INTRODUCTION
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Chalcones, or 1,3-diaryl-2-propen-1-ones, are natural/synthetic compounds belonging to the flavonoid family. They are the first isolable compounds from flavonoid biosynthesis in plants, but do not necessarily accumulate to any appreciable degree unless the enzyme chalcone isomerase, which catalyses the cyclisation of chalcone to flavanone is absent. They are characterized by the opening of oxygenated ring (C) that is present in the other classes of flavonoids, leading to the formation of a double bond between carbons α and β with respect to the carbonyl function. Contrary to the majority of the flavonoids, the nucleus A of chalcone is numbered with cardinal numbers followed by a line (') whereas nucleus B is numbered with cardinal numbers alone.

Basic nucleus of chalcones
Flavonoids are polyphenolic compounds found in rich abundance in all plants (Bors, 1997). Chemically flavonoids are \( C_6 - C_3 - C_6 \) compounds in which the two \( C_6 \) groups are substituted benzene rings, and the \( C_3 \) is an aliphatic chain which contains a pyran ring (Bravo, 1998). In plants, flavonoids and isoflavonoids are synthesized from hydroxychalones which are synthesized from p- coumaroyl- CoA and malonyl- CoA by enzyme catalyzed condensation and cyclization reactions.

\[
p\text{- coumaroyl CoA} + 3 \ (\text{malonyl CoA}) \rightarrow \text{chalcone} \rightarrow \text{naringenin} \rightarrow \text{flavonoids.}
\]

Owing to their polyphenolic nature, flavonoids often exhibit strong antioxidant properties, akin to \( \alpha \)-tocopherol, which they structurally resemble and can replace in some model systems (Bors, 1997; van Acker, 2000). One estimate has the average daily consumption of flavonoids by humans as 1gm, an amount much greater than that of other dietary antioxidants such as ascorbate or \( \alpha \)-tocopherol (Bravo, 1998). Flavonoids possess both excellent iron chelating and radical scavenging properties (Havsteen, 1983; Hanen, 1993).
The main constituents of flavonoids are:

**Carbon numbers**
4 3 2

**Chalcones / Flavones**
\[
\begin{array}{c}
\text{C—CH}=\text{C—H} \\
\text{O}
\end{array}
\]

**Flavanones**
\[
\begin{array}{c}
\text{C—CH}_2—\text{C—H} \\
\text{O}
\end{array}
\]

**Catechins**
\[
\begin{array}{c}
\text{CH—CH}=\text{C—H} \\
\text{OH}
\end{array}
\]

**Flavanonols**
\[
\begin{array}{c}
\text{C—CH—C—H} \\
\text{O—OH}
\end{array}
\]

**Anthocyanidins**
\[
\begin{array}{c}
\text{C=}\text{C—C—H} \\
\text{OH}
\end{array}
\]

Chalcones occur in a wide variety of plants, and are found in different parts (heartwood), bark, leaf, flower, fruit and root (Dewick, 1997). Chalcones in many cases serve in plant defense mechanism to counteract reactive oxygen species (ROS) in order to survive and
prevent molecular damage and damage by microorganisms, insects and herbivores. Chalcones play an important role in ecological system due to colors they produce in plants, as attractants of insects and/or birds necessary for polinization (Zuanazi, 2001). Biological activities exhibited by chalcones include: anti-bacterial (Nielson, 2004) and bacteriostatic (Lin, 2002), antiviral (Al-Nakib, 1987), antimalarial (Domínguez, 2005), trypanosomicidal (Lunardi, 2003), antileishmanial (Bergmann, 2004), antiulcerogenic (Yamamoto, 1992), antioxidant (Mukherjee, 2001), immunomodulatory (Barford, 2002), cytotoxic (Sabzevari, 2004), angiogenesis inhibition (Robinson, 2005), anti-inflammatory (Ko, 2003), antinociceptive, antioedematogenic (Buzzi, 2006),

In agriculture certain chalcones are used to destroy phytopathogenic organisms, whereas in industry they are used in controlling corrosion of steel, as dyes and as U.V. absorbers in cosmetics (Augusto, 2006). Chalcones are also effective in vivo as chemopreventive agents in several rat carcinogenesis models (Baba, 2002; Wattenberg, 1994; Makita, 1996). Induction of phase II enzymes and increasing glutathione levels are major chemopreventive strategies for preventing chemical carcinogenesis. Various chalcones incubated with
cultured cells readily induced NAD(P)H: quinone reductase (NQO1) (Dinkova- kostava, 2001; Miranda, 2000) as well as glutathione –S-transferase (GST) (Fiander, 2000) and would be expected to alleviate oxidative stress and detoxify mutagenic xenobiotics. Also some chalcones such as metochalcone (a cholretic drug) and sofalcone (an anticancer agent) have been approved for clinical use (Ni, 2004).

The entire evolution of living organisms is characterized by the mutual rivalry of free radicals and antioxidants. Both free radicals and antioxidants have developed and they have always influenced the viability of living organisms. Many disease and pathological syndromes are caused by oxidative stress, i.e., by an imbalance in favour of free radicals. The most frequent consequences of oxidative stress are atherosclerosis, diabetes mellitus, tumors, cataract and ageing. The nervous system, due to enriched concentrations of polyunsaturated fatty acids, is particularly susceptible to the deleterious effects of oxidative stress (Beckman, 1991; Cini et. al., 1994; DeLeo et. al., 1986, Eldjarn and Pihl, 1960). Reactive oxygen species (ROS) are produced in the brain during cellular respiration and their rate is accumulated during brain insults (Dandekar, 1997) and measured in Alzheimer’s (Lovell,
1995) and Parkinson’s (Fahn, 1992) disease, epilepsy (Rokyta, 2001),
trauma and amyotropical lateral sclerosis.

Anxiety disorders in a modern society have a relatively high
prevalence and command considerable financial resources, and also are
the most common illness associated with unreasonable and disturbing
sensation of fear and tension. Stress increases vulnerability to anxiety
and depression. The development of anxiety/ stress- related disorders
involves complex interactions among various body mechanisms
involving the limbic system and the hypothalamo-pituitaryadrenal
axis; their interactions play a significant role in the manifestation of
disease pathology (Chrousos and Gold, 1992; Ray et. al., 1993).
Recently, oxidative stress has been shown to be associated with anxiety
and cognitive defects in different behavioral models (Hovatta et. al.,
2005; Gingrich, 2005; Berry et. al., 2007). Recent studies have also
shown that social phobia, depression, anxiety, and other neuropsychiatric
disorders result in signs of oxidative stress such as increased reactive
oxygen generation and decreased antioxidant capacity (Arranz et. al.,
2007; Bouayed et. al., 2007). There is increasing evidence that oxidative
stress in neurons is involved in pathological manifestations of many
neurological disorders. Further, it is becoming increasingly clear that
cognitive and emotional biases play an important role in the development and maintenance of depression, especially in response to stress (Anisman, 2005; Coles, 2002).

Stress is a state of threatened homeostasis that causes a variety of changes in the central nervous, endocrine and immune systems and in peripheral tissue metabolism (Black, 1994; Sothman & Kastello, 1997). Stress is known to induce alterations in various physiological responses even leading to pathological states (Chrousos, 1998). Immobilization-stress approximates emotional stress in animals, and associates with greater oxidative stress: increased free-radical production, decreased anti-oxidant enzyme levels, and increased oxidized lipids in tissues, including brain (Yokoi et al., 1999; Liu et al., 1996). Oxidative modification of lipids in brain clearly associates with impaired cognitive function (Stark-Reed et al., 1991; Forster et al., 1996). Immobilization for a single eight-hour period increased brain lipid oxidation, which associated with decreased memory and behavior (Radak et al., 2001), and lesser oxidation associated with improved cognitive function (Butterfield et al., 1997; Radak et al., 2001). Reactive oxygen species (ROS) and free radicals induce membrane damage, DNA base oxidation,
DNA strand breaks, chromosomal aberrations, and protein alterations (Olinski, 2002).

Chalcones continue to attract considerable scientific attention because of their association with a variety of biological activities. Besides of their association with a number of pharmacological properties, no study has been conducted regarding the effect on anxiety, cognition, and immobilization-stress induced changes in behavior and biochemical parameters. The pathogenesis of several neuropsychiatric disorders, including anxiety and depression, has been linked to oxidative stress, in part via alternations in cyclic nucleotide signaling. PDE2 inhibition by increasing cGMP signaling is able to inhibit oxidative-stress induced anxiety in mice (Masood et. al., 2008). Chalcones caused an increase in cGMP and cAMP (Yu et. al., 1995a; Yu et. al., 1995b). Many attempts have been made to reverse cognitive deficits by increasing brain cholinergic activity via acetylcholinesterase inhibitors (AChEIs), acetylcholine precursors, or cholinergic agonists (Davies and Maloney, 1976; Perry et. al., 1978) and also BChE inhibitors may also be effective for the treatment of Alzheimer and related dementias (Yu et. al., 1999). Chalcones have been shown to be the inhibitors of AChE and BChE (Ansari et. al., 2005). Chalcones have been reported to have the
property to scavenge free radicals and have the antioxidative capacity
toward LDL oxidation and lipid peroxidation (Vaya, 1997; Haraguchi,
1998; Cheng, 1998; Anto, 1995). Thus, this consideration has induced us
to explore the possible effects of chalcones on anxiety and cognition.

The effect of free radicals, as one of the oldest chemical stimuli, in
the phytogenetically youngest and the best-organized biological
structure- the central nervous system (CNS) has been relatively seldom
studied. Living cells protect themselves from oxidative damage by low
molecular weight antioxidants including enzymatic and non-enzymatic
antioxidant system (Halliwell and Cross, 1994). These cellular defenses
reduce the steady- state concentrations of free radical species and repair
oxidative cellular damage. The antioxidant defense system includes
enzymes such as superoxide dismutase (SOD), catalase (CAT) and
glutathione peroxidase (GPx), which decrease the concentration of the
most harmful oxidants. Immobilization stress is a good model for
investigating the alterations occurring in oxidant-antioxidant balance in
tissues of rats. Anti-oxidant nutrients blunt the increase in oxidized
lipids when administered before or after immobilization- stress (Zaidi,
2003, 2005). Therefore, keeping in view the antioxidant properties of
chalcones, we also tested them for their possible antioxidant effects
against restraint - stress induced oxidative damage to central nervous system.

Thus, in the present study we attempted to investigate:

(1) Evaluation of chalcones for their anxiolytic effects using elevated plus maze and open field behavior tests.

(2) Inhibitory potential of chalcones on brain parts and spinal cord acetylcholinesterase activity and memory in rats using Y-maze.

(3) Modulation of restraint-stress induced neurobehavioral and oxidative changes in antioxidant enzymes by chalcones.

(4) Protective effects of chalcones against oxidative damage to lipids, proteins and DNA induced by restraint stress.

(5) Differential effects of chalcones on glycogen contents of liver, brain, and spinal cord in rats.