1. Introduction

Several epidemiological findings on mental disorders throughout the world have convergently shown with some variability by diagnoses that in each year approximately 1/3rd population suffers from a mental disorder (Kessler and Üstün, 2008). Neuropsychiatric disorder mainly Schizophrenia, depression, bipolar disorder affecting millions of people around the world. Amongst these, major depression is the leading cause of disability worldwide among persons with age of five and older (World Health Organization, "Global Burden of Disease," 1996; Kokare et al., 2010). Depression is one of the most common neuropsychiatric disorders characterized by low mood and aversion to activity that can affect a person’s thoughts, behavior, feelings and sense of well-being. As per Diagnostic and Statistical Manual of Mental Disorders, depression is defined as “a mental health disorder condition characterized by anhedonia, the presence of loss of pleasure or interest in usually pleasurable activities, together with an array of other features, including anergia, changes in sleep and appetite, sadness, and suicidal ideation”. The depressive patients are predominantly vulnerable to negative psychological feedback, which has a disproportionately disruptive effect on subsequent performance (Elliott et al., 1996). Monoamine hypothesis proposed by Schildkraut in 1965 proposed that the insufficiency of monoamines serotonin (5HT), dopamine (DA), norepinephrine (NE), and epinephrine (E) are responsible for the corresponding features of depressive behavior. Based on various pharmacological evidences of depressed patients, it is believed that, each neurotransmitters exhibit particular effect on depressive behavior. For example NE may be related to alertness and energy as well as anxiety, attention, and interest in life; 5HT is related to anxiety, obsessions, and compulsions; and dopamine to attention, motivation, pleasure, and reward, as well as interest in life.

The NE also called noradrenaline (NA), a catecholamine functions as a hormone and neurotransmitter in body. In the brain, NE is released by cell neurons or nuclei which exert its effects on other brain regions. The noradrenergic neurons are involved in the alertness, arousal, and readiness for action. The NE from locus coeruleus (LC), augments processing of sensory inputs, attention, formation and retrieval of long term and working memory, and the ability of the brain to react to inputs by altering the activity pattern in the prefrontal cortex and other areas (Sara and Susan, 2015). The attention has motivated mainly on the LC, the
neuron is silent during the sleep, and their activity drastically higher with behavioral arousal. The CNS stimulants which releases NE in brain increases alertness and exploratory activity, that are found to be impaired in depressive disorders while antidepressant agents improve such behavioral alterations (Sara and Susan, 2015). It is further understood that there is a close connection of mood/depressive state with state of arousal; depressed individuals are often lethargic and insensitive to external stimuli. The monoamine hypothesis of affective disorders suggested that depression due to functional deficiency of NE in certain brain areas, while mania results due excessive activity of NE.

In fact, it is suggested that serotonin (5-HT) might be more potent than NE in depressive state. The considerable facts are available to validate the hypothesis that changes in serotonergic neuronal function in the CNS have effect in patients with major depression. In CNS, 5-HT is thought to act predominantly as excitatory neurotransmitter. The reductions in CSF concentrations of monoamine metabolites are generally believed to reflect reduced neuronal activity (Owens et al., 1994). Roy et al., (1989) in their studies showed depressed patients reduced 5-hydroxyindoleacetic acid (5-HIAA) in CSF in those patients most likely to attempt suicide. Drugs like reserpine and p-chlorophenylalanine liable to precipitate depression by reducing 5-HT concentration. Since tryptophan hydroxylase is not ordinarily saturated by its substrate, altered tryptophan level might results into consequent changes in the ability of neurons to release 5-HT.

Deficient levels of dopamine (DA) activity in the brain can also cause depressive disorders. This DA deficient depression (DDD) is described as low energy, demotivated state. The depressive like behavior associated with low energy with a complete lack of motivation. Mesolimbic/mesocortical dopaminergic pathways occur in midbrain and limbic system mainly in nucleus accumbens and the amygdaloid nucleus and to the frontal cortex which is involved in the behavioral regulation. Amphetamine treatment in rats resulted into release of DA, NE and induced a cessation of ratty behavior, exploration and grooming (behavioral effects in depressive state). Amphetamine induces motor impairment in rats mainly reflect hyperactivity in the nigrostriatal dopaminergic pathway. The DAIs often called "the happy neurotransmitter" as it elicits major effects mainly feelings of pleasure, feelings of attachment/love, and integration of thoughts and feelings, whereas deficiencies of DA in brain produces anhedonia (lack of pleasure), lack of ability to feel love, sense attachment to another, lack of remorse about actions and distractibility clinically.
There exist good correlation between depressive behavior and role of oxidative stress and/or impaired monoaminergic neurotransmission along with role of oxidant-antioxidant system (Gonçalves et al., 2012). Due to high rate of oxygen consumption, the brain is more vulnerable to free radicals formation in terms of reactive oxygen species (ROS) and consequently neuronal damage which perhaps leads to depressive like behavior (Zafir et al., 2009). Oxidative stress in brain probably might be attributed to presence of iron in brain, low endogenous antioxidant enzymatic activity, production of excessive free radicals, and presence of high content of polyunsaturated fatty acids (Muley et al., 2012). Lipid peroxidation is an important phenomenon of injury of cells/tissues during oxidative stress. A significant amount of ROS formation during stress is responsible for lipid peroxidation process as demonstrated in terms of increased MDA formation an end product of lipid peroxidative process (Niki, 2012; Lang and Borgwardt, 2013). The patients with depressive episodes are found to exhibit elevated plasma peroxide levels and higher malondialdehyde (MDA) formation due to oxidative stress (Sarandol et al., 2007). These free radicals can be neutralized and/or quenched by endogenous antioxidant enzymes viz superoxide dismutase (SOD), catalase (CAT), which are however, impaired in depression (Sarandol et al., 2007; Thakare and Patel, 2015).

Stress occurs whenever an endogenous or exogenous challenge is perceived as unpleasant, aversive, or threatening for the homeostasis or survival of an individual. Stressful life experiences are considered to be the major culprit in the development of neuropsychiatric diseases including depression. Various documented findings demonstrated the relationship between stressful life, and subsequent depressive like symptoms (Musazzi et al., 2010; Hammen, et al., 2009). The behavioral and biochemical alterations rest on severity of stress, type and duration of stressful events subsequently resulting into dysfunctioning of the central nervous system (Burri et al. 2013). There are mainly two forms of stress responses, acute and chronic. Acute stress is the more common form of stress characterized by emotional anguish, headaches, back pains and general muscular problems. Acute stress mainly combines both emotional and physical components in addition to affecting the brain’s intra-cellular redox status (Buynitsky and Mostofsky, 2009). Acute stress mainly results into induction of depressive like behavior associated with impairment of in vivo antioxidant defense associated with cognitive dysfunctions (Budni et al., 2013). Similarly, Balk et al (2010) and Garcia-Fernandez et al (2012) showed that acute stress in rodents induce neurochemical and
hormonal abnormalities that are often associated with an imbalance in the brain’s intracellular redox state, and enhanced lipid peroxidation and impaired antioxidant enzymes mainly superoxide dismutase, catalase in brain regions mainly cerebral cortex and hippocampus, structures involved in the induction of depressive like behavior.

It is generally believed that chronic stress is a key factor in the development and acceleration of depressive state. Chronic stress is accompanied with reduction in hippocampal excitability and memory, long-term potentiation, inflammatory reactions, loss of pleasure, and impaired in neurogenesis (Joels et al., 2007; Lucassen et al., 2014). Chronic stress induce sustain activation of HPA axis associated with an abnormally high blood glucocorticoid level, which may eventually lead to depressive behavior (Johnson et al., 2006). Further, chronic stress demonstrated to exhibit increased inflammatory cytokines mainly TNF-α and IL-6, which are known to participate in depression (Lotrich et al., 2011). Several studies have reported that chronic stressful conditions lead to oxidative stress and damage to macromolecules mainly lipid, proteins and nucleic acids by excessive production of reactive oxygen species (ROS), impaired BDNF in hippocampus and prefrontal cortex causing neuronal dysfunction and development of depressive disorders (Zhao et al., 2008; Ng et al., 2008; Jindal et al., 2013; Deng et al., 2015). The above mentioned studies point towards the fact that chronic stress conditions can lead to depression predominantly; however, acute stress may also lead to depressive like conditions. Hence, there is need to find novel agents for both the conditions.

Apart from aforementioned pathological aspects of depression, brain-derived neurotrophic factor also known as BDNF, is a member of the neurotrophin family of growth factors, which are related to the nerve growth factor helping to support the survival of neurons, and encourage the growth and differentiation of new neurons and synapses thereby helps in neurogenesis (Acheson et al., 1995). BDNF plays a significant role in neurogenesis. Exposure to stress and the stress hormone corticosterone is known to decline the BDNF in rats and produce atrophy of the hippocampus. Additionally, it has been shown that BDNF plays an important role in the pathogenesis of psychiatric disorders including depression. Postmortem investigation of brain tissues from patients with major depression showed a reduction in brain BDNF levels (Castren et al., 2007). Clinical findings suggested that a decrease serum BDNF level was observed in depressive patients and subsequent antidepressant therapy was able to restored the BDNF level to normal (Aydemir et al., 2007;
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Bas et al., 2009; Aydemir et al., 2005; Matrisciano et al. 2009; Fernandes et al. 2011). It was documented that BDNF levels have been found to be reduced in the postmortem samples of brains of depressed patients (Dunham et al., 2009; Thompson Ray et al., 2011). Moreover, BDNF levels are also reduced in the brains of suicide victims, several of which suffer from depressive disorders (Chen et al., 2001; Dwivedi et al., 2003; Tripp et al., 2012). The impaired BDNF in hippocampus and prefrontal cortex of experimental animals induced depressive like symptoms when exposed to chronic stressful conditions which was subsequently reversed with antidepressant treatment (Mao et al. 2014b). Furthermore, it was noted that impaired BDNF expression in hippocampus and prefrontal cortex of experimental animals when exposed to chronic stressful conditions results into depressive like symptoms which were subsequently reversed with antidepressant treatment (Shen et al., 2016; Mao et al., 2014). Administration of clinical antidepressants in rats mainly venlafaxine (Czuba et al., 2009), escitalopram (Ladea and Bran, 2013), duloxetine (Ball et al., 2013), and imipramine (Gislaine et al., 2011) increases BDNF levels in the hippocampus and cortex, plasma and serum respectively, thus, indicating BDNF is implicated in the antidepressant like activity. Olfactory bulbectomized (OBX) experimental model of exhibited significant reduction in BDNF level (Yang et al., 2014) and similar changes were observed in hippocampus (Rinwa et al., 2013) and brain (Song et al., 2009). Further, BDNF expression was also found to be reduced in the hippocampus and PFC in chronic stress studies (Chiba et al., 2012). Thus, it is presumed that BDNF may be the potential target of antidepressants and participate in the molecular mechanism of depression.

Proinflammatory cytokines are the cytokines that are important in cell signaling and inflammation. They are produced predominantly by activated macrophages and are involved in the up regulation of inflammatory reactions and have the ability to influence neurocircuitry and neurotransmitter systems to induce behavioral alterations. Studies have reported that proinflammatory cytokines mainly tumor necrosis factor-α (TNF-α) and interleukin ((IL)-1β, IL-6, IL-12 etc. are involved in psychiatric disorder mainly depression (Lotrich et al., 2011; Dowlati et al., 2010). Elevated levels of these cytokines were found in patient with depressive disorders (Janssen et al., 2010). The animals subjected to OBX surgery were found to exhibit increased inflammatory reactions with increased levels of IL-1β and TNF-α, in several brain regions (Rinwa and Kumar, 2013). In chronic unpredictable mild stress (CUMS) induced depression model using rats reported to induce marked elevation of TNF-α, IL-1β, and IL-6
inflammatory markers and a decrease in the IL-10 level (López et al., 2016). Rats subjected to OBX showed higher IL-6 and TNF-α in hippocampus and cerebral cortex, suggesting inflammatory reaction accompanied by neuronal damage. Moreover, effective antidepressants have been shown to attenuate proinflammatory cytokines, protein levels for TNF-α and IL-6 in the prefrontal cortex and hippocampus due to CUMS in rats. Similarly, Deng et al (2015), demonstrated mice exposed to CUMS paradigms elicited increase in IL-6 and TNF-α in hippocampus which were subsequently prevented after administration of thymol. Inhibitory effects of various studied drugs on these cytokines are documented to exhibit significant antidepressant like activity in other chronic stress models of depression (Jiang et al., 2013; Deng et al; 2015).

Studies have been proposed that the glutamatergic system contributes to the pathophysiology of depression (Crane, 1959; Pittenger et al., 2007). D-cycloserine, a partial NMDA glutamate receptor agonist, has antidepressant effects. Many reports have highlighted alterations in glutamate signaling as well as changes in the expression of AMPA or NMDA receptor subunits in depression, although there are significant variations across brain areas, and the functional significance of these changes remains unclear (Feyissa et al., 2009; Karolewicz et al., 2009; Sanacora et al., 2008). Although, no clinical antidepressant drug is currently approved targeting the glutamatergic system, the glutamatergic agent riluzole is sometimes administered for its antidepressant effects and the non-competitive NMDA receptor antagonist, ketamine, produces a rapid and sustained antidepressant response in patients with treatment resistant depression (Berman et al., 2000; Zarate et al., 2006). Yu et al., (2008) have demonstrated that NMDAR-dependent long-term potentiation and long-term depression can be reliably induced in the amygdala, an important region suggested to be implicated in the depressive behavior. In addition, Zhang et al (2013) investigated the effects of curcumin on depressive-like behavior with a focus upon the possible contribution through NMDA subtype glutamate receptors in this antidepressant-like effect of curcumin. The studies further documented that NR2B-containing NMDA subtype glutamate receptor antagonist Ro25-6981 prevents the behavioral stress facilitated hippocampal long-term depression (Wang et al; 2006), and attenuated the depressive-like behaviors in chronically stressed rodents (Li et al., 2010). Recently, the lamotrigine, an anticonvulsant agent showed antidepressant properties in forced swim test mediated through the involvement of NMDA receptors and nitric oxide-cyclic guanosine monophosphate (Ostadhadi et al., 2016).
Current antidepressants include tricyclic antidepressants (TCAs, e.g. Imipramine, desipramine, amitriptyline, doxepin etc.), serotonin-norepinephrine reuptake inhibitors (SNRIs, e.g. Venlafaxine, Duloxetine Desvenlafaxine), Selective serotonin reuptake inhibitors (SSRIs e.g. Fluoxetine, fluvoxamine, paroxetine, citalopram, escitalopram sertraline etc.), monoamine oxidase inhibitors (MAOIs); irreversible, non-competitive MAO- A and MAO-B inhibitors (e.g. Phenelazine, tranylcypromine); reversible, selective MAO-A inhibitors (e.g. Maclobamide) and atypical agents (e.g. Trazadone, mirtazapine and mianserin etc.)

The major effect of TCAs is to prevent the uptake of amines by nerve endings, probably by competition for binding site of amine transporter. TCAs also seem to enhance transmitter release indirectly through blocking presynaptic α-2 adrenergic receptors. The important actions of TCA are to inhibit NA and 5-HT uptake by brain synptosomes and to lesser extent DA uptake. It has been thus argued that improvements of emotional symptoms are related mainly to enhancement of 5-HT neurotransmission, while NA neurotransmitter release results into relief of biological symptoms. However, apart from these effects, TCA exert their effects on cholinergic, neurologic and cardiovascular systems and thus rendering them for undesirable effects (Dipiro et al., 2005). The antimuscarinic actions of TCAs do not involve in the antidepressant effects but is accountable for undesirable effects. The major side effects of TCAs are xerosotomia, blurred vision, constipation, and urinary retention. At higher doses, TCA might cause ventricular dysrhythmias associated with prolongation of QT intervals(Dipiro et al., 2005). TCAs are known to interact with alcohol to produce synergistic effect as they potentiate the effect of alcohol and anesthetic agents (Dipiro et al., 2005).

The MAOIs (phenelazine, tranylcypromine and iproniazid) produce irreversible inhibition of monoamino oxidase (MAO). Various findings have proposed a reduction in platelet MAO activity in certain groups of depressed patients; although there is no clear evidence that abnormal MAO activity results in the induction of depressive like behavior. MAO-A is a substrate specific for 5-HT and MAO-B is specific for phenylethylamine, however, both enzymes act on NE and DA. The MAO-A is the main target for antidepressant activity for the class of MAOIs. The inhibition of MAO-A gene results into prevention of degradation of serotonin and thus concentration in synaptic cleft is increased (Shish et al., 1999). Moreover, MAOIs are not specific for their action, inhibit other enzymes too and this explains the side effects. In addition, use of MAOIs should be restricted with foods and beverages containing tyramine, as large amounts of tyramine consumptions, might results into
hypertensive crisis, which can be fatal. Examples of foods and beverages with potentially high levels of tyramine include liver and fermented substances, such as alcoholic beverages and aged cheeses. Tyramine leads to hypertensive crisis by increasing the release of norepinephrine (NE), which causes blood vessels to constrict (through binding to alpha-1 adrenergic receptors). Ordinarily, MAO-A would destroy the excess NE. When MAO-A is inhibited, NE levels get too high, leading to dangerous increases in blood pressure. Furthermore, biochemical studies on depressed patients do not reinforce the role of monoamines hypothesis. However, it is simple form of explanation of depression and pharmacological approach of monoamine hypothesis remains the most successful therapeutic strategy.

The SSRIs exerted high selectivity for 5-HT reuptake and thus is widely established as antidepressants. These agents inhibit selectively serotonin reuptake at a greater extent as that of noradrenaline, and showed lesser effects on cholinergic system, less dangerous at overdose too, and further in contrast to MAOIs, the SSRIs do not cause ‘cheese reactions (Rang et al., 2007). The SSRIs widely employed in obsessive compulsive disorder- one of type of anxiety disorder. Loss of libido and failure of orgasm are the important side effects associated with SSRIs. Additionally, SSRIs in combination of MAOIs can induce ‘serotonin syndrome’ characterized by tremor, hyperthermia, and cardiovascular collapse and finally death may occur. Fluoxetine, a SSRI is liable to produce aggression and violence. Under age of 18, the use of SSRIs is prohibited due to its lack of efficacy and likely to produce side effects like excitement, insomnia and aggression along with chances of higher suicidal tendency in this age group. Thus, from above mentioned studies, it is evident that currently used antidepressant have several limitations and side effects. This points out towards that fact that novel drugs acting on multiple targets is the need of hour.

Various natural polyphenols are known to affect various physiological and biochemical functions in the body. These polyphenols modulate monoaminergic neurotransmission in the brain. Various experimental and clinical studies reveals antidepressant potential of some important polyphenols such as amentoflavone (Ishola et al., 2012), curcumin (Xu et al., 2009), ferulic acid(Zeni et al 2012), hesperidin (Souza et al., 2013), quercetin (Bhutada et al., 2010), naringenin(Yi et al., 2010), resveratrol (Hurley et al., 2014), ellagic acid (Dhingraa and Chhillar, 2012), and nobiletin (Yi et al., 2012).
Protocatechuic acid ethyl ester (Ethyl 3,4-dihydroxybenzoate, PCA) is a phenolic compound of *Hibiscuss abdariffa* and *Eucommi aulmoides* (Lin et al., 2003; Pacheco-Palencia at al., 2008.). PCA elicited neuroprotection through promotion of the endogenous antioxidant enzymatic activity in hydrogen peroxide induced oxidative damage (Shi et al., 2006). Neuroprotective effects of PCA against H$_2$O$_2$-induced oxidative damage on PC12 cells was thought to be by improved the cognition of aged rats, reduced lipid peroxide content, and increased the activity of GSH and SOD Kim et al (2012) demonstrated that PCA isolated from *Gardenia jasminoides* showed significant antidepressant activity by inhibition of MAO-A and MAO-B in animal model of depression. Furthermore, ethanolic extract of *Gardenia jasminoides* increased 5-HT in the brain tissues of rats along with decreased MAO-B activity (Kim et al., 2012). The PCAtreatment prevented the elevation of MDA formation, and restored the depleted antioxidants in cerebral ischemic rats and thus improved behavioral and biochemical alterations (Muley et al., 2013; Muley et al., 2012). Further, PCA pre-treatment results in modulation of cellular redox status with the restoration of endogenous antioxidant enzymes, and decreased MDA formation (Zhang et al., 2015). In addition, PCA treatments suppressed cyclooxygenase (COX)-2 expressions and abated COX-2 activity, thus reduce the production of PGE2 and inflammatory cytokines. These findings indicate that the anti-inflammatory effects of PCA in brain were partially due to this compound inhibit COX-2 and subsequently the PGE2.

Silymarin and protocatechuic acid ethyl ester are the polyphenols known to exhibit various effects on central nervous system disorders. Silymarin, a polyphenolic flavonoid antioxidant obtained from fruits and seeds of the *Silybum marianum* commonly called as ‘Milk thistle’ used clinically for the management of hepatic disorders (Koksal et al., 2009; Sangeetha et al., 2010). Silymarin crosses blood brain barrier (Pradhan and Girish, 2006), and were evaluated the effects on enzymatic and non enzymatic antioxidant defensive systems in rat brain in acetaminophen APAP-induced brain damage and found to protect the *substantia nigra compacta* by oxidative damage for its ability to prevent lipid peroxidation and replenishing the GSH levels (Nencini, et al., 2007). In another studies, silymarin evaluated for their effect on biochemical parameters in aged and young rat brain by measuring total oxyradical scavenging capacity through the concentration of ROS, lipid peroxidation as TBARS, proteins oxidation. The findings showed that, silymarin treatment (400 mg/kg) decreased TBARS in hippocampus. Hence, silymarin treatment significantly prevented
Pharmacological evaluation of PCA and Silymarin in experimentally induced depression in rodents

Drug therapy has been the mainstay of treatment for depression in humans. However, current treatment options are not entirely effective in the treatment of depression, especially in chronic forms [1]. Therefore, the use of herbal medicine, such as Silymarin, a potent antioxidant, and a neuroprotective agent, has been an area of great interest. Silymarin, the main constituent of the milk thistle plant, has been extensively studied for its pharmacological properties. It is known to improve the antioxidant properties of the brain, which can be useful in the treatment of depression [2].

In this study, we investigated the effect of PCA and Silymarin on various behavioral parameters in experimental animals. PCA and Silymarin are known to enhance monoamines level and attenuate oxidative stress in brain areas. The effects on depressive behavior are not reported in acute and chronic stressful conditions. In light of above mentioned facts, the main objectives of the present study are

- To study the effect of PCA and silymarin on various behavioral parameters in experimental animals
- To evaluate the PCA and silymarin for its possible role in experimentally induce depressive like behavior in rodents.
- To understand the underlying mechanism(s) of action of PCA and silymarin in acute and chronically induced depression in experimental animal.
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