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Vol. 7(46), pp. 2960-2971, 15 December, 2013
DOI: 10.5897/AJPP2013.3928
ISSN 1996-0816 © 2013 Academic Journals
http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Role of serotonergic and dopaminergic neurotransmission in the antidepressant effects of malt extract

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Accepted 11 December, 2013

The benefit of malt extract in clonidine-induced depression was previously reported. The present study aimed to explore its mechanism of action. Animals were classified into normal and depressed rats. Induction of depression was done by i.p. injection of clonidine (0.8 mg/kg) daily for 7 days. Depressed rats were sub-classified into 6 groups treated for one week as follows: Group I received 1% tween 80 p.o. (control group); the remaining groups received malt extract (1250 mg/kg; p.o) alone or preceded (30 min) by i.p. injection of spiperone (0.03 mg/kg), sulpiride (7.5 mg/kg), phentolamine (5 mg/kg) or propranolol (7.5 mg/kg), respectively. Forced swimming test (FST) was carried out 24 h thereafter. Brains were isolated for estimation of serotonin, dopamine and norepinephrine contents as well as inflammatory and oxidative stress biomarkers. Clonidine increased total immobility time and decreased struggling time in FST parallel to alterations in brain neurotransmitters, inflammatory and oxidative stress markers. Treatment with malt extract reversed clonidine-induced behavioral and neurochemical changes. Such effects were partly antagonized in groups pre-treated with spiperone or sulpiride. Serotoninergic and dopaminergic transmission are involved in the antidepressant effects of malt extract in addition to its antioxidant and anti-inflammatory effects.

Key words: Depression, malt extract, spiperone, sulpiride, neurotransmitters, cytokines.

INTRODUCTION

Depression is a common prevalent and major disorder nowadays, often associated with psychological, behavioral and physiological symptoms (Cryan et al., 2002). It is characterized by altered mood and intellectual functions associated with frequent thoughts of death or suicide (Paykel, 2006). Pathogenesis of depression is thought to be related to deficiency in serotonin (5-HT) and/or norepinephrine (NE) availability (Rampello et al., 2000) accompanied by induction of oxidative/nitrosative stress pathways and reduced antioxidant status (Ng et al., 2008; Chung et al., 2012; Aboul-Fotouh, 2013). Moreover, patients with major depressive disorders (MDD) have been consistently shown to have altered levels of pro- and anti-inflammatory cytokines in circulation (Miller et al., 2009; Dowlati et al., 2010; Janelidze et al., 2011). The increasing burden of MDD makes the

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search for additional treatments highly significant. The use of herbal medicine in psychiatric disorders has increased immensely in the recent years owing to their efficiency and lower side effects as compared to traditional drugs (Beaubrun and Gray, 2000).

Malt extract is prepared from barley grains (*Hordeum vulgare* L). It is rich in maltose, dextrin, glucose as well as other carbohydrates and proteins (Qi et al., 2005). The antioxidant effects of hordein, the main storage protein fraction in barley seeds (Qi et al., 2005) have been reported (Bamdad and Chen, 2013). Malt extract contains several other antioxidants including proanthocyanidins, carotenoids, tocopherols and melatonin (Goupy et al., 1999; Badria, 2002; Bonoli et al., 2004; Qingming et al., 2010). Interest in barley products increased because of their high content of phenolic antioxidants that can reduce the risk of coronary heart disease, cancer and the aging processes (Qingming et al., 2010; Omwamba et al., 2013). In addition, beneficial effects of barley grains were shown in diabetes and hypercholesterolemia (Ikegami et al., 1996; Hong and Maeng, 2004).

Behavioral depression induced by clonidine has been suggested to be a suitable animal model for depression disorder (Parale and Kulkarni, 1986; Enginar and Eroglu, 1990). In a previous investigation (Rizk et al., 2009), malt extract was shown to be effective in treatment of clonidine-induced depression. However, the exact mechanism of antidepressant effects of malt extract remains unclear.

The present study was designed to explore the possible role of serotonergic, adrenergic and dopaminergic mechanisms in the antidepressant effect of malt extract using adrenergic, serotonergic and dopaminergic receptor antagonists. Moreover, the study aimed to inspect the role of oxidative stress and inflammatory cytokines in the pathophysiology of depression as well as the action of malt extract.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 120 ± 10 g were used in the present experiments. They were purchased from National Cancer Institute (Cairo, Egypt) and kept in the animal house of the faculty of Pharmacy, Cairo University at a temperature of 25 ± 1°C, humidity of 60 ± 5% and natural lighting conditions (12 h light and 12 h dark cycles). The study was carried according to the guidelines of Ethics Committee, Faculty of Pharmacy, Cairo University.

Chemicals

Malt extract was obtained from Marine Chemicals, India. Clonidine, spiperone, sulpiride, phentolamine and propranolol were got from Sigma Chemical Company, USA. Each agent was suspended in 1% tween 80 prior to administration. Kits for determination of tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and interleukin-10 (IL-10) were purchased from R & D Systems, USA. All other used chemicals were of analytical grade.

Experimental design

Animals were classified into two main groups: normal (n = 8) and depressed rats (n = 48). Depression was induced by i.p. injection of clonidine (0.8 mg/kg) daily for 7 successive days (Enginar and Eroglu, 1990); meanwhile the normal control group received saline i.p. for the same period. Thereafter, depressed rats were sub-classified into 6 groups each consisted of 8 rats. The 1st group received 1% tween 80 p.o. for 7 days (depressed control); whereas the remaining 5 groups were orally treated for one week with malt extract (1250 mg/kg, p.o.; Rizk et al., 2009) alone or preceded (30 min) by i.p. injection of spiperone (0.03 mg/kg), sulpiride (7.5 mg/kg), phentolamine (5 mg/kg) or propranolol (7.5 mg/kg), respectively (Khalifa, 2003). During the same 7 days, normal control group received 1% tween 80, p.o. Forced swimming test (FST) was carried out 24 h after the last treatment. One hour later, animals were sacrificed; brains were isolated and homogenized to be used for biochemical measurements.

Forced swimming test

The test was carried out in a swimming pool specified according to Porsolt et al. (1978) consisting of a plexiglass cylinder of 40 cm height and 22 cm diameter with a rounded lid. The tank was filled up to 25 cm with tap water thermostatically controlled at 25°C (Cryan et al., 2002). FST is based on the fact that rats develop an immobile posture when placed in an inescapable chamber of water; accordingly rats were placed into the chamber and left for 6 min (Vergoni et al., 1995) during which their behavior was videotaped. The evaluated parameters included latency to first immobility; total immobility time and struggling time. Immobility indicates the floating of rat with its head above the water surface without making vigorous movements (Lozano–Hernández et al., 2010). Struggling time is defined as the time in seconds during which the animal tries to jump out of the tank, climb the walls or dive into the tank (Einat et al., 1999).

Tissue sampling

Rats were decapitated and their brains were carefully removed. The forebrain was dissected through the mid line into two hemispheres and each one was weighed. One of the hemispheres was homogenized in 50 mM phosphate buffer (pH 7.4) to prepare 10% homogenate that was used for the assessment of brain contents of lipid peroxides, reduced glutathione (GSH), nitric oxide (NO), TNF-α, IL-1β, IL-6 and IL-10. The other hemisphere was homogenized in ice cold 70% methanol for determination of brain contents of NE, dopamine (DA) and 5-HT.

Determination of brain lipid peroxides

Brain lipid peroxides were determined according to the method of Mihara and Uchiyama (1978) using malondialdehyde (MDA) as a standard and expressed as nmol/g wet tissue.

Determination of brain reduced glutathione

Brain content of GSH was determined using Ellman's reagent according to the method described by Ahmed et al. (1991) and expressed as mg/g wet tissue.

Determination of brain nitric oxide

Brain NO content was determined as total nitrate/nitrite (NO_x) using Griess reagent according to the method described by Miranda et al. (2001) and expressed as µmol/g wet tissue.

Determination of brain cytokines

Brain contents of TNF-α, IL-1β, IL-6 and IL-10 were estimated by enzyme linked immunosorbent assay (ELISA) using rat specific kits (Quantikine; R & D Systems, USA) and expressed as pg/g wet tissue.

Determination of brain norepinephrine, dopamine and serotonin

Brain homogenates were centrifuged for 15 min at 5,000 rpm. The supernatant was used for assessment of brain contents of NE, DA and 5-HT by high performance liquid chromatography (HPLC) as described by Pagel et al. (2000) and they were expressed as µg/g wet tissue.

Statistical analysis

All the values are expressed as means ± standard error (SE). Comparisons between different groups were carried out using the one-way analysis of variance (ANOVA) followed by least significant difference (LSD) multiple comparisons test. Difference was considered significant when *p* was less than 0.05. SPSS (version 11) program was used to carry out these statistical tests.

RESULTS

Effect of pretreatment with spiperone, phentolamine, propranolol or sulpiride on the behavior of depressed rats treated with malt extract in forced swimming test

Data of the present study revealed that clonidine significantly decreased latency to first immobility of rats by 45.83% as compared with normal group (50.00 ± 3.16 s) while treatment of depressed rats with malt extract increased the time to first immobility by 63.33% as compared with that of control depressed rats (Figure 1A). The effect of malt extract was not changed by its combination with phentolamine, propranolol or sulpiride. On the other hand, combination of malt extract with spiperone antagonized its effect on latency time (Figure 1A).

Clonidine increased the immobility time by 24.62% when compared to that of normal control rats (158.66 ± 0.84 s). Treatment of depressed rats with malt extract reduced clonidine-induced effect by 33.57% to be (139.83 ± 7.64 s). Administration of phentolamine, propranolol or sulpiride 30 min before malt extract partially antagonized the effects of the latter, by 22, 18 and 35%, respectively. Moreover, spiperone completely abolished the effect of malt extract on immobility time (Figure 1B).

Clonidine reduced struggling time of the normal control rats (141.33 ± 0.84 s) by 37%. Treatment of depressed rats with malt extract increased the struggling time by 79% to be (160.16 ± 7.64 s) when compared to control depressed group. Pretreatment of depressed rats with spiperone, phentolamine, propranolol or sulpiride, 30 min before administration of malt extract, decreased struggling time by 37, 19, 16 and 30%, respectively as compared to the group receiving malt extract alone (Figure 1C).

Effect of pretreatment with spiperone, phentolamine, propranolol or sulpiride on brain neurotransmitters of depressed rats treated with malt extract

In clonidine-induced depressed rats, brain 5-HT content decreased by 14% when compared with normal control rats (0.21 ± 0.01 µg/g wet tissue). Treatment of depressed rats with malt extract normalized 5-HT content. Malt extract effects were not changed in groups pretreated with propranolol or sulpiride. On the other hand, prior treatment with spiperone or phentolamine prevented malt extract-induced changes where 5-HT contents in both groups were comparable to that of control depressed group (Figure 2A). Similarly, brain NE content decreased by 19% to be (0.91 ± 0.03 µg/g wet tissue) in depressed rats when compared to that of normal control group (1.07 ± 0.02 µg/g wet tissue). Treatment of depressed rats with malt extract increased NE content by 7% (to be 0.97 ± 0.04 µg/g wet tissue) as compared with control depressed rats. Prior administration of spiperone or phentolamine in malt-treated depressed rats abolished such effect; meanwhile prior administration of sulpiride potentiated malt extract effect by 7%. Propranolol, on the other hand, did not alter the effect of malt extract on the measured parameter (Figure 2B). In addition, there was no significant change in the brain DA content of depressed rats as compared with the normal group (Figure 2C). Similarly, all the used treatments did not affect this parameter except the group treated with malt extract and sulpiride where DA content was increased by 4 and 5% as compared to control depressed and malt extract-treated rats, respectively (Figure 2C).

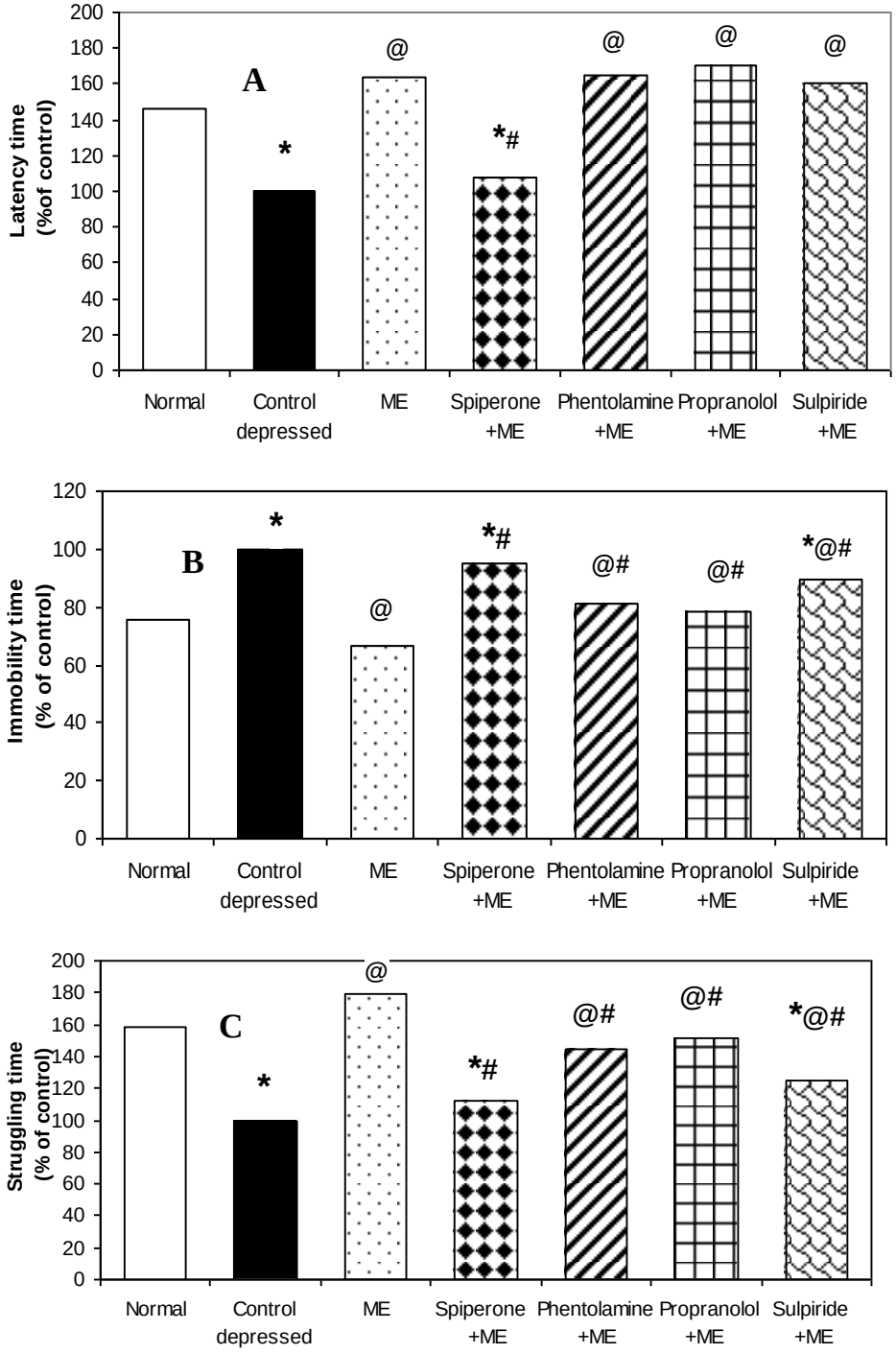

Figure 1. Effect of pretreatment with spiperone (0.03 mg/kg; i.p.), phentolamine (5 mg/kg; i.p.), propranolol (7.5 mg/kg; i.p.) or sulpiride (7.5 mg/kg; i.p.) on: A. Latency time, B. Immobility time, and C. Struggling time of depressed rats treated with malt extract (ME; 1250 mg/kg; p.o.) in forced swimming test (FST). Depression was induced by daily i.p. injection of clonidine (0.8 mg/kg) for 7 successive days. Statistical analysis was carried out by one way ANOVA followed by LSD test.Values represent mean ± SE (n= 6 to 8 rats).*Significantly different from normal control group at p <0.05. $^{@}$Significantly different from control depressed group at p < 0.05.$^{\#}$Significantly different from ME-treated group at p < 0.05.

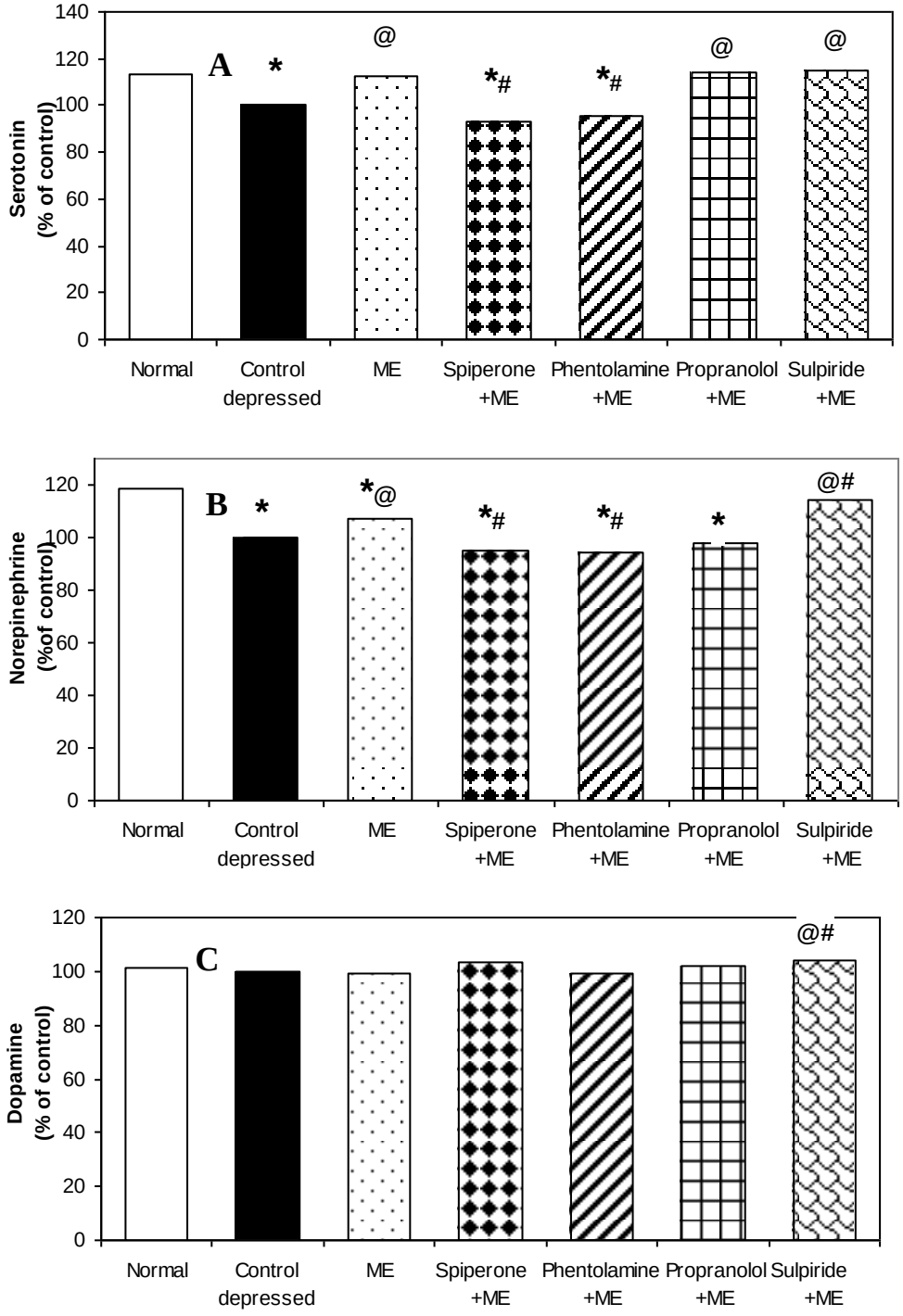

Figure 2. Effect of pretreatment with spiperone (0.03 mg/kg; i.p.), phentolamine (5 mg/kg; i.p.), propranolol (7.5 mg/kg; i.p.) or sulpiride (7.5 mg/kg; i.p.) on brain contents of: A. Serotonin, B. Norepinephrine, and C. Dopamine in depressed rats treated with malt extract (ME; 1250 mg/kg; p.o.). Depression was induced by daily i.p. injection of clonidine (0.8 mg/kg) for 7 successive days. Statistical analysis was carried out by one way ANOVA followed by LSD test. Values represent mean ± SE (n= 6-8 rats).*Significantly different from normal control group at p <0.05. [@]Significantly different from control depressed group at $p < 0.05$.
[#]Significantly different from ME-treated group at $p < 0.05$.

Effect of pretreatment with spiperone, phentolamine, propranolol or sulpiride on brain malondialdehyde, reduced glutathione and total nitrate/nitrite contents of depressed rats treated with malt extract

Induction of depression in rats resulted in an increase in MDA brain content by 60% (to reach 88.17 ± 4.74 nmol/g wet tissue) as compared with normal rats (34.96 ± 2.17 nmol/g wet tissue). Treatment of depressed rats with malt extract reduced clonidine-induced increase in MDA by 42% to be (50.55 ± 2.86 nmol/g wet tissue). Prior administration of spiperone antagonized the effect of malt extract on brain MDA content. This did not occur in groups pretreated with phentolamine or propranolol. Moreover, sulpiride administration before malt extract increased the effect of the latter by 28% (Figure 3A). GSH brain content of normal rats (8.14 ± 0.61 mg/g wet tissue) was reduced by 59% after induction of depression to be (3.31 ± 0.27 mg/g wet tissue). Treatment of depressed rats with malt extract increased this content by 82% as compared with control depressed rats. Spiperone and phentolamine did not significantly change malt effect on brain GSH; meanwhile, propranolol and sulpiride potentiated such effect leading to normalization of GSH content (Figure 3B). Depression increased NO_x content (744.41 ± 31.72 µmol/g wet tissue) by 40% when compared to normal rats (441.44 ± 22.74 µmol/g wet tissue). Treatment with malt extract decreased the elevated NO_x content by 47% (to be 394.52 ± 29.29 µmol/g wet tissue) as compared with control depressed rats. Prior administration of phentolamine, propranolol or sulpiride in malt-treated rats did not alter the effect of malt extract on the NO_x brain content. However, pretreatment with spiperone reduced malt effects by 48% (Figure 3C).

Effect of pretreatment with spiperone, phentolamine, propranolol or sulpiride on brain tumor necrosis factor-alpha, interleukin-1beta and interleukin-6 contents of depressed rats treated with malt extract

Induction of depression by clonidine caused no significant change in brain content of TNF-α (354.12 ± 20.52 pg/g wet tissue) when compared to normal control group. Treatment of depressed rats with malt extract decreased brain TNF-α content by 24% as compared to control depressed group. This effect was antagonized only in the group pre-treated with spiperone (Figure 4A).

Depression increased IL-1β brain content by 33% (to be 623.78 ± 55.45 pg/g wet tissue) as compared with normal control group (419.76 ± 24.06 pg/g wet tissue). Treatment of depressed rats with malt extract decreased IL-1β content by 24% as compared with control depressed rats. This effect was abolished by pre-treatment of malt extract treated rats with spiperone and propranolol,respectively;meanwhile,sulpiridepretreatment did not alter the decreased effect of malt extract on the IL-1β content of depressed rats (Figure 4B).

Brain content of IL-6 was increased by 70% in depressed rats (to reach 1042.874 ± 82.862 pg/g wet tissue) when compared to the normal group (313.551 ± 24.115 pg/g wet tissue). Treatment with malt extract decreased the elevated IL-6 content by 54% (to be 480.7114 ± 48.434 pg/g wet tissue) as compared with that of depressed rats. The effect of malt extract was partly antagonized in groups pretreated with propranolol or sulpiride. Moreover, spiperone not only antagonized malt extract effect but also increased IL-6 content by 194 and 36% when compared to malt extract-treated and control depressed groups, respectively (Figure 4C).

Effect of pretreatment with spiperone, phentolamine, propranolol or sulpiride on brain interleukin-10 content of depressed rats treated with malt extract.

Clonidine decreased the content of IL-10 (333.33 ± 23.49 pg/g wet tissue) by 29% as compared with normal rats (430.33 ± 27.42 pg/g wet tissue). Treatment with malt extract alone or combined with spiperone, phentolamine or propranolol did not significantly affect the reduced level of IL-10 in depressed rats. Sulpiride, on the other hand, increased IL-10 content by 45 and 36% as compared with control depressed rats and malt extract-treated depressed rats, respectively (Figure 5).

DISCUSSION

In the present experiment, clonidine-induced depression resulted in an increase of total immobility time parallel to a decrease of struggling time in FST. The latter is a commonly used animal model for depression (Rojas et al., 2011). Hence, the test has been used as a tool to evaluate the behavioral activity of rats to predict the effectiveness of different antidepressants (Porsolt et al., 1977; Cryan et al., 2002).

The current results are in accordance with the study that showed that clonidine induced a dose dependent behavioral despair in mice revealed by the significant increase in total immobility time (Parale and Kulkarni, 1986). Increased immobility time and decreased swimming or struggling time clearly indicates depressive like symptoms (Andrade et al., 2007). In the present investigation, induction of depression was coupled by significant decreases in brain 5-HT and NE contents compared to normal control rats. These results are in agreement with the results of previous investigators (Tang et al., 1979; Warsh et al., 1981; Kostowski and Krzqścik, 2003).

Previous studies indicated that depression-induced by clonidine is associated with decreased noradrenergic

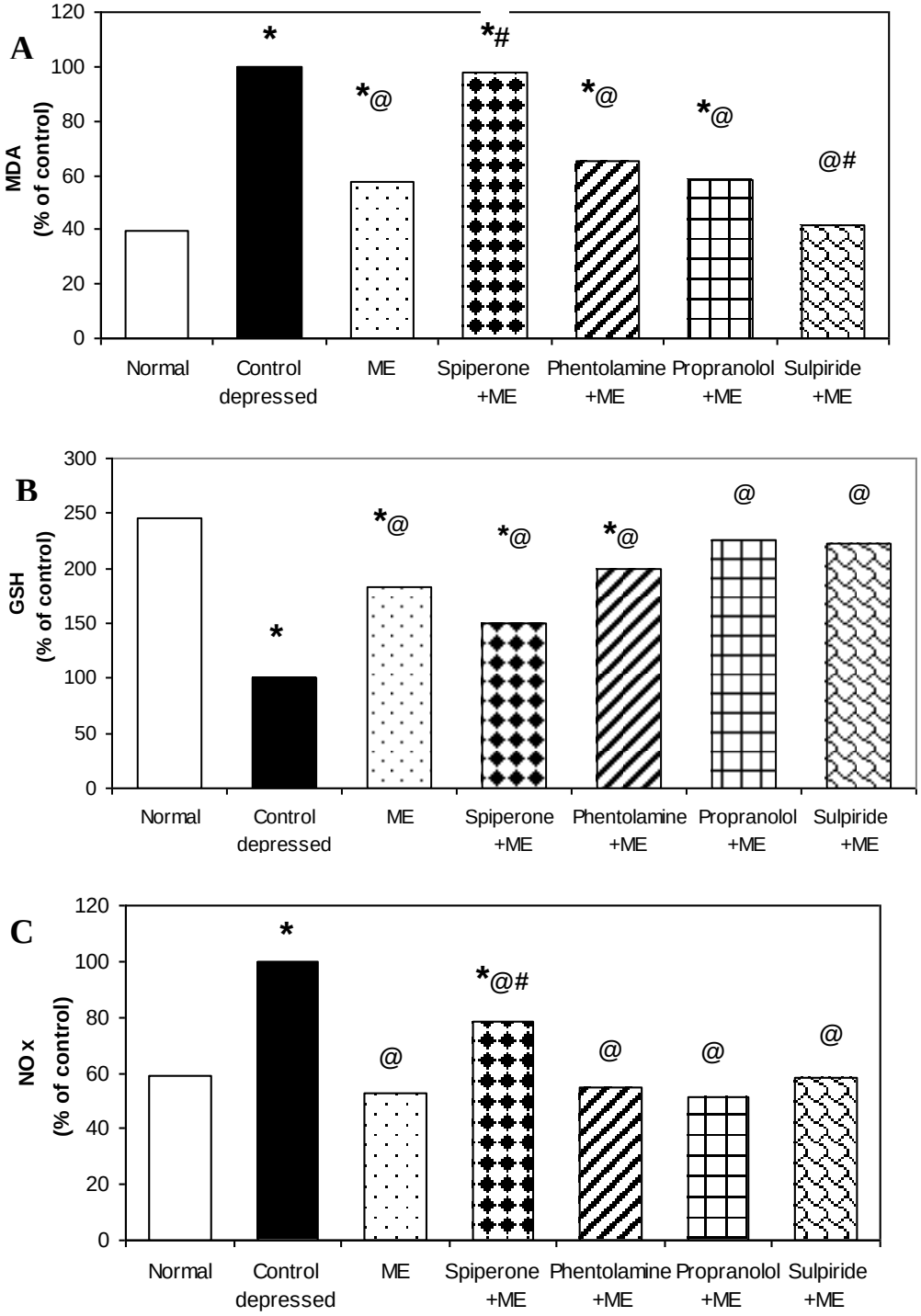

Figure 3. Effect of pretreatment with spiperone (0.03 mg/kg; i.p.), phentolamine (5 mg/kg; i.p.), propranolol (7.5 mg/kg; i.p.) or sulpiride (7.5 mg/kg; i.p.) on brain contents of: A. Malondialdehyde (MDA), B. Reduced glutathione (GSH), and C. Total nitrate/nitrite (NO_x) in depressed rats treated with malt extract (ME; 1250 mg/kg; p.o.). Depression was induced by daily i.p. injection of clonidine (0.8 mg/kg) for 7 successive days. Statistical analysis was carried out by one way ANOVA followed by LSD test. Values represent mean ± SE (n= 6-8 rats).*Significantly different from normal control group at p <0.05. [@]Significantly different from control depressed group at $p < 0.05$. [#]Significantly different from ME-treated group at $p < 0.05$.

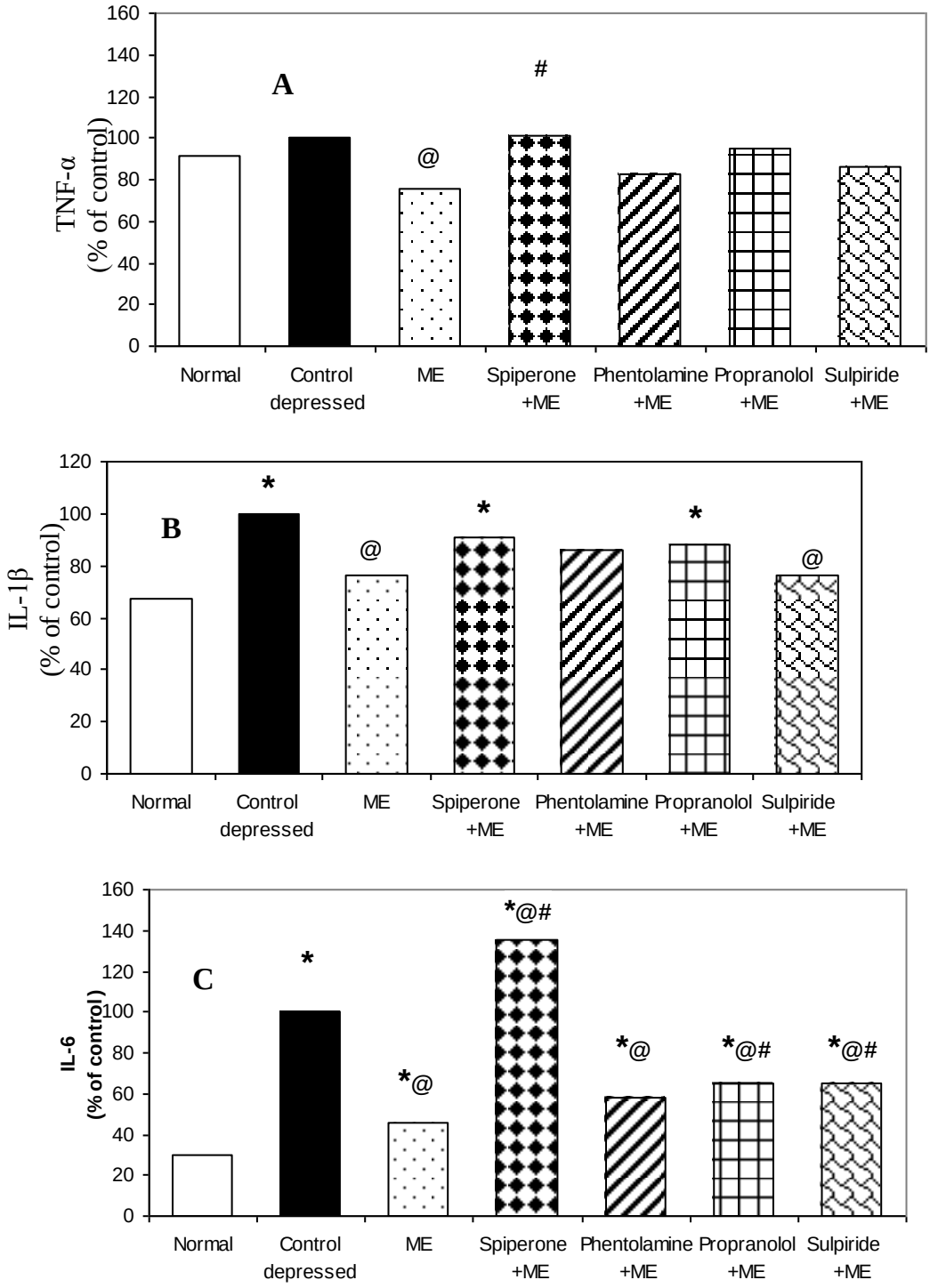

Figure 4. Effect of pretreatment with spiperone (0.03 mg/kg; i.p.), phentolamine (5 mg/kg; i.p.), propranolol (7.5 mg/kg; i.p.) or sulpiride (7.5 mg/kg; i.p.) on brain contents of: A. Tumor necrosis factor-alpha (TNF-α), B. Interleukin-1 beta (IL-1β), and C. Interleukin-6 (IL-6) in depressed rats treated with malt extract (ME; 1250 mg/kg; p.o.). Depression was induced by daily i.p. injection of clonidine (0.8 mg/kg) for 7 successive days. Statistical analysis was carried out by one way ANOVA followed by LSD test.Values represent mean ± SE (n= 6-8 rats).*Significantly different from normal control group at p <0.05. $^{@}$Significantly different from control depressed group at $p < 0.05$. $^{\#}$Significantly different from ME-treated group at $p < 0.05$.

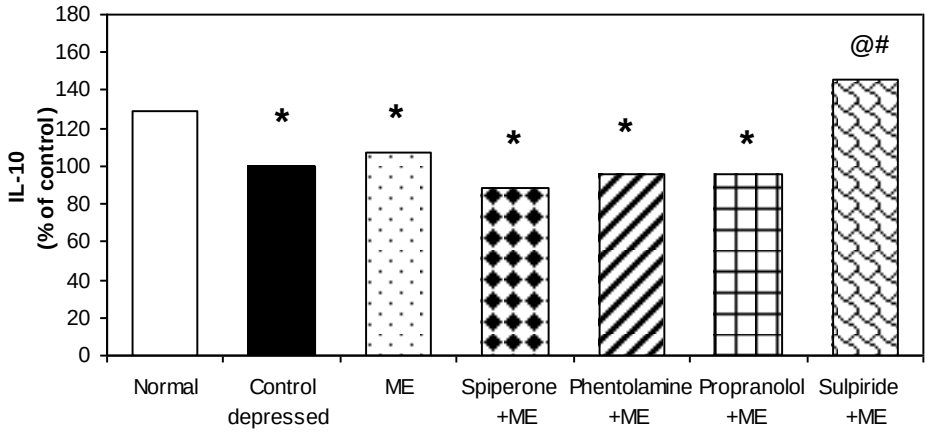

Figure 5. Effect of pretreatment with spiperone (0.03 mg/kg; i.p.), phentolamine (5 mg/kg; i.p.), propranolol (7.5 mg/kg; i.p.) or sulpiride (7.5 mg/kg; i.p.) on brain interleukin-10 (IL-10) content in depressed rats treated with malt extract (ME; 1250 mg/kg; p.o.). Depression was induced by daily i.p. injection of clonidine (0.8 mg/kg) for 7 successive days. Statistical analysis was carried out by one way ANOVA followed by LSD test.Values represent mean ± SE (n= 6-8 rats).*Significantly different from normal control group at p <0.05. $^{@}$Significantly different from control depressed group at $p < 0.05$.$^{\#}$Significantly different from ME-treated group at $p < 0.05$.

activity. Decreased brain 3-methoxy-4-hydroxyphenylglycol (MHPG), a metabolite of NE, concentrations in rats treated with clonidine, suggested reduced NE turnover (Tang et al., 1979; Warsh et al., 1981). Clonidine stimulates presynaptic α_2 receptors at low doses and acts on postsynaptic sites at higher doses. The dose of clonidine used in the present study (0.8 mg/kg) affects presynaptic receptors since clonidine was shown to stimulate presynaptic α_2 adrenergic receptors at same and even higher doses (2.5 mg/kg) (Enginar and Ergluo, 1990). Thus, the biochemical basis of clonidine–induced behavioral changes observed in the present experiment is probably due to decrease in NE release in the central nervous system (Dennis et al., 1987).

There is also mounting evidence that depressive symptoms are associated with reduction in 5-HT neurotransmission (Kostowski and Krzqścik, 2003). A close inter-relation was shown to exist between NE and 5-HT (Massé et al*.,* 2006). This relation seems to be expressed through α_2-adrenoceptors and 5-HT receptors. Both receptors are present in the same brain structures as the cortex, hippocampus and hypothalamus (French, 1995). Low doses of clonidine (0.002 to 0.01 mg/kg) were shown to enhance 5-HT neurotransmission through action on α_2-somatodendritic autoreceptors. On the other hand, higher doses of clonidine (0.1 to 0.4 mg/kg) decrease 5-HT transmission by direct activation of α_2-heteroreceptors on 5-HT terminals (Rénéric et al., 2002). In the present work, treatment of clonidine-induced depressed rats with malt extract decreased total immobility time and increased struggling time in the FST. Thus malt extract made rats more active and less depressed, which could be regarded as antagonism to the behavior despair caused by clonidine (Cryan et al., 2002).

The increased activity caused by malt extract was accompanied by a significant increase in 5-HT and NE brain contents compared with control depressed rats. The crucial role of NE and 5-HT in the development of depression syndrome was previously discussed by several investigators (Russo et al., 2005; Nutt, 2006). Ciulla et al. (2007) reported that the efficacy of antidepressant drugs appears in their ability to reduce immobility time and increase activity in FST. Moreover, enhancement of neurotransmission of 5-HT, NE, or both is the main mechanism of most agents used to treat depressive disorders (Blier and Abbott, 2001). Hence, malt extract can be considered an effective antidepressant based on its results in FST as well as its effect on brain monoamines.

Hordeins in barley seeds are degraded into several amino acids including tyrosine and tryptophan (Davy et al., 2000), both of which are essential amino acids in human and act as precursors for catecholamines and serotonin synthesis (Jin and Lin, 2004). Furthermore, it was reported that barley is rich in phytate (myo-inositol hexaphosphate) which is hydrolysed to myo-inositol (Rimsten et al., 2002). Myo-inositol is involved in the phosphatidylinositol (PIP) cycle which results in diacylglycerol (DAG) formation. DAG, in turn, activates protein kinase C, and inositol triphosphate (IP_3) leading to enhanced intracellular calcium flow. Such second

messengers can modulate cholinergic, noradrenergic, serotoninergic and dopaminergic systems (Einat et al., 1999).

In the present study, combination of spiperone with malt extract abolished most of the effects of the latter on rats' behavior and brain neurotransmitters. Spiperone is a potent 5-HT_{1A} and 5-HT_{2A} antagonist (Metwally et al., 1998). Hence, this could indicate involvement of 5-HT receptors in the antidepressant effect of malt extract.

Combination of malt extract with propranolol did not significantly change malt extract effects on neurotransmitters content but partly reduced malt extract effects in some of the behavioral experiments. On the other hand, phentolamine, a non-selective alpha blocker, abolished malt extract effects on brain neurotransmitters and partially affected its behavioral response.

In a study performed by Souza et al. (2013), propranolol, prazosin, a selective α_1 blocker, and yohimbine, a selective α_2 blocker did not abolish the antidepressant effect of hesperidin. The noted differences between these results and that of the present study could indicate that malt extract effects are partly mediated via actions on α_1 receptors but further studies may be needed to confirm that.

In the present study, combination of malt extract with sulpiride potentiated its antidepressant effects on brain neurotransmitters including DA. Several studies have shown the effectiveness of D_2 antagonists as sulpiride in different types of depression (Niskanen et al., 1974; Benkert and Holsboer, 1984; Tsukamoto et al., 1994). The dose of sulpiride used in the current experiment (7.5 mg/kg/day) is even much lower than those used in the other studies (100 to 300 mg/day). The mechanism underlying the antidepressant action of sulpiride appears to involve blocking of dopaminergic system and a possible D_1 and D_2 receptors sensitization in the mesolimbic area (Tsukamoto et al., 1994). Moreover, using low single doses of DA antagonists as sulpiride was shown to increase striatal DA content by inhibiting presynaptic DA autoreceptors (Imperato and Di Chiara, 1985; Levant, 1997). In the present study, clonidine-induced depression resulted in marked oxidative imbalance evidenced by decrease of brain GSH stores parallel to increased MDA and NO_x contents.

Involvement of reactive oxygen species (ROS) in stress-induced depression has been shown (Bilici et al., 2001). Moreover, one of the mechanisms of free radical generation in tissues is mediated via autoxidation of catecholamines released excessively in stress (Alptekin et al., 1996).

Depression is also characterized by activation of the inflammatory response and consequent production of pro-inflammatory cytokines (Andreasson et al., 2007; Maes et al., 2012). This was evident in the current study by the increase in brain contents of IL-1ß and IL-6 coupled with a decrease in IL-10. Activation of immune cells by pro-inflammatory cytokines leads to over production of reactive oxygen species (ROS), which leads to an increase of the levels of lipid peroxides as MDA (Eren et al., 2007).

In the present study, the antioxidant and anti-inflammatory effects of malt extract were evidenced from its effects on oxidative stress and inflammatory biomarkers. Malt extract contains several antioxidants (Goupy et al., 1999; Bonoli et al., 2004; Qingming et al., 2010). In addition, the effects of malt extract could also be attributed to its high content of melatonin (Badria, 2002), a well-known antioxidant. Parallel to the observed effects on neurotransmitters and the behavioral responses, the combination of malt extract with spiperone antagonized its effects on all investigated oxidative stress and inflammatory biomarkers. This again confirms that malt extract antidepressant actions are mainly mediated via 5-HT receptors. Moreover, the observed synergistic effect of both malt extract and sulpiride on brain lipid peroxidation could be also attributed to the reported antidepressant effects of both agents (Tsukamoto et al., 1994; Risk et al., 2009); and consequently reduction of depression-induced stress and lipid peroxidation.

Conclusion

The present study illustrated involvement of both 5-HT and DA receptors in the antidepressant effects of malt extract in addition to its antioxidant and anti-inflammatory effects. Further studies may be needed to establish a more precise relationship concerning malt extract and alpha or beta blockers.

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