Plant Archives Volume 20 No. 2, 2020 pp. 7465-7477 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

MOLECULAR MECHANISM UNDERLYING HISTOLOGY, IMMUNOLOGICAL AND BIOCHEMICAL EFFECTS OF CAFFEINE AGAINST CARDIOVASCULAR DISEASES IN DEPRESSIVE RATS – INDUCED BY RESERPINE

Hanaa K. Mohamed[1]*, Najat Jabbar Ahmed[2], Waffa A. Hammad[3] and Marwa T. Hassen[4]

[1*,3 and 4]Department of Zoology, Faculty of Women for Arts, Science and Education, Ain Shams University, Cairo, Egypt.

[2] Erbil Technical Health College, Erbil Polytechnic University, Erbil, Iraq.

Abstract

Depression is a mood disorder which affects one's quality of life and is commonly associated with neurotransmitter dysfunctional mechanisms. The present study was planned to investigate the therapeutical activity of caffeine against depression induced in the experimental model in attempt to clarify its mode of action. This study was conducted on 30 adult male albino rats divided into 3 groups n=10 Gp.(1) normal control group, Gp.(2) reserpine group received reserpine in a dose of 0.2 mg/kg/ day, Gp.(3) reserpine plus caffeine group treated with caffeine at dose 30 mg/kg. Serum neurochemical changes (the levels of serotonin, dopamine and cortisol), level of interleukin-10 (IL-10), C-reactive protein (CRP), the value of brain proinflammatory cytokine (TNF-α), activity of acetylcholinesterase and lipid peroxidation(MDA), cardiac activity of Na+, K+, ATPase enzyme, histopathological changes in brain, heart and DNA fragments in were carried out. In comparison with the normal control group, the reserpinized group recorded significant decrease in serum serotonin and dopamine contents and significant increase in the plasma level of cortisol, significant increase in the brain content of TNF-α, CRP, MDA and acetylcholinesterase. Moreover, significant decrease in levels of IL-10 and cardiac activity of Na+, K+ and ATPase enzyme was detected in the reserpinized group compared with the normal control. Histological analysis of the reserpinized community brain tissue parts of rats revealed moderate cortical edema, scattered degenerated neurons and diffuse gliosis. On the other hand, induced depression by reserpine caused a significant effect on the cardiac muscles and increased the collagen fibers around coronaries when compared with normal group.

Key words: Depression, reserpine, neurotransmitters, cardiac muscles, collagen fibers.

Introduction

Depression is a major health concern worldwide and by 2030 depression is expected to be one of the third disorders adding to the universal burden of disease (Mathers and Loncar, 2006 and Ozerov *et al.,* 2106). According to the current World Health Organization (WHO) estimation, the number of people suffering from depressive disorders worldwide is 350 million. Moreover, researchers have projected that depression will be the second civilization disorder producing incapacity by 2020. In addition, WHO has revealed that depression is the fourth largest contributor to life-years adjusted for disability, a metric reflecting the amount of years lost due to injuries, illness, impairment and premature death (Reddy, 2010 and Park *et al.*, 2018).

***Author for correspondence :** E-mail: drhanaa_eta@yahoo.com

Generally, depression is a mood disorder affecting one's quality of life and is commonly associated with dysfunctional mechanisms of neurotransmitters (Cui *et al.,* 2012). It is also characterized by feelings of unpleasantness, helplessness, sadness and despair. Such feelings are often accompanied by symptoms such as sleep disturbance, loss of appetite and decreased concentration or hindered decision making, feeling guilty or worthless, decreased energy and fatigue, thoughts of harming one's self, suicidal contemplations (Park *et al.,* 2018), which can substantially impact the patient's quality of life and social functioning.

Depression can be divided into several types, including major depressive disorder (MDD), dysthymic disorder,

psychotic depression, postpartum depression, seasonal affective disorder (SAD) and bipolar disorder. However, MDD and dysthymic disorder are the most common types of depression (Gilbert, 2017).

The causes of depression are not known exactly, however, as with many other psychiatric illnesses, it is believed that there are several biochemical, genetical and environmental factors that may cause depression (Hyman, 2010). There is evidence that people who suffer from depression have physical changes in the brain. In addition, hormonal imbalance can also cause this disorder. Some studies have shown that depression is more common in people with family members with history of the disease, encouraging researchers to seek out the genes that cause the disease (Dunn *et al.,* 2015). Environmental factors also play a critical role in depression. Depression may occur as a result of drug addiction, certain medications, or as a result of stressful life events (Caspi *et al.,* 2003). On the other hand, diseases, such as cancer, thyroid disease and chronic pain, are associated with an increased risk of depression. Both environmental and genetic factors are likely to interact to facilitate the development of the disease in a complete and rapid manner. Under the monoamine depression hypothesis, the brain depletion of serotonin, norepinephrine and/or dopamine could underlie the depression's etiology and pathogenesis (Schechter *et al.,* 2005 and Gao *et al.,* 2016). In the 1960, a neurochemical model of depression was proposed based on reports that monoamine depletion induced by reserpine.

Reserpine is a Rauwolfia indole alkaloid that acts as a sympatholytic and sedative agent and was once used as a primary treatment for hypertension (Holt, 1961 and Guo *et al.,* 2015). Several researches have shown that reserpine has a serious side-effect, causing major depression after chronic use of the medication in a percentage of the drug's users (Leith and Barrett, 1980). The mechanism of Reserpine is the irreversible binding to storage vesicles in monoaminergic neurons (Minor, 1990). The vesicle can then leak, resulting in the seepage of transmitter into the cytoplasm where it is then destroyed by intraneuronal monoamine Oxidase. This causes a severe decrease or total depletion of active transmitter needed to be released at the synapse after depolarization (Hanff *et al.,* 2010 and Greenwood *et al.,* 2018). In addition, several lines of evidence indicate the involvement of oxidative and nitrosative stress in the pathophysiology of depressive disorders (Maes *et al.,* 2011).

Caffeine (1, 3, 7 trimethylxanthine) is a psychoactive drug, used in different ways worldwide. The most commonly consumed psycho-stimulant drug known to humans is caffeine, used in tea, coffee, energy drinks and others. Recently, it was estimated that 90% of U.S. population and 80% of the world's population consume caffeine on a daily basis (Fredholm *et al.,* 1999). It has been reported that caffeine can enhance memory in both animal models and humans (Ardais *et al.,* 2014). In fact, while healthy people can tolerate moderate intakes of caffeine, heavy caffeine consumption has been associated with severe adverse health effects (Gallagher *et al.,* 1993). Caffeine intake was proved to decrease anxiety and depression in humans in low doses, while high doses exacerbate anxiety (Yamada *et al.,* 2014). Due to its lipophilicity, caffeine crosses the blood-brain barrier easily exerting its stimulant effect (Ferré, 2016). Caffeine exerts its activity on the central nervous system (CNS) by counteracting most of the inhibitory effects of adenosine on neuroexcitability (Fredholm *et al.,* 1999), arousal and spontaneous activity (Kuzmin *et al.,* 2006). Also, the changes induced by caffeine in CNS functions were mediated by reducing phosphodiesterase activity, blocking GABA-A receptor activity and increasing intracellular calcium (Garrett and Griffiths, 1997). The stimulating effect of caffeine has been attributed to its antagonistic effect on the activity of endogenous adenosine, which inhibits the release of several CNS neurotransmitter systems, including GABA, acetylcholine, glutamate, dopamine, norepinephrine and serotonin (El-Yacoubi *et al.,* 2001; Fisone *et al.,* 2004 and Williams, 1987) and thus the effect of caffeine on depression has been taken into consideration (Khadrawy *et al.,* 2018).

Therefore, the current study aims to evaluate the effect of caffeine on neurochemical changes (the levels of serotonin, corticosterone, dopamine), the activities of acetylcholinesterase and Na+, K+, ATPase, levels of inflammatory cytokines tumor necrosis factor (TNF-α), lipid peroxidation, histopathological changes in brain and heart and DNA fragments in depressive-like rats induced by reserpine.

Table 1: Effect of daily caffeine treatment (30 mg/kg) on dopamine and serotonin levels in depressive rat-induced by reserpine.

Groups / Parameters	Normal control (NC) group	Reserpine (RES) group	Reserpine +Caffeine (RES+CAAF) group
Dopamine (ng/ ml)	26.7±0.43	8.66±0.43 *-67.56%	18.73±0.56 *#116.28%
Serotonin (ng/ ml)	35.96±1.21	16.8±0.62 *-53.28%	30.56±0.45 #81.90%

Values are presented as mean ±SEM; *: statistically significant compared to corresponding value in control group (P<0.05); #: statistically significant compared to corresponding value in reserpine (RES) group (P < 0.05).

Table 2: Effect of daily caffeine treatment (30 mg/kg) on cortisol levels in depressive rat-induced by reserpine.

Parameters / Group	Cortisol (ng/ ml)
Normal control (NC)group	3.53±0.91
Reserpine (RES) group	10.1±0.67 *186.11%
Reserpine +Caffeine (RES+CAAF) group	6.03±0.36 #-40.29%

Values are presented as mean ±SEM; *: statistically significant compared to corresponding value in control group (P<0.05); #: statistically significant compared to corresponding value in reserpine (RES) group (P < 0.05).

Material and Methods

Experimental Animals

Thirty adult male albino rats were employed in the present study. They were obtained from the Serum and Antigen Laboratories at Helwan. Their weight ranged between (230-250g). Animals were allowed a one-week pre-experimentation period to adapt to laboratory conditions in order to avoid any complications along the course of the experiment. They were housed in metabolic cages and received food and water ad-libitum with fresh supplies presented daily.

Chemicals

Reserpine was purchased from sigma (crystallized, ≥99.0% (HPLC), was dissolved in glacial acetic acid (1 μg/μl) and then completed to 25 ml with distilled water. Caffeine was also purchased from sigma. It was dissolved in 0.9% saline (15 mg/ml). All other reagents were obtained from Sigma Chemical Co.

Experimental design

At the beginning of the experiment, the rats were divided randomly into control rats (normal control (NC)) that feeding basal diet and reserpine-treated rats that were injected intraperitoneally (i.p.) with reserpine (0.2 mg/ kg/ day) for 15 days to establish the animal model of depression according to Antkiewicz-Michaluk *et al.,* (2014). On the 16th day, the reserpine-treated rats were further divided into two groups:(reserpine group (RES)) rat model of depression that continued receiving an i.p. injection of reserpine (0.1 mg/kg/ day) for further 15 days to keep the state of depression and (reserpine +caffeine (RES+CAAF)) rat model of depression in which rats were treated with an i.p. injection of caffeine (30 mg/kg) for 15 days after reserpine induction.

At the end of the experiment, blood samples were withdrawn from the retro-orbital vein of each rat, under light anesthesia by diethyl ether, according to the method described by Moore, (2000). Blood was allowed to coagulate and then centrifuged at 3,000 rpm for 15 minutes. The obtained serum was used to estimate of serotonin (5-hydroxytryptamine; 5-HT), cortisol and dopamine (DA) according to the fluorometric method described by (Ciarlone, 1978).

Dissection and tissue preparation

Immediately after blood extraction, the cervical dislocation sacrificed animals and removed brain and heart tissues, washed in ice cooled saline, plotted dray and weighed them. A weighed part of each brain and heart was homogenized, using a homogenizer, with ice-cooled saline at 4,000 rpm for 5 minutes. The supernatant was used for biochemical analysis. For histopathology, brain and heart tissues were fixed in neutral buffered formalin (10%).

Biochemical analysis

Homogenized brain tissue samples were analyzed for malondialdehyde (MDA) levels by the method of Ohkawa *et al*., (1977), acetylcholinesterase (AChE) activity by the method of Ellman *et al.,* (1961) and tumor necrosis factor-α (TNF-α) content was determined by ELISA technique using TNF-α assay kit purchased from Assay Pro.,Co., USA according to the method described by Taylor, (2001). TNF-α levels were measured using a standard quantitative sandwich ELISA and C-reactive protein level (CRP) was measured according to Kadhem *et al.,* (2016). Interleukin-10 (IL-10) level was measured using commercially available ELISA kits for IL-10 (ab100765) according to Hassan *et al.,* (2019). The

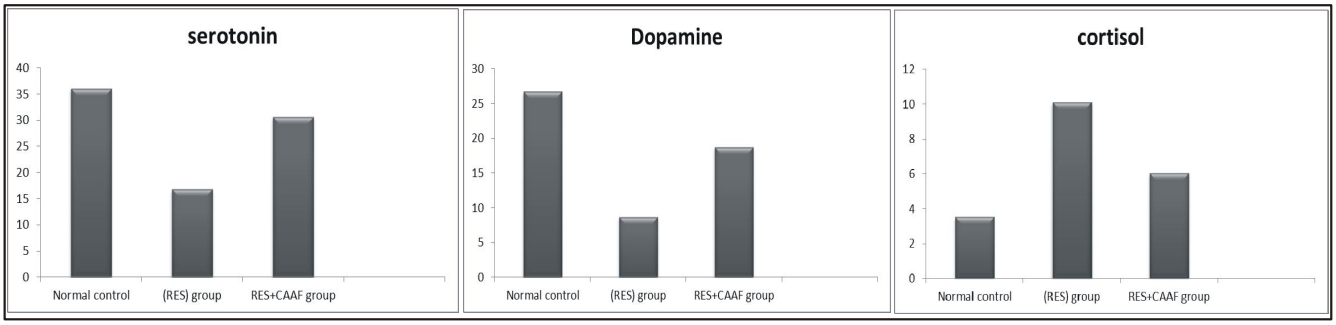

Fig. 1: Effect of caffeine (CAAF) on the concentration of plasma dopamine, serotonin and cortisol in rat with reserpine-induced depression.

Table 3: Effect of daily caffeine treatment (30 mg/kg) on cytokines levels in depressive rat-induced by reserpine.

Groups / Parameters	Normal control (NC) group	Reserpine (RES) group	Reserpine +Caffeine (RES+CAAF) group
TNF-α(pg/ml)	2.73±3.89	5.43±27.38 *98.9%	3.11±3.52 #-42.72%
CRP(µg/mL)	77.03 ±0.02	371.12±.31 *381.79%	82.06±1.06 #-77.88%
IL -10(pg/ml)	151.01±021	71.16±1.06 *-52.88%	141.06±0.8 *#98.22%

Values are presented as mean ±SEM; *: statistically significant compared to corresponding value in control group (P<0.05); #: statistically significant compared to corresponding value in reserpine (RES) group (P < 0.05).

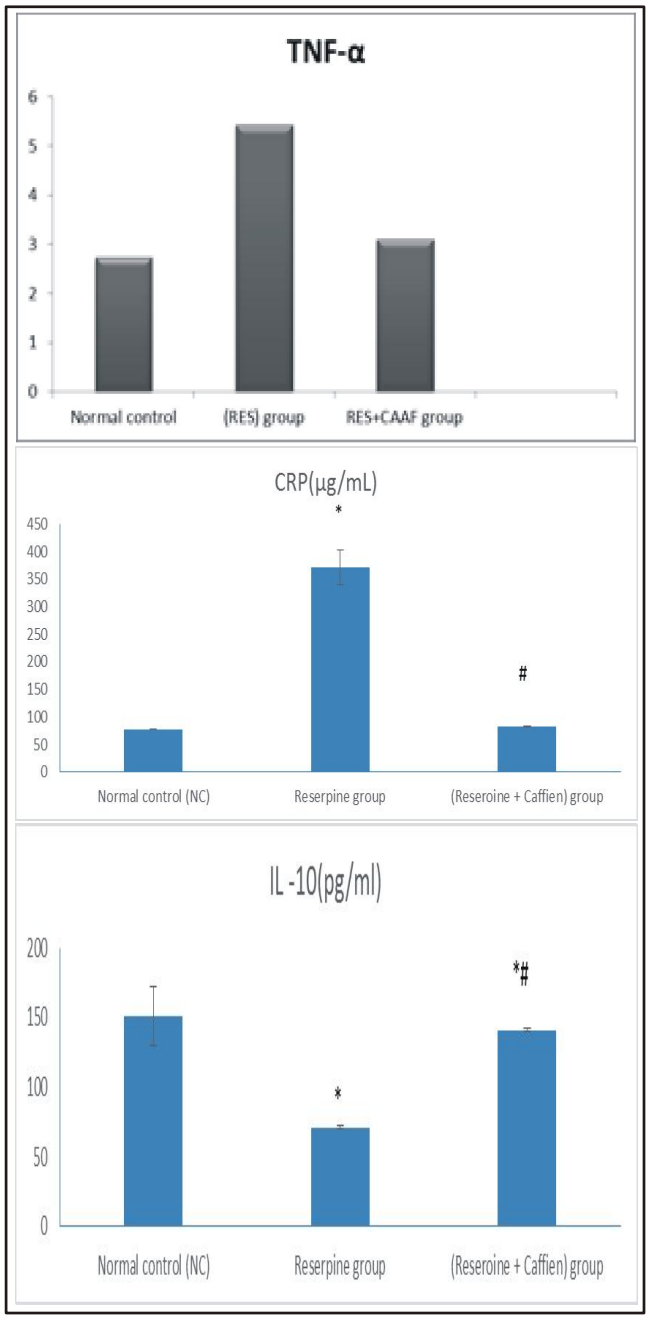

Fig. 2: Effect of daily caffeine treatment (30 mg/kg) on cytokines levels TNF-α, CRP and IL-10 in the brain of depressive rats-induced by reserpine.

supernatant of heart tissue were also used for determination of ATPase enzyme. Na+-stimulated Na, K-ATPase activity was determined by measuring ATP hydrolysis. Released inorganic phosphate (P.i.) was detected using a malachite-based Biomol Green reagent (Biomol AK-111, Enzo Life Sciences) as previously described (Juel *et al.,* 2013).

Histopathological evaluation

Histopathological slides of the brain and heart tissues were prepared and staining was done using haematoxylin and eosin (Slaoui and Fiette, 2011 and Cardiff *et al.,* 2014). Also, the heart tissues were stained with Masson Trichrome was used for collagen fibers. Slides prepared from all groups were examined and photographed (Bancroft and Gamble, 2008).

Detection of DNA damage by the comet assay

The portion of the heart was minced and suspended in chilled homogenizing buffer (pH 7.5) 0.075µ NaCl and 0.024µ Na2EDTA and the homogenized gently using

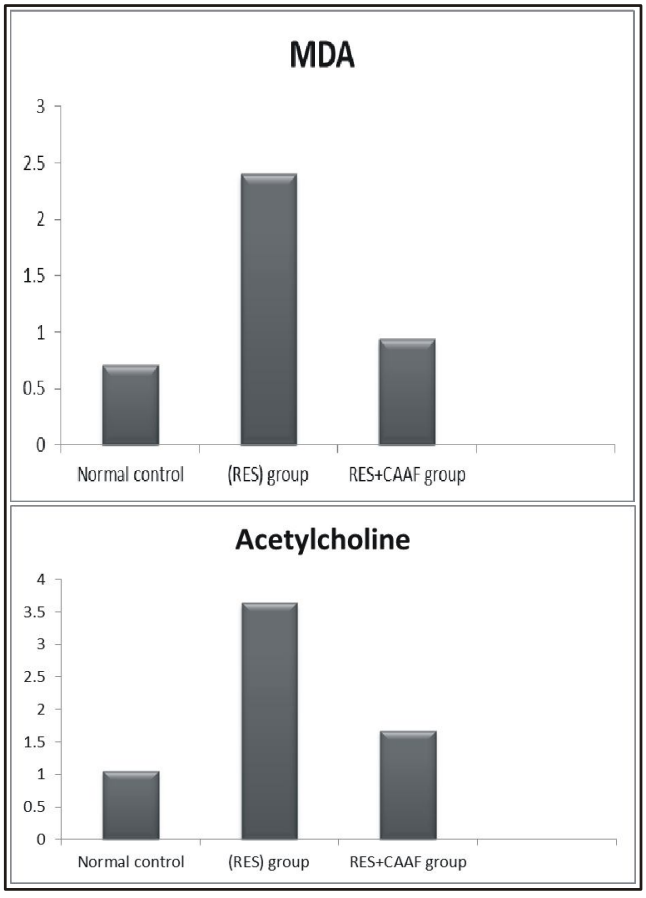

Fig. 3: Effect of daily caffeine treatment (30 mg/kg) on MDA, and the activity of acetylcholinesterase (AchE) in the brain of depressive rats-induced by reserpine.

Table 4: Effect of daily caffeine treatment (30 mg/kg) on MDA, the activity of acetylcholinesterase (AchE) and TNF- α in the brain of depressive rats-induced by reserpine.

Groups / Parameters	Normal control (NC) group	Reserpine (RES) group	Reserpine +Caffeine (RES+CAAF) group
Acetylcholine (M mosh/g/min)	1.05±2.48	3.64±14.77 *246.66%	1.67±5.73 #-54.12%
MDA (m.mol/l)	0.71±0.02	2.40±0.31 *238.02%	0.94±0.05 #-60.83%

Values are presented as mean ±SEM; *: statistically significant compared to corresponding value in control group (P<0.05); #: statistically significant compared to corresponding value in reserpine (RES) group (P < 0.05).

homogenizer in ice. The cells suspension was centrifuged at 40°C, 700×9 for 10 min. The cells were suspended in the cold buffer (Sasaki *et al*., 1997). The comet assay was carried out under alkaline conditions, basically as described by Singh *et al.,* (1988). Slides were stained with 50 A of ethidium bromide (2 mg/ml) and observed at 400 magnifications using a lexica DFC 425 camera.

Analysis of comet parameters was performed using TriTek cometscore, version 1.5. The DNA damage was quantified by measuring the displacement between the genetic material of nucleus (comet head) and the resulting (tail). The number of tailed cells and tail DNA% are the two most commonly used parameters to analyze the result of the comet assay (De Boeck *et al.,* 2000).

Statistical analysis

Data have been coded and entered using the (Social Sciences Statistical Software SPSS), version 25 (IBM Corp., Armonk, NY, USA). For quantitative variables, the data was summarized using mean and standard mean error (SEM).

Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test for comparison between each 2 groups (Chan, 2003). P-values less than 0.05 were considered as statistically significant.

Results

Neurochemicals results

1. Serotonin (5-HT):

The data presented in table 1 and fig. 1 revealed significant decline (P<0.05) in plasma serotonin level of the reserpine group when compared with that of the normal control group (35.96 ng/ml vs. 16.8 ng/ml) with the percent of difference -53.28%. In contrast, treatment of the reserpine groups with caffeine led to significant elevation (*P*<0.05) in plasma serotonin level as compared to that of reserpine group. Serum serotonin level of the reserpine group treated with caffeine was (30.56 ng/ ml vs. 16.8 ng/ ml) for the reserpine group with the percent of difference 81.90%.

2. Dopamine (DA):

The results of dopamine level in the normal control and other studied groups are illustrated in table 1 and fig. 1. The recorded value of plasma dopamine concentration in the reserpine group revealed significant decrease (*P*<0.05) as compared to that in the control group (8.66 ng/ ml vs. 27.7 ng/ ml with the percent of difference-67.56%). However, treatment with caffeine attenuated these decreases (RES+CAAF: 18.73 ng/ml, p<0.05).

3. Cortisol:

The data of serum cortisol level of the different groups under investigation are recorded in table 2 and fig 1. Serum cortisol level displayed significant increase (*P*<0.05) in the reserpine group as compared to that in the normal control group 10.1 ng/ ml vs 3.53 ng/ ml representing a percent of difference 186.11%. In contrast, treatment of the with caffeine led to significant decline (*P*<0.05) in serum cortisol level as compared to that of reserpine group. Serum cortisol level of the reserpine group treated with caffeine at dose (30 mg/kg) was 10.1 ng/ ml vs 6.03 ng/ ml for the depressive group with the percent of difference -40.29%.

Proinflammatory Cytokines

1. Tumor necrosis factor-α (TNF-α):

Table 3 and fig. 2 represented significant effects of depression on levels of inflammatory cytokines tumor necrosis factor (TNF-α) in brain. Significantly higher

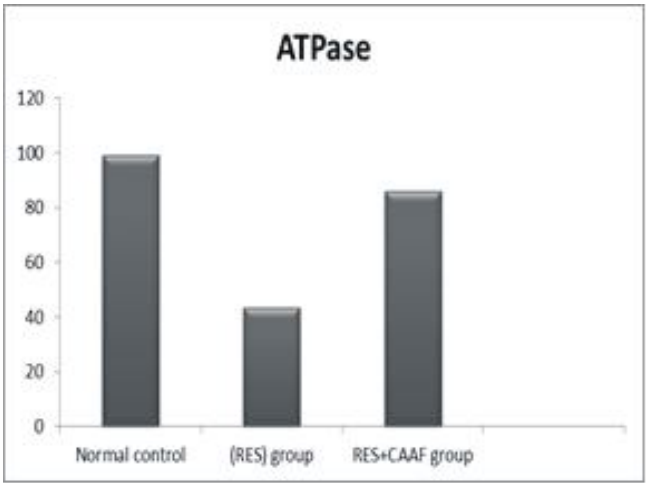

Fig. 4: Effect of daily caffeine treatment (30 mg/kg) on cardiac Na^+, K^+, ATPase activity of depressive rats-induced by reserpine.

Table 5: Effect of daily caffeine treatment (30 mg/kg) on cardiac Na^+, K^+, ATPase activity of depressive rats-induced by reserpine.

Parameters / Group	Na^+-K^+-ATPase (M mol pi / min/g)
Normal control (NC)group	99.06±1.43
Reserpine (RES) group	43.36±3.05 *-56.22%
Reserpine +Caffeine (RES+CAAF) group	86.20±2.19 *#98.80%

Values are presented as mean ±SEM; *: statistically significant compared to corresponding value in control group (P<0.05); #: statistically significant compared to corresponding value in reserpine (RES) group (P < 0.05).

levels (P<0.05) of TNF-α was observed in the depressive rats when compared with the normal control (5.43 pg/ml vs. 2.73 pg/ml) with the percent of difference 98.9%. Treatment with caffeine at dose (30 mg/kg) reduced the levels of TNF-α as compared to depressive control. Brain content of TNF-α in the reserpine group treated with caffeine was (3.11 pg/ml 5.43 pg/ml with the percent of difference -42.72%), These data indicate that CAFF may help to regulate immune and endocrine dysfunction associated with depression.

2. C-Reactive protein (CRP):

The results of CRP level in the control and other studied groups are illustrated in table 3 and fig 2. The recorded value of serum CRP concentration in the reserpine group revealed significant increase (P<0.05) as compared to that in the control group (371.12 µg/mL vs. 77.03 µg/mL with the percent of difference 381.79%). However, treatment with caffeine attenuated these decreases (RES+CAAF: 82.06 µg/mL, p < 0.05).

Anti-inflammatory interlukine-10(IL-10)

The data presented in table 3 and fig. 2 revealed significant decline (P<0.05) in plasma IL-10 level of the reserpine group when compared with that of the normal control group (151.01 pg/ ml vs. 71.16 pg/ ml) with the percent of difference -52.88%. In contrast, treatment of the reserpine groups with caffeine led to significant elevation (P<0.05) in plasma IL-10 level as compared to that of reserpine group. IL-10 level of the reserpine group treated with caffeine was (71.16 pg/ ml vs. 141.06 pg/ ml) for the reserpine group with the percent of difference 98.22%.

Brain Malondialdehyde (MDA) as a lipid peroxidation marker

On measuring of the level of MDA in the brain of the different studied groups, the data revealed that MDA concentration exhibits significant increase (P<0.05) in the reserpine group when compared to that in the normal control group (2.40 m.mol/l vs. 0.71 m.mol/l) with the percent of difference 238.02%. In contrast, the treatment of the reserpine groups with caffeine resulted in significant reduction (P<0.05) in brain MDA level with respect to that in reserpine group (Table 4 and Fig. 3).

Brain Acetylcholine (AchE) activity

Table 4 represented the results of brain of AchE activity in the normal control group and the other studied groups. Significant increase (P<0.05) in AchE was detected in the reserpine group when compared with that in the control group (3.64 M mosh/g/min) protein vs. 1.05 M mosh/g/min) with the percent of difference 246.66%. On the other hand, significant reduction (P<0.05) in AchE activity was recorded in the groups treated with caffeine. AchE activity of the reserpine group treated with caffeine was 1.67 M mosh/g/min) vs. 3.64 M mosh/g/min) for the reserpine group with the percent of difference -54.12%.

Cardiac activities of Na^+-K^+-ATPase results

The results of Na^+-K^+-ATPase in the normal control and other planned groups are clarified in table 5. The recorded value of Na^+-K^+-ATPase in the reserpine group revealed significant decrease (P<0.05) as compared to that in control group (43.36 M mol pi / min/g vs 99.06 M mol pi / min/g with the percent of difference-56.22%). However, significant increase (P<0.05) in Na^+-K^+-ATPase activity was detected in the reserpine group treated with caffeine as compared to that in the depressive rats. Na^+-K^+-ATPase activity of the reserpine group treated with caffeine was 86.20 M mol pi / min/g vs 43.36 M mol pi / min/g for the reserpine group with the percent of difference 98.80%.

Table 6: Comet assay parameters by image analysis of cells isolated from heart of treated groups.

Groups / Parameters	Normal control (NC) group	Reserpine (RES) group	Reserpine +Caffeine (RES+CAAF) group
% of tailed cells (damaged cells)	8.71 ± 0.42	49.71 ± 2.31*470.72%	8.86 ± 0.45*#172.21%
% of DNA in tail	3.78 ± 0.68	18.57 ± 1.25*391.26%	4.25 ± 0.85*#-77.11%

Values are presented as mean ±SEM; *: statistically significant compared to corresponding value in control group (P<0.05); #: statistically significant compared to corresponding value in reserpine (RES) group (P < 0.05).

Histological examination of the brain (haematoxylin and eosin)

Sections from group I (control) indicated that white matter showing average neurons, average glial cells in fibrillary background and average intra-cerebral blood vessels. Induction with reserpine showed important degenerative changes in brain tissue showed mild cortical edema, scattered

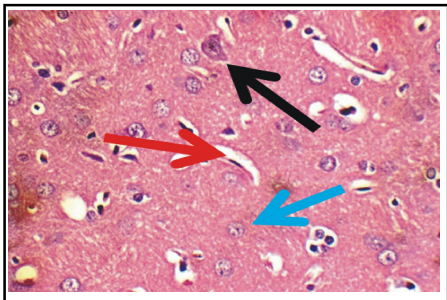

Fig. 1: (Control): white matter showing average neurons (black arrow), average glial cells in fibrillary background (blue arrow) and average intra-cerebral blood vessels (red arrow) (H&E X 400).

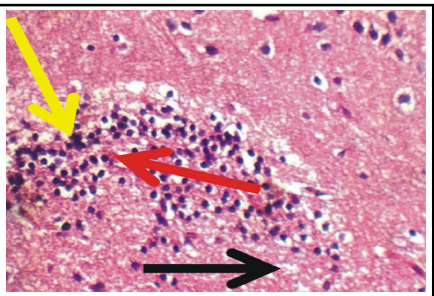

Fig. 2: (RES Group): showing mild cortical edema (black arrow), scattered degenerated neurons (blue arrow), and diffuse gliosis (yellow arrow) (H&E X 400).

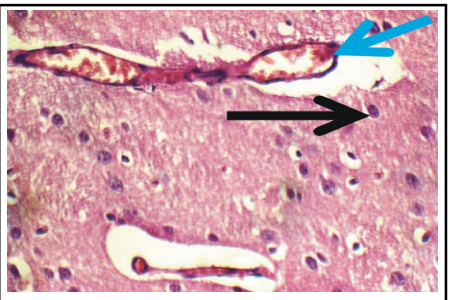

Fig. 3: (RES+CAAF Group): white matter showing average glial cells in fibrillary background (black arrow), and mildly dilated congested intra-cerebral blood vessels (blue arrow) (H&E X 400).

Fig. (1-3): Photomicrographs showing histopathological changes of brain in (depression model (RES) and treated (RES+ CAAF) compared to control group (NC).

degenerated neurons and diffuse gliosis. Rats treated with caffeine showed less degenerative changes. White matter showing average glial cells in fibrillary background and mildly dilated congested intra-cerebral blood vessels (Fig. 1-3).

Histological examination of cardiac muscle (haematoxylin and eosin)

Transverse section (TS) of cardiac muscles of controlled rats showed normal cardiac fibers. Reserpine group showed cardiac muscle fibers with indistinct cell borders, pale cytoplasm, small pyknotic nuclei and intra-cytoplasmic vacuoles and mildly congested myocardial blood vessels. Caffeine-treated group showed cardiac muscle fibers with indistinct cell borders and mild intra-cytoplasmic vacuoles (Fig. 4-6).

Masson stain and collagen

Masson trichrome stain was used to demonstrate the changes in collagen content around coronaries and any fibrotic changes in cardiac muscles. Reserpine leads to increase in collagen fibers around coronaries compared with normal group. In group 3 caffeine prevent the increase in collagen fibers around coronaries (Fig. 7-9).

DNA analysis, single-cell gel electrophoresis (Comet assay)

The results in table 6 and Figs. (a, b and c) showed that reserpine induced a significant concentration dependent increase in the tail length and % of DNA in tail in the heart. Normal rats showed more or less constant levels during the course of the study. Where in the reserpine rats group, a significant elevation in the tail length and % of DNA in tail were recorded. The percentage of increment was 470.72% and 391.26% respectively at the end of the experiment as compared to their corresponding animals in the normal control. Additionally, the supplementation of caffeine to reserpine rats group led to a significant decrease in the tail length and% of DNA in tail (Table 5 and Fig.). The percentages of these changes were 172.21% and -77.11%.

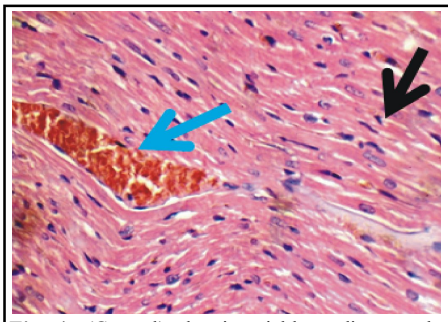

Fig. 4: (Control): showing viable cardiac muscle fibers with distinct cell borders and central oval\elongated nuclei (black arrow), and average myocardial blood vessel (blue arrow) (H&E X 400).

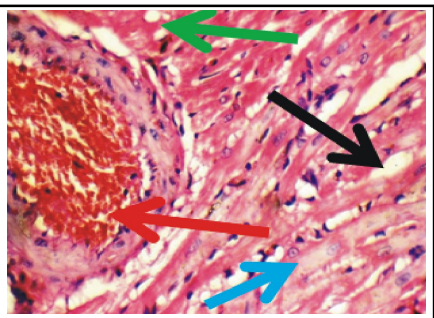

Fig. 5: (RES Group): high power view showing cardiac muscle fibers with indistinct cell borders (black arrow), pale cytoplasm (blue arrow), small pyknotic nuclei (yellow arrow) and intra-cytoplasmic vacuoles (green arrow) and mildly congested myocardial blood vessel (red arrow) (H&E X 400).

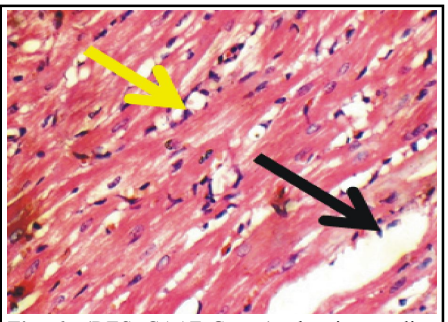

Fig. 6: (RES+CAAF Group): showing cardiac muscle fibers with indistinct cell borders (black arrow), and mild intra-cytoplasmic vacuoles (yellow arrow) (H&E X 400).

Fig. (4-6): Photomicrographs showing histopathological changes of the myocardium of heart in (depression model (RES) and treated (RES+ CAAF) compared to control group (NC).

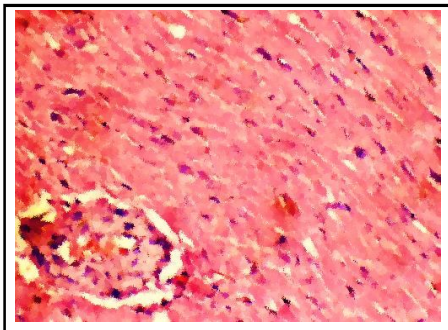

Fig. 7: (Control): high power view showing normal distribution of collagen fibers.

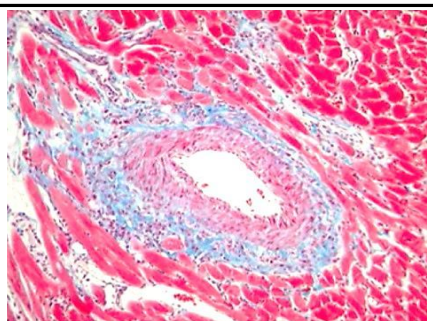

Fig. 8: (RES Group): high power view showing highly distribution of collagen bundles.

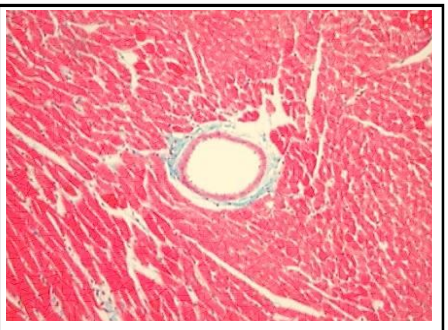

Fig. 9: (RES+CAAF Group): high power view showing somewhat normal appearance of distribution of collagen fibers.

Fig. (7-9): Photomicrographs showing distribution of collagen fibers in heart of (depression model (RES) and treated (RES+ CAAF) compared to control group (NC) (Masson's trichome).

Discussion

In the current study, we investigated the therapeutic effect of reserpine induced depression on caffeine in rats. Antihypertensive drugs, such as reserpine, are naturally extracted indole alkaloids, acting as a dopamine depletion agent, that inhibits several neurotransmitters (Yaffe *et al.,* 2018). Several studies suggested that the depletion of cerebral monoamines characterizes the main cause for the pathogenesis of depression (Dale *et al.,* 2015). Reserpine could deplete monoamines by inhibiting the activity of vesicular monoamine transporter-2 (VMAT-2) (Erickson *et al.,* 1992). This effect prevents the reuptake of monoamines into the synaptic vesicles exposing them to oxidative catabolism by the cytosolic enzyme monoamine oxidase (Antkiewicz-Michaluk *et al.,* 2014). This mechanism could explain the decreased monoamine levels reported in the present study. Concurrent results revealed significant decrease in dopamine and serotonin levels. These results are in agreement with Khadrawy *et al.,* 2018. They reported that as a consequence to the oxidative catabolism of cytosolic dopamine, norepinephrine and serotonin by monoamine oxidase, the cellular oxidant, hydrogen peroxide is produced (Youdim *et al.,* 2006). In addition, monoamines, especially dopamine and norepinephrine, can undergo spontaneous cytoplasmic oxidation leading to the destruction of cellular structures (Wasik *et al.,* 2009). These reactions produce several potentially neurotoxic byproducts, such as hydrogen peroxide (Barros-Miñones *et al.,* 2015). Hydrogen peroxide can result in the generation of reactive oxygen species and induce neuronal apoptosis due to mitochondrial damage (Bortolato *et al.,* 2008). Dopamine (DA) and serotonin (5-HT) are monoamine neurotransmitters in the brain. In the pathogenesis of depression, the DA and 5-HT hypotheses are recognized. Studies have also shown that the DA and 5HT levels in the hypothalamic tissue of patients with depression have decreased, indicating that depression is closely related to the low DA and 5-HT functions (Segura-Aguilar, 2014 and Li *et al.,* 2015).

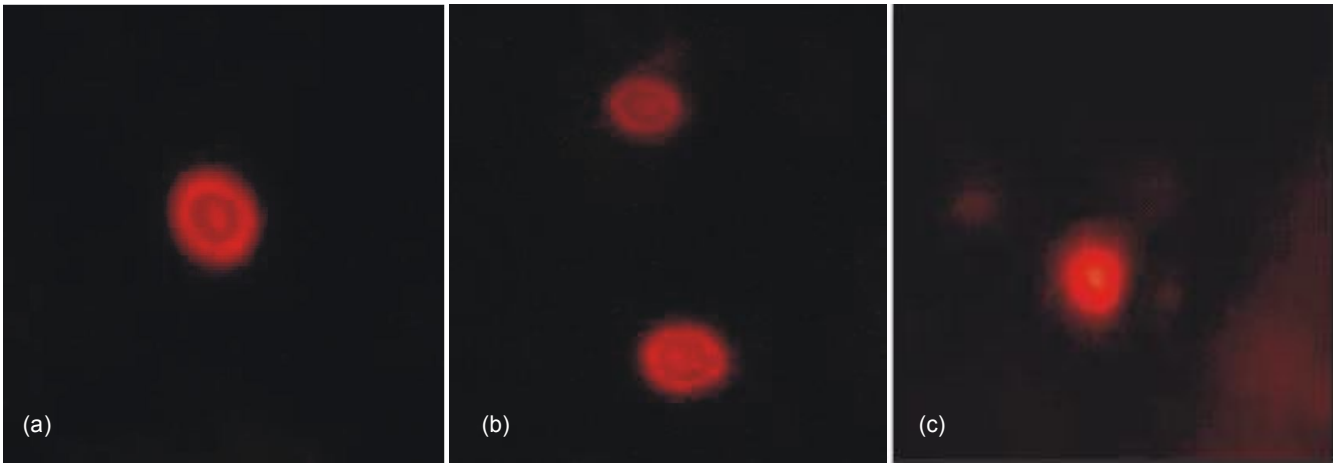

Photo: Photograph of fragments DNA migration pattern by comet assay evaluated with a fluorescence microscope for liver cells Fig. (a): control group showing intact cells; most of DNA is located in the head of the comet. Fig. (b): reserpine group showing tailed cells as a marker of DNA fragmentations; fragmented DNA migrated from the comet head and formed a tail. Fig. (c): reserpine rats group treated with caffeine, showing the intact DNA without migration.

The levels of DA and 5-HT in the brain tissue of the rat model of depression established in this study were significantly lower than those in normal rat, indicating that DA and 5-HT play an important role in the pathogenesis of depres-sion. After treatment with caffeine, the levels of DA and 5-HT of depressive rats were significantly increased.

In the recent study, reserpine administration produced significant increase in serum level of cortisol and when compared with the control group. This is another document for creating reserpinized animal as experimental model of depression. Depression is often associated with HPA axis hyperactivity, which is characterized by hypercortisolaemia in human (Watanabe *et al.,* 1992). Whereas hyperactivity of the HPA axis may be prevented by means of an inhibitory feedback mechanism as the dysregulation of this feedback mechanism appears to occur in depressive disorders (Young *et al.,* 1991).

The present findings revealed a state of oxidative stress after reserpine administration. This was evident from the significantly increased lipid peroxidation (MDA). Moreover, the observed increased MDA levels may arise from the attack of the neuronal membrane phospholipids by free radicals produced from monoamine catabolism. Malondialdehyde is produced from decomposition of products of lipid peroxidation (Gaweł *et al.,* 2004).

A common feature of depressive patient is the activation of inflammatory pathway resulting in elevated plasma levels of proinflammatory cytokines which subsequently induce acute phase proteins, such as CRP (Trongtorsak *et al.,* 2018). These inflammatory mediators are associated with risk and severity of depression (Schiepers *et al.,* 2005 and Pasco *et al.,* 2010). In our work, reserpine was able to induce inflammation as indicated by increases in plasma levels of TNF-α and CRP but, the concentration of IL-10 was reduced. CRP is an acute-phase protein of hepatic origin that increases following IL-6 secretion by macrophages and T cells. TNF-α is produced mainly by monocytes and macrophages and also by B-cells, T-cells and fibroblasts (Dayer *et al.,* 1985). The primary role of TNF-α is the regulation of immune cells and induction of apoptotic cell death and inflammation. TNF-α also stimulates mesenchymal cells, to release substances for tissue degradation like matrix metalloproteinase (MMP-1, 2, 3, 9, 13) that leads to the damage of the cartilage thereby increasing the production of super oxide radicals and prostaglandin (Cassim *et al.,* 2002). Currently, administration of reserpine increases the levels of inflammatory cytokines tumor necrosis factor (TNF-α) in brain tissue. Dowlati *et al.,* (2010) has demonstrated that peripheral rise in pro-inflammatory cytokines stimulated by chronic stress can cause depression through transmitting immune-mediated signals from the periphery to the CNS. Additionally, in the CNS, cytokines can also be generated by neural cells, microvessel endothelial cells, astrocytes and microglia in the brain and can induce inflammatory responses affecting neurotransmitter systems to ultimately affect neurocircuits regulating motivation and increased anxiety behaviors related to depression (Miller and Raison, 2016). In addition, cytokines can activate the (hypothalamic-pituitary-adrenal) HPA, promote the secretion of (corticotrophin releasing hormone) CORT and cause damage to neurons in the hippocampus.

Under treatment by (30 mg/kg caffeine for 21 days) treated groups showed decrease in TNF-α and CRP levels but increase IL-10 level. Several authors recorded that methylxanthine derivatives are non-selective adenosine antagonists responsible for inhibition of adenosine receptor leading to the inhibition of enzyme phosphodiesterase (PDE) that ultimately increases cAMP activates PKA thereby suppressing the production of proinflammatory cytokine TNF-α (Horrigan *et al.,* 2004). Caffeine is a methylxanthine derivative, it owns anti-inflammatory effects which inhibit the secretion of the TNF-α and IL-1β from stimulated monocytes and furthermore increases the production of anti-inflammatory IL-10 from lymphocytes (Van Furth *et al.,* 1995 and Shan *et al.,* 2015).

Acetylcholinesterase (AchE) is the enzyme responsible for catabolism of acetylcholine (Ach) to terminate its action. Therefore, the increased AchE activity observed in the present study may be attributed to the increased ACh, AchE substrate. Also, this increase in AchE may represent a compensatory mechanism to attenuate the increase in cholinergic activity that has been reported in depression. Charles *et al.,* (1994) found an increase in choline, the rate-limiting precursor in the synthesis of acetylcholine in the brains of depressed patients compared to normal controls. Higley and Picciottop, (2014) reported that the enhancement of ACh signaling can induce depressive symptoms in humans and animal models. Using human imaging, Saricicek *et al.,* (2012) suggested that the levels of ACh are increased in patients with active depression, as assessed by the occupation of nicotinic receptors all over the brain and remain elevated in patients with a history of depression. This may support the hypothesis relating adrenergic-cholinergic balance with depression and mania (Janowsky *et al.,* 1972). This theory suggests that elevated cholinergic tone and reduced noradrenergic tone may

cause depressive symptoms and has been supported by several lines of evidence from rodent and human studies (Mineur *et al.,* 2013).

The Na^+, K^+-ATPase (NKA) or Na^+ pump is found in the plasma membrane of practically all eukaryotic cells. This enzyme couples the energy released in the intracellular hydrolysis of ATP to the export of three intracellular Na+ ions and the import of two extracellular K^+ ions. Thus the Na^+, K^+-ATPase maintains the transmembrane ion balance needed to establish and control the membrane potential, *i.e*., it plays an important role in cell survival (Skou and Esmann, 1992). The Na^+ pump is a major cellular transport system that controls Na^+ homeostasis (O'Donnell and Owen, 1994) and membrane potential (Hermsmeyer and Erne, 1990), both key factors in the regulation of vascular tone and blood pressure (Marin and Redondo, 1999), in the present study, we found that as a result of induction of depression by reserpine; the activity of Na^+, K^+-ATPase enzyme in cardiac tissue was reduced. Moreover, recent evidence directly indicates that reduced NKA level may induce cardiomyocyte death and cardiac dysfunction (Liu *et al*., 2012). Also, decreased NKA activity and expression have long been associated with heart failure in both animal models and human patients (Ishino *et al.,* 1999 and Hua *et al.,* 2018).

The histopathological study of structure and chemical composition of tissues of animals are linked to their role. The primary aim is to understand how tissues are ordered at all structural levels, including the molecular and macromolecular, the entire cell and intercellular substances and the tissues and organs. Compared to other organ systems, the nervous system is often referred to as the most sensitive to reserpine. Cerebral cortex is a sheet of neural tissue which is the outer-most part of the cerebrum of a mammalian brain. Its key roles are in memory, attention, perceptual awareness, thought, language and consciousness (Notor *et al.,* 2001). From the histopathological results in this current study, the brain of the depressed rats showed alterations in the histopathological structure of brain, with slight cortical edema, scattered degenerated neurons and diffuse gliosis. While the degree of degeneration was reduced after treatment with caffeine. These findings were in agreement with the reports of Park *et al.,* (2018) stated that an overproduction of proinflammatory cytokines in depressive rats can impair neuronal structure and function, leading to deficits in neuroplasticity (Calabrese *et al.,* 2014).

Microscopic examination of cardiac muscles of rats given reserpine showed that cardiac muscle fibers with indistinct cell borders, pale cytoplasm, small pyknotic nuclei and intra-cytoplasmic vacuoles and mildly congested myocardial blood vessels. These findings are in agreement with those of (Roest *et al.,* 2010; Colquhoun *et al.,* 2013 and Kessler *et al.,* 2005) who stated that depression may also be an independent risk factor for heart health problems. Furthermore, they informed that depression affects recovery and increases the risk of further heart-related incidents, such as another heart attack and slower recovery from heart surgery.

Conclusion

The current data shed light on the therapeutical effect of caffeine. This effect was evidenced by the activation of serotoninergic and dopaminergic system, inhibition of cortisol levels, suppression of proinflammatory cytokine markers in the brain.

References

Antkiewicz-Michaluk, L., A. Wąsik, E. Możdżeñ, I. Romañska and J. Michaluk (2014). Antidepressant-like effect of tetrahydroisoquinoline amines in the animal model of depressive disorder induced by repeated administration of a low dose of reserpine: behavioral and neurochemical studies in the rat. *Neurotox Res*., **26:** 85-98.

Ardais, A.P., M.F. Borges, A.S. Rocha, C.R. Sallaberry, A. Cunha and L.O. Porciúncula (2014). Caffeine triggers behavioral and neurochemical alterations in adolescent rats. *Neuroscience*., **270:** 27-39.

Bancroft, J.D. and M. Gamble (2008). Theory and practice of histological techniques, 6th edn.

Bortolato, M., K. Chen and J.C. Shih (2008). Monoamine oxidase inactivation: from pathophysiology to therapeutics. *Adv. Drug. Deliv. Rev.*, **60:** 1527-1533.

Barros-Miñones, L., B. Goñi-Allo, V. Suquia, G. Beitia, N. Aguirre, and E. Puerta (2015). Churchill Livingstone, Philadelphia. Contribution of dopamine to mitochondrial complex I inhibition and dopaminergic deficits caused by methylenedioxymethamphetamine in mice. *Neuropharmacology*., **93:** 124-133

Colquhoun, D.M., S.J. Bunker and D.M. Clarke *et al*., (2013). Screening, referral and treatment for depression in patients with coronary heart disease. A consensus statement from the National Heart. Foundation of Australia. *Med. J. Aust.,* **198 (9):** 483-484.

Calabrese, F., A.C. Rossetti, G. Racagni, P.M. Gass, A. Riva and R. Molteni (2014). Brain-derived neurotrophic factor: a bridge between inflammation and neuroplasticity," *Frontiers in Cellular Neuroscience*, **vol. 8, article 430**.

Cardiff, R.D., C.H. Miller and R.J. Munn (2014). Manual hematoxylin and eosin staining of mouse tissue sections. *Cold Spring Harb. Protoc.,* **2014:** 655-8.

Caspi, A., K. Sugden, T.E. Moffitt, A. Taylor, I.W. Craig and H.

Harrington *et al*., (2003). Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science*., **301(5631):** 386-9.

Cassim, B., G. Mody and K. Bhoola (2002). Kallikrein cascade and cytokines in inflamed joints. *Pharmacol. Ther*., **94(1, Suppl2):** 1-34.

Charles, H.C., F. Lazeyras, K.R. Krishnan, O.B. Boyko, M. Payne and D. Moore (1994). Brain choline in depression: *in vivo* detection of potential pharmacodynamics effects of antidepressant therapy using hydrogen localized spectroscopy. Prog. Neuropsychopharmacol. *Biol Psychiatry.,* **18:** 1121-1127.

Chan, Y.H. (2003). Biostatistics 102: Quantitative Data-Parametric & Non-parametric Tests. *Singapore Med. J*., **44(8):** 391-396.

Chiu, Y.T., S.K. Liu, M. Liu, S.P. Chen, Y.H. Lin, S.J. Mao and R. Chu (1999). Characterization and quantitation of extracellular collagen matrix in myocardium of pigs with spontaneously occurring hypertrophic cardiomyopathy. *Cardiovasc Pathol*., **8:** 169-175.

Ciarlone, A.E. (1978). Further modification of a fluoromertric method for analyzing brain amines. *Microchem Journal.*, **23:** 9-12.

Cui, J., L. Jiang and H. Xiang (2012). Ginsenoside Rb3 exerts antidepres-sant-like effects in several animal models. *J. Psychopharma-cology*., **26(5):** 697-713.

Dale, E., B. Bang-Andersen and C. Sánchez (2015). Emerging mechanisms and treatments for depression beyond SSRIs and SNRIs. *Biochem. Pharmacol*., **95:** 81-97.

Dayer, J.M., B. Beutler and A. Cerami (1985). Cachectin/tumor necrosis factor stimulates collagenase and prostaglandin E2 production by human synovial cells and dermal fibroblasts. *J. Exp. Med.,* **162(6):** 2163-8.

De-Boeck, M., N. Touil, G. De Visscher, P.A. Vande and M. Kirsch-Volders (2000). Validation and implementation of an internal standard in comet assay analysis. *Mutat. Res*., **469:** 181-197.

Dowlati, Y. *et al*., (2010). A meta-analysis of cytokines in major depression. *Biological psychiatry*., **67:** 446-457.

Dunn, E.C., R.C. Brown, Y. Dai, J. Rosand, N.R. Nugent and A.B. Amstadter *et al*., (2015). Genetic determinants of depression: recent findings and future directions. *Harvard Review of Psychiatry*., **23(1):** 1-18.

Erickson, J.D., L.E. Eiden and B.J. Hoffman (1992). Expression cloning of a reserpine sensitive vesicular monoamine transporter. *Proc. Natl. Acad. Sci. U.S.A*., **89:** 10993-10997.

Gao, Z.Y., P. Yang, Q.J. Huang and H.Y. Xu (2016). The influence of dizocilpine on the reserpine-induced behavioral and neurobiological changes in rats. *Neuroscience Letters*., **614:** 89-94.

Gilbert, P. (2017). Depression : the evolution of powerlessness, Routledge, Taylor & Francis Group, London, UK, Classic edition.

Guo, Z., X. Liu and H. Huang (2015). Kinetics and Thermodynamics of Reser-pine Adsorption onto Strong Acidic Cationic Exchange Fiber. *PLoS ONE*., **10(9):** e0138619.

Greenwood, R., J. Zhao, B. Ludrick, T. Golden, N.A. Wu1 (2018). Practical animal model for depression by reserpine-administered mice. *Theranostics Brain Disord*., **3(2):** 555609.

Gallagher, M., R. Burwell and M. Burchinal (1993). Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze, *Behav Neurosci*., **107:** 618-626.

Garrett, B.E. and R.R. Griffiths (1997). The role of dopamine in the behavioral effects of caffeine in animals and humans. *Pharmacol Biochem Behav*., **53:** 533-541.

Gaweł, S., M. Wardas, E. Niedworok and P. Wardas (2004). Malondialdehyde (MDA) as a lipid peroxidation marker. *Wiad Lek*., **57:** 453-455.

Hua, F., Z. Wu, X. Yan, J. Zheng, H. Sun, C. Xu and J.S. Bian (2018). DR region of Na+-K+-ATPase is a new target to protect heart against oxidative injury. *Scientific Reports*., **8:** 13100.

Hanff, T.C., S.J. Furst and T.R. Minor (2010). Biochemical and anatomical sub-strates of depression and sickness behavior. *Isr. J. Psychiatry. Relat. Sci*., **47(1):** 64-71.

Higley, M.J. and M.R. Picciotto (2014). Neuromodulation by acetylcholine: examples from schizophrenia and depression. *Curr. Opin. Neurobiol*., **29:** 88-95.

Holt Mary, C. (1961). Methoserpidine: A Non-Depressant Isomer of Re-serpine. *The British Medical J*., **2(5249):** 415-418.

Horrigan, L.A., J.P. Kelly and T.J. Connor (2004). Caffeine suppresses TNF-α production via activation of the cyclic AMP/protein kinase a pathway. *Int. Immunopharmacol*., **4(10, Suppl 11):** 1409-17.

Hyman, S.E. (2010). The genetics of mental illness: implications for practice. Bulletin of the World Health Organization. **78(4):** 455-63.

Ellman, G.L., K.D. Courtney and V. Jr. andres (1961). Feather Stone RM A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol*., **7:** 88 95.

El-Yacoubi, M., C. Ledent, M. Parmentier, R. Bertorelli, E. Ongini, J. Costentin and J.M. Vaugeois (2001). Adenosine A2A receptor antagonists are potential antidepressants: evidence based on pharmacology and A2A receptor knockout mice. *Br. J. Pharmacol*., **134:** 68-77.

Ferré, S. (2016). Mechanisms of the psychostimulant effects of caffeine : implications for substance use disorder. *Psychopharmacology.,* **233(10):** 1963-1979.

Fisone, G., A. Borgkvist and A. Usiello (2004). Caffeine as a psychomotor stimulant: mechanism of action. *Cell. Mol. Life Sci*., **61:** 857-872.

Fredholm, B.B., K. Bättig, J. Holmén, A. Nehlig and E.E. Zvartau

(1999). Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol. Rev*., **51:** 83-133.

Ishino, K., H.E. Botker, T. Clausen, R. Hetzer and J. Sehested (1999). Myocardial adenine nucleotides, glycogen and Na, K-ATPase in patients with idiopathic dilated cardiomyopathy requiring mechanical circulatory support. *Am. J. Cardiol.,* **83:** 396-399.

Janowsky, D.S., M.K. El-Yousef, J.M. Davis and H.J. Sekerke (1972). A cholinergic-adrenergic hypothesis of mania and depression. *Lancet*., **2:** 632-635.

Juel, C., N.B. Nordsborg and J. Bangsbo (2013). Exercise-induced increase in maximal *in vitro* Na, K-ATPase activity in human skeletal muscle. *Am. J. Physiol. Integr. Comp. Physiol*., **304:** R1161-R1165.

Khadrawy, Y.A., H.G. Sawie, E.N. Hosny and H.H. Mourad (2018). Assessment of the antidepressant effect of caffeine using rat model of depression induced by reserpine. *Bulletin of the National Research Centre*., **42:** 36.

Leith, N.J. and R.J. Barrett (1980). Effects of chronic amphetamine or reser-pine on self-stimulation responding: *Animal model of depression? Psy-chopharmacology*., **72(1):** 9-15.

Li, X., Y. Fan, S. Xiao, S. Peng, X. Dong, X. Zheng, C.C. Liu, H. Li and Z. Xiao (2015). Decreased platelet 5-hydroxytryptamin (5-HT) levels: A response to antidepressants. *J. Affect Disord.*, **187:** 84-90.

Liu, C. *et al*., (2012). Reduction of Na/K-ATPase potentiates marinobufagenin-induced cardiac dysfunction and myocyte apoptosis. *J. Biol. Chem.*, **287:** 16390-16398.

Mathers, C.D. and D. Loncar (2006). Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med*., **3:** e442.

Maes, M., P. Galecki, Y.S. Chang and M. Berk (2011). A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro) degenerative processes in that illness. *Prog Neuro-Psychopharmacol Biol Psychiatry*., **35:** 676-692.

Marin, J. and J. Redondo (1999). Vascular sodium pump: endothelial modulation and alterations in some pathological processes and aging. *Pharmacol. Ther*., **84:** 249-271.

Miller, A.H. and C.L. Raison (2016). The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nature reviews. *Immunology*., **16:** 22-34.

Mineur, Y.S., A. Obayemi, M.B. Wigestrand, G.M. Fote, C.A. Calarco, A.M. Li and M.R. Picciotto (2013). Cholinergic signaling in the hippocampus regulates social stress resilience and anxiety- and depression-like behavior. *Proc. Natl. Acad. Sci. USA*., **110:** 3573-3578.

Moore, D.M., B.F. Feldman, J.G. Zinkl and N.C. Jain editors (2000). Hematology of rabbits. Philadelphia, USA: Lippincott Williams and Wilkins.

Notor, S.C. *et al*., (2001). Neuron derived from radial glial cells establish radial units in neocortex. *Nature.,* **409(6821):** 714-720.

Kessler, R.C., W.T. Chiu, O. Demler, K.R. Merikangas and E.E. Walters (2005 june). prevalence, severity and comorbidity of 12-month DsM-Iv disorders in the National Comorbidity survey Replication. *Arch. Gen. Psychiatry*., **62(6):** 617-27.

Kuzmin, A., B. Johansson, L. Gimenez, S. Ögren and B.B. Fredholm (2006). Combination of adenosine A1 and A2A receptor blocking agents induces caffeine-like locomotor stimulation in mice. *Eur. Neuropsychopharmacol*., **6:** 129-136

O'Donnell, M. and N.E. Owen (1994). Regulation of ion pumps and carriers in vascular smooth muscle. *Physiol. Rev*., **74:** 683-721.

Ohkawa, H., N. Ohishi and K. Yagi (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.,* **95:** 351-8.

Ozerov, A.A., V.V. Bagmetova, V. Chernysheva Yu and I.N. Tyurenkov (2016). Comparison of the Efficiency of Adeprophen and Antidepressants of Various Groups on the Model of Reserpine- Induced Depression in Rats. *Bull Exp Biol Med*., **160(5):** 649-652.

Park, B.K., Y.R. Kim, Y.H. Kim, C. Yang, C.S. Seo, I.C. Jung, I.S. Jang, S.H. Kim and M.Y. Lee (2018). Antidepressant-Like Effects of Gyejibokryeong-hwan in a Mouse Model of Reserpine-Induced Depression. *Bio. Med. Research International*., **2018:** 12.

Pasco, J.A., G.C. Nicholson, L.J. Williams, F.N. Jacka, M.J. Henry and M.A. Kotowicz *et al*., (2010). Association of high-sensitivity C-reactive protein with de novo major depression. *Br. J. Psychiatry*., **197:** 372-7.

Reddy, M. (2010). Depression: the disorder and the burden," *Indian Journal of Psychological Medicine*., **32(1):** 1-2.

Roest, A.M., E.J. Martens and P. de Jonge *et al*., (2010). Anxiety and risk of incident coronary heart disease. A meta-analysis. *J. Am. Coll. Cardiol.,* **56:** 38-46.

Saricicek, A., I. Esterlis, K.H. Maloney, Y.S. Mineur, B.M. Ruf, A. Muralidharan, J.I. Chen, K.P. Cosgrove, R. Kerestes, S. Ghose, C.A. Tamminga, B. Pittman, F. Bois, G. Tamagnan, J. Seibyl, M.R. Picciotto, J.K. Staley, Z. Bhagwagar (2012). Persistent β2*-nicotinic acetylcholinergic receptor dysfunction in major depressive disorder. *Am. J. Psychiatry*., **169:** 851-859.

Sasaki, Y.F., S. Tsuda, F. Izumiyama and E. Nishidate (1997). Detection of chemically induced DNA lesions in multiple mouse organs using the alkaline single cell gel electrophoresis (Comet assay). *Mutation research*., **388:** 33-44.

Schiepers, O.J., M.C. Wichers and M. Maes (2005). Cytokines and major depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry.,* **29:** 201-17.

Segura-Aguilar, J., I. Paris, P. Muñoz, E. Ferrari, L. Zecca and F.A. Zucca (2014). Protective and toxic roles of dopamine

in Parkinson's disease. *J. Neurochem.*, **129:** 898-915.

Shan, K.M., P. Dadhanya and V.R. Trivedi (2015). Effect of caffeine in experimental model of rheumatoid arthritis in rats. *Int. J. Pharm. Sci.*, **7(3):** 364-367.

Singh, N.P., M.T. McCoy, R.R. Tice and E.L. Schneider (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell. Res.*, **175:** 184-191.

Slaoui, M. and L. Fiette (2011). Histopathology procedures: From tissue sampling to histopathological evaluation. *Methods Mol. Biol.,* **691:** 69-82.

Skou, J.C. and M. Esmann (1992). The Na, K-ATPase. *J. Bioenerg. Biomembr.*, **24:** 249-261.

Schechter, L.E., R.H. Ring, C.E. Bayer, Z.A. Haghes, X. Khawaja, J.E. Malberg and S. Rosenzweig-Lipson (2005). Innovative approaches for the development of antidepressant drugs: current and future strategies. *J. Am. Soc. Exp. Neurotherap.*, **2:** 590-611.

Taylor, P.C. (2001). *Mol. Biotechnol.*, **19, 2:** 153-68.

Trongtorsak, P., W. Olankijanunt, A. Trongtorsak and U. Intamaso (2018). Antidepressant and anti-inflammatory effects of a combined fluoxetine and celecoxib treatment in a rat model of depression. *Chula. Med. J.,* **62(4):** 653-65

Van Furth, A.M., E.M. Seijmonsbergen, J.A. Langermans, P.H. van der Meide and R. van Furth (1995). Effect of xanthine derivates and dexamethasone on Streptococcus pneumoniae-stimulated production of tumor necrosis factor alpha, interleukin-1 beta (IL-1 β) and IL-10 by human leukocytes. *Clin. Diagn. Lab. Immunol.*, **2(6):** 689-92.

Watanabe, Y., E. Gould and B.S. McEwen (19920.) Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. *Brain Res.*, **588:** 341-345.

Wasik, A., I. Romañska and L. Antkiewicz-Michaluk (2009). 1-Benzyl-1,2,3,4- tetrahydroisoquinoline, an endogenous parkinsonism-inducing toxin, strongly potentiates MAO-dependent dopamine oxidation and impairs dopamine release: *ex vivo* and *in vivo* neurochemical studies. *Neurotox. Res.*, **15:** 15-23.

Williams, M. (1987). Purine receptors in mammalian tissues: pharmacology and functional significance. *Annu Rev Pharmacol Toxicol.*, **27:** 315-345.

Yaffe, D., L.R. Forrest and S. Schuldiner (2018). The ins and outs of vesicular monoamine transporters. *The J. General Physiology.*, **150(5):** 671-82.

Yamada, K., M. Kobayashi and T. Kanda (2014). Involvement of adenosine A2A receptors in depression and anxiety. *Int. Rev. Neurobiol.*, **119:** 373-393.

Youdim, M.B., D. Edmondson and K.F. Tipton (2006). The therapeutic potential of monoamine oxidase inhibitors. *Nat. Rev. Neurosci.*, **7:** 295-309.

Young, E.A., R.F. Haskett, V. Murphy-Weinberg, S.J. Watson and H. Akil (1991.) Arch. Loss of glucocorticoid fast feedback in depression. *Gen.Psychiatry.*, **48(8):** 693- 699.