expression and activity of CYP3A4, by clearance of concomitantly applied substrates and modifying the first-pass metabolism [39]. The efflux transporter P-glycoprotein (P-gp, ABCB1, MDR1), where treatment with St. John's wort extracts increased intestinal ABCB1 expression,

whereby explaining the reduced bioavailability of ABCB1 substrates. The underlying mechanism is the activation of the Pregnane X Receptor (PXR) [71].

From the results of the present study, it was concluded that experimental SCH could result in depression-like behavior accompanied by the elevation of serum cortisol, TNF alpha, and reduced hippocampal monoamines and BDNF levels. Treatment with *H. Perforatum* extract resulted in marked improvement of these behavioral and biochemical changes, in a comparable manner to the standard antidepressant drug Milnacipran. Also, an improvement was observed with Agomelatine but to a lesser extent than that of Milnacipran and *H. Perforatum* extract.

In this work, *H. Perforatum* showed marked alleviation of the level of TNF alpha and serum cortisol levels at a comparable level to Milnacipran and superior to Agomelatine. Considering H perforatum extract, action on the OFT and FST behaviours.

From the results of the present study, it was concluded that experimental SCH could result in depression-like behavior accompanied by the elevation of serum cortisol and TNF alpha, and reduced hippocampal monoamines and BDNF levels. Treatment with *H. Perforatum* extract resulted in marked improvement of these behavioral and biochemical changes, in a comparable manner to the standard antidepressant drug Milnacipran. Also, an improvement was observed with Agomelatine but to a lesser extent than that of Milnacipran and *H. Perforatum* extract.

Conclusion:

In conclusion, experimental Subclinical Hypothyroidism (SCH) could result in depression-like behavior accompanied with elevation of serum cortisol, TNF alpha and hippocampal monoamines and BDNF levels reduction. Treatment with H. *Perforatum* extract resulted in marked improvement of behavioral and biochemical changes, in compare with the manner to the standard antidepressant drug Milnacipran. In addition, Agomelatine was improved but to a lesser extent than that of Milnacipran and H. *Perforatum* extract.

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