Medicinal plants as potential source of therapeutic aids has attained a significant role in health system all over the world for both humans and animals not only in diseased condition but also as potential material for maintaining proper health (Khan Nazeerullah, 2012).

Traditional herbal drugs have proven to be a better choice when compared to modern synthetic drugs. These drugs have a few or no side effects and are claimed to be safer ones (Biren NS, 2010).

That is why the researchers of the world are actively busy in screening of plants for bioactivities with therapeutic usefulness. The selection of the plants for such a study is based on the traditional therapeutic claim. The treatment of many of the diseases is mentioned in the traditional medical system. The Ayurveda has emphasized the importance of food in the management of diseases. It has been seen that the practitioner of the modern system encourages the use of dietary items in the management of chronic diseases (Elizabeth MW, 1996).

Our traditional literature of Unani and Ayurvedic medicine contains a vast knowledge and information about these herbal drugs. The Charaka Samhita (1000 B.C.), one of such ancient literatures, contains the medical information about 2000 herbal drugs. Some of the very important lifesaving drugs has been obtained from these herbs (Goyal BR, 2007).

In American health care, herbal drugs are a major component. There has been an exponential increase in the sales of herbal medicines. Worldwide sales of herbal
DISCUSSION

medicines have exceeded a staggering US dollars 40 billion per annum (Gilani A, 2002).

Herbal drugs are easily available in the local market and these drugs are being prescribed by local practitioners who are part of the community so the patient feels very comfortable in the presence of these practitioners. A large number of our modern medicines descend directly or indirectly from higher plants. No doubt, there has been a great advancement in the field of modern medicines but herbal drugs are still in practice of modern practitioners (Muhammad Aslam, 2013).

Oxidative stress is one of the leading cause of chronic diseases due to internal and external free radicals attack to the body homeostasis in both developed and developing countries. Some drugs which are uses to treatment of disease themselves causes side effect. Hence it is imperative to find out drugs with minimum side effect and for its effective antioxidant activity.

Plants are the source of antioxidants due to the presence of poly phenols, flavonoids and tannins which possess wide range of biological activities. Recent studies reveal that many flavonoids, polyphenols and tannins shows a significant total antioxidant activity, which further helps as organ protectives.

Lagenariasiceraria (Bottle gourd) is an important member of the Cucurbitaceae family. Lagenariasiceraria (Molina) Standley is commonly known as Bottle gourd (English) and lauki (Hindi), an excellent fruit in the nature having composition of all the essential constituents that are required for normal and good health of humans. The use of Lagenariasiceraria in Indian traditional medicine system is known for ages but there is paucity of information regarding on its antioxidant property in chronic diseases induced oxidative stress.

The fruits were collected and extract was prepared using continuous hot percolation, thus prepared extract was subjected to preliminary phytochemical studies, the results indicated that fruits possess carbohydrates, proteins, alkaloids, amino acids, steroids, glycosides, flavonoids, saponins, terpenoids, tannins, vitamin C (Ascorbic acid) and polyphenolic compounds.Since ethanolic extract of fruits showed dose dependent
antioxidant properties in all the in vitro models. Antioxidants having organ protective properties. Therefore, the present research work was carried out the protective role and antioxidant activity of ethanolic extract of the fruit of Lagenariasiceraria with the following pharmacological actions (Deshpande JR, 2007).

Before screening the test extract for in vivo antidiabetic, hepatoprotective, antidepressive and antioxidant activities, the extract was subjected to the acute toxicity studies as per OECD guidelines no. 420 (fixed dose method). There was no mortality found amongst the graded dose groups of animals and they did not show any toxicity or behavioral changes at a dose level of 2000 mg/kg. This finding suggests that the EELS were safe or non-toxic to rabbits. Hence, the LD50 dose was calculated by taking 1/10th of the maximum dose, i.e. 200 mg/kg and the other 2 doses which were half and double of the 1/10th dose, i.e. 100 mg/kg and 400 mg/kg, p.o were selected for the study.

**Effect of EELS on Alloxan induced Diabetes and its associated oxidative stress:**

In this work we studied the potential antidiabetic effect of EELS using the alloxan model, the most widely used and well described technique for induction of diabetes. Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetrione) is an oxygenated pyrimidine derivative and was originally isolated in 1818 by Brugnatelli and got its name in 1838 by Friedrich Wöhler and Justus von Liebig (Bhattacharya S, 2012). This is one of the usual substances used for the induction of diabetes mellitus apart from streptozotocin.

Alloxan acts as a cytotoxin for beta-cells of the islet of langerhans, causes diabetes by inducing cell necrosis (JornsA, 1997; LedouxSP, 1986). The Reactive Oxygen Species (ROS) mediates the cytotoxic action with the increase in cytosolic calcium concentration, leading to rapid beta-cells destruction (Szkudelski T, 2001). This results into decreased insulin secretion and elevated blood glucose level (DeewanjeeS, 2008). This causes an insulin-dependent diabetes mellitus (called "Alloxan Diabetes")
in these animals, with characteristics similar to type-1 diabetes in humans (Bhattacharya S, 2012).

Oxidative stress depicts the existence of products called free radicals and reactive oxygen species (ROS) which are formed under normal physiological conditions but become deleterious when not being quenched by the antioxidant systems (Fang YZ, 2002). There are convincing experimental and clinical evidences that the generation of reactive oxygen species is increased in both types of diabetes and that the onset of diabetes is closely associated with oxidative stress (Johansen JS, 2005; Rosen P, 2001).

Free radicals are formed disproportionately in diabetes by glucose autoxidation, polyol pathway and non-enzymatic glycation of proteins (Obrosova IG, 2002). Abnormally high levels of free radicals and simultaneous decline of antioxidant defense systems can lead to the damage of cellular organelles and enzymes, increased lipid peroxidation and development of complications of diabetes mellitus (Maritim AC, 2003).

The increase in the level of ROS in diabetes could be due to their increased production and/or decreased destruction by nonenzymic and enzymic catalase (CAT), reduced glutathione (GSH), and superoxide dismutase (SOD) antioxidants. The level of these antioxidant enzymes critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of complications in diabetes (Lipinski B, 2001).

Plant derived natural products such as flavonoids, terpenoids and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including antidiabetic and antioxidant activity. Realizing the fact this study was carried out to evaluate the antidiabetic activity of L. siceraria extract and vitamin C and zinc in this direction.

In present study, rabbits were selected as experimental animals as they were easy to handle because of their size and temperament, economical, large volume of blood can be taken from them at any stage of the experiment, the physiology of the rabbits is similar to human being.
Rabbits were made diabetic with a single dose of alloxan monohydrate (150mg/kg b.w.) infused via the ear vein of rabbit (Akhtar MS, 2002). After three days blood glucose level of surviving rabbits was measured and rabbits with blood glucose levels between 250-300 mg/dl were used for further study (Olajide OA, 1999; Shani J, 1974).

Normal control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight at 7th and 14th days. Alloxan mediated body weight reduction was significantly reversed by the ethanolic extract in dose dependant fashion (at 100, 200 and 400mg/kg). The effect of combination therapies on body weight of the animals was also found statistically significant. Results are shown in Table 22.

Proteolysis, lipolysis and acute fluid loss during diabetes pave the way for weight loss (Alberti KG, 1998). The weight gain in extract treated groups reflects the correction of body metabolism.

Glucose measurement is useful in the diagnosis and treatment of pancreatic islet cell carcinoma and of carbohydrate metabolism disorders, including diabetes mellitus, neonatal hypoglycemia, and idiopathic hypoglycemia.

The changes in blood glucose and insulin levels reflect abnormalities in β-cells function and its structure. There are studies which show that Alloxan causes a glucoseoxidation and reduction in insulin release by the destruction of β-cells of the islets of langerhans (Siyem et al., 2002).

From the results obtained, it is evident that the diabetic rabbits had much higher levels of fasting blood glucose, malondialdehyde and decreased levels of superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) when compared with normal control rabbits. These adverse changes were reversed to near normal values in ethanolic extract of the fruit of Lagenariasiceraria monotherapy and combination therapy (Table 17-21). The possible mechanism by which ethanol extract brings back its normoglycemic action may be by induction of pancreatic insulin secretion from β cells of islets of langerhans or due to enhanced transport of blood glucose to peripheral tissue (Hakkim FL, 2007). Earlier many plants have been
studied for their hypoglycemic and insulin release stimulatory effects (Kota KR, 2015). It is well known that CAT, SOD and GSH play an important role as protective enzymes against free radical formation in tissues (Oberly WR, 1974).

Enzymatic antioxidant such as SOD and CAT are considered primary enzymes since they are involved in the direct elimination of ROS (Arulselvan P, 2007). SOD is an important defense enzyme and scavengers O$_2$ anion from H$_2$O$_2$ and hence diminishes the toxic effects due to this radical or other free radicals derived from secondary reaction (Manonmani G, 2005). CAT is a haemoprotein, which catalyzes the reduction of hydrogen peroxides (Punitha ISR, 2005). The antioxidant enzymes such as SOD and CAT are known to be inhibited in diabetes mellitus as a result of non-enzymatic glycosylation and oxidation (Al-Azzawie H, 2006).

GSH is by far the most important antioxidant in most mammalian cells. This ubiquitous tripeptide, γ-Glu-Cys-Gly, performs many cellular functions. In particular, the thiol containing moiety is a potent reducing agent (Klaus Apel, 2004). GSH has the important function of destroying reactive oxygen intermediates and free radicals that are constantly formed in metabolism (Alton Meister, 1983).

In the present study, the activities of SOD, CAT, and GSH decreased in diabetic rabbits as reported earlier, which could be due to inactivation caused by alloxan generated ROS (Sepici A, 2004). The ethanol extract of Lagenariasiceraria had reversed the activities of these enzymatic and non-enzymatic antioxidants, which might be due to decreased oxidative stress as evidenced by decreased lipid peroxidation.

The preliminary phytochemical investigation of the EELS revealed the presence of alkaloids, flavonoids, saponins, cardiac glycosides, triterpenoids, phenolic compounds and tannins. However, glycosides, flavonoids, tannins, organic sulphur compounds, catechol and alkaloids are active ingredients of hypoglycemic plant (Oliver B, 1980). Flavonoids are reported to regenerate the damaged pancreatic beta cells (Chakravarthy BK, 1980). Phenols have found to be effective antihyperglycemic agents (Manickam M, 1997).
HPTLC analyses (Spectral analysis) of plant extract exhibiting Oleanolic acid, βsitosterol, Campesterol, Isoquercitrin, quercertin, kaempferol. Quercetin is an important flavonoid known to possess an enormous array of pharmacological activities. The main mechanism of action of flavonoids, as a rule, is antioxidant activity, and a number of quercetin’s effects appear to be due to its antioxidant activity.

Quercetin probably acting either by the insulinomimetic activity or increasing the insulin secretion. This assumption was supported by the earlier study, grape seed derived procyanidins, a bioflavonoid produced the anti-hyperglycemic effect in streptozotocin induced diabetic rats by insulinomimetic activity in insulin sensitive cell lines. Quercetin scavenges oxygen radicals (Saija et al., 1995), and lipid peroxidation in vitro (Chang et al., 1993; Chen et al., 1990). Quercetin, a well known bioflavonoid with promising antioxidant property, it might have reduced the oxidative stress and improved the antioxidant defense status in diabetic rabbits. In this study, the extract presumably exhibited protective effects by interfering with alloxan-mediated oxidative stress through insulinomimetic activity or increasing the insulin secretion and antioxidant activity.

**Effect of EELS on CCl₄ induced hepatotoxicity and its associated oxidative stress:**

The liver may be considered as the most important organ in drug toxicity for two reasons: first it is functionally important site of absorption and the systemic circulation and second it is a major site of metabolism and elimination of foreign substances; but on the other hand these features also render it a preferred target for drug toxicity.

A number of chemicals including various environmental toxicants and clinically useful drugs can cause severe cellular damages in different organs of our body through the metabolic activation to highly reactive substances such as free radicals. CCl₄ is one of such extensively studied environmental toxicant (WebwrlW, 2003). Up to the present time, the etiology and treatment of most liver diseases are not known. The liver is the commonest site affected during the toxic manifestation of
many drugs. Toxicity in liver due to CCl$_4$ and other chemicals is attributed to the toxic metabolites formed, responsible for the initiation of CCl$_4$ dependent lipid peroxidation, the nature of which is not yet unambiguously determined. The most likely candidate is the trichloromethyl radical (Recknagel RO, 1989).

In the liver, CCl$_4$ is metabolized by the cytochrome P450-dependent monooxygenase systems to produce the trichloromethyl free radicals, which in turn covalently binds to cell membrane and organelles to elicit lipid peroxidation (Recknagel RO, 1989). It has been evident that several phytoconstituents have the ability to induce microsomal enzymes either by accelerating the excretion of CCl$_4$ or by inhibition of lipid peroxidation induced by CCl$_4$(Mehta RS, 1999). Thus, the present study was conducted to evaluate the hepatoprotective effect of the L. siceraria against CCl$_4$ induced hepatic damage and their associated oxidative stress in rabbits. So, that various biochemical and antioxidant parameters were used to assess the liver damage.

Carbon tetra chloride was obtained from market. Since CCl$_4$ is a hepatotoxic agent, it induces hepatitis in the animals. Hepatitis produces anorexia and decreases in the body weight, Therefore assessment of weight loss was done in all the groups.

The effects were more prominent in EELS monotherapy and combination therapy pre-treated groups. Graph 17 shows the effect of EELS on body weight of CCl$_4$ induced hepatotoxic rabbits. The disease control group exhibit gradual weight loss as compared with the normal group while weight gain was observed in extractpre-treated groups. 18.07% and 20.11% weight gain was noticed in combination therapy groups such as 9 and 10$^{th}$ groups. Our findings regarding body weight of animals were supporting the earlier reports (Deshpande JR, 2008).

Results of the present work indicate that CCl$_4$ raised the levels of SGPT, SGOT, ALP, bilirubin (TB & DB) and reduced the total protein in rabbits that received CCl$_4$ as compared to the normal control group. Such elevation suggests that toxification was able to reach the liver and induce a detectable damage, as previously reported by Hukkeri and his colleagues., (2002) who proved the elevation in the plasma level of cytoplasmic and mitochondrial enzymes due to liver injury induced by CCl$_4$(HurkeriVI, 2002). This elevation could potentially be attributed to the release of
these enzymes from the cytoplasm into the blood circulation after rupture of the plasma membrane and cellular damage (ShaarawySM, 2009).

Administration of silymarin or EELS in the present work significantly reduced the activity of liver enzymes (SGPT, SGOT, ALP), bilirubin and significantly increased the total protein in CCl₄ induced rabbits, a finding which is consistent with those shown before by Pradeep et al., (2007) and are almost definitely suggestive of protection of the structural integrity of the hepatocytes membrane or regeneration of damaged liver cells by test samples (Pradeep K, 2007; Patrick-IwuanyanwuKC, 2007).

Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and free radical scavenger i.e. reduced glutathione (GSH) protect the biological systems from oxidative stress. The current study showed a significant decrease in SOD, CAT and GSH activity in rabbits intoxicated with CCl₄ as compared to pre-treated groups. On the other hand, there was a significant increase in SOD, CAT and GSH activities in groups that received silymarin and EELS as compared to the intoxicated group. This improvement in the antioxidant status approached or even exceeded the control counterparts, a finding that may explain the modulatory effect of silymarin and EELS involves the maintenance of antioxidant capacity in protecting the hepatic tissue against oxidative stress (ShaarawySM, 2009). This suggests that EELS can reduce ROS that might lessen the oxidative damage to the hepatocytes and improve the activities of the liver antioxidant enzymes, thus protects the liver from carbon tetrachloride.

Hepatoprotective activity of EELS noticed in the present study might be due to its property of reducing oxidative stress. The possible mechanism may be by prevention of process of lipid peroxidation and stabilization of the hepatocellular membrane which may be due to its various antioxidant constituents like flavonoids, vitamin C, and beta carotene as well as due to the presence of phenolic compounds, terpenoid compounds and especially due the fucosterol and compesterol (Deshpande, 2008; Singh S, 2012). In other studies Lagenariasiceraria showed hepatoprotective effect as it significantly prevented derangement of biochemical parameters. The results of the present study are in accordance with the findings of Deshpande et al, Lakshmi BVS et
al and Elisha EE et al although they studied effect of EELS against carbon tetrachloride (CCl₄) induced liver damage.

**Effect of EELS on depression and its associated oxidative stress:**

Depression is a common, debilitating, life-threatening illness with a high incidence associated with lot of morbidity. Hence, it is very important to address this problem and find effective remedies. Even though several drugs are available, they associated with side effects including tricyclics, selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs) and atypical antidepressants.

Therefore, there is an urgent need for alternative medications for the control of depression-related disorders. The present work was subjected to investigation for the evaluation of the anti-depressant activity of ethanolic extract of the fruit of L. siceraria in animal models.

On the basis of the clinical association of depressive episodes and stressful life events, many of the animal models for the evaluation of antidepressant drug activity assess stress-precipitated behaviors.

In the present study forced swimming test (FST) and tail suspension test (TST) were carried out which are widely used, accepted and simple models for screening the antidepressant activity. The immobility is noted as a measure of antidepressant action also swimming behaviour corresponds to the behavioural despair as seen in human depression (Wankhede S, 2009). It was reported that the major depressive disorder involves disturbances of lifestyle, emotional changes, autonomic and endocrine functions affecting about 20% of population (Mayur B, 2011).

In phytochemical study we observed the presence of flavonoids (flavonolglycosides) in L. siceraria fruit extract. Recently several studies have suggested the antidepressant effect of flavonol glycosides (Lei A, 2008). Therefore one of the antidepressant mechanisms of L. siceraria fruit extract is thought to involve flavonoids, which exerts an antidepressant effect.
The oral administration of EELS was found to be effective in reducing the mean immobility time in rats, indication potential as antidepressant activity. It is accepted that immobility seen in rodents during swimming reflects behavioral despair as seen in human depression and that the antidepressant drugs are able to reduce the immobility time in mice (Wankhede S, 2009).

The Forced swim test is a simple, reliable and specific model to screen antidepressant activity. This model shows a positive response to acute antidepressant treatments. The mean duration of immobility time is noted as a measure of antidepressant activity. Many antidepressants and compounds possessing antidepressant activity show decrease in immobility time (Rosa AO, 2003; Cryan JF, 2002). The oral administration of EELS showed a positive effect in reducing the mean immobility time in FST, which is evident from the significant reduction in the immobility time by treatment of zinc (Rodrigues AL, 2005). We have explored the acute and subchronic antidepressant effects of Lagenariasieraria using the FST and TST animal models. After the acute treatment with Lagenariasieraria extract, the decrease in mean immobility time was observed in the FST and TST tests. The rats were treated with extracts for 7 days to investigate the subchronic effect of Lagenariasieraria extract and then subjected to FST and TST.

In the present study positive control group exhibit gradual weight loss as compared with the normal group while weight gain was observed in extract pre-treated groups of FST. Our findings regarding body weight of animals were supporting and correlate with the previous report of Deshpande JR. Whereas TST did not show any significant changes regarding body weight reduction during 7 days.

We observed that following pre-treatment of test formulations of ethanolic extract of the fruit of Lagenariasieraria demonstrated significant (compared to vehicle treated group) a dose dependant reduction in duration of immobility.

In FST in acute administration of sample under investigations demonstrated a dose dependant, statistically significant reduction in duration of immobility that was comparable to imipramine (15mg/kg). Such observations correlates with report of Brocco et al., that imipramine was found to be more effective in rat upon single
administration (Brocco M, 2006). The effect of combination therapy treated groups such as 7, 9 and 10th groups were had better than 15mg/kg imipramine (group 2).

However, in case of EELS treated animals at dose 400mg/kg (EELS) shows significant reduction in duration of immobility which was nearly equal to 15mg/kg imipramine in rat forced swim test, although 400mg/kg with imipramine was insignificant. The same results are reflected in antioxidant parameters also. The highly significant values were observed in animals received reference standard imipramine (15mg/kg) along with EELS 200 mg/kg.

In case of EELS treated animals, effect at dosage 400mg/kg with imipramine was insignificant when compared to vehicle treated group. This indicates that EELS at high dose (400mg/kg) along with imipramine (combined) formulations did not reduce duration of immobility and did not improve the antioxidant parameters in rat forced swim test. Hence, this test formulation may not have antidepressant effect and probably showing window effect.

In mouse tail suspension test it has been argued that the TST is less stressful than FST and has greater pharmacological sensitivity (Thierry B, 1986). In this study, rats are suspended by their tail for defined period of time (usually 6min.) and duration of their immobility is assessed. Typically, animal’s immediately engaged in escape oriented behaviour followed by progressive increasing period of immobility. We observed that following acute administration of test formulations demonstrated significant (compare to vehicle treated animals) a dose dependant reduction in duration of immobility. Furthermore the effect of L. siceraria extract in TST was similar to the effect produced by the oral administration of imipramine.

The effect of 100, 200 and 400mg/kg (EELS) was statistically significant when compared to vehicle treated animals. Effect of 400mg/kg (EELS) was nearly equal to 15mg/kg imipramine and 100mg/kg (EELS) shown minimize effect compared to imipramine (15mg/kg). This indicates that the effect of high dose was better than low dose of EELS test formulations in TST.

While EELS treated animals, effect at dosage 400mg/kg with imipramine (combination therapy) was insignificant when compared with vehicle treated group.
This indicates that EELS at high dose (400mg/kg) along with imipramine (combined) formulations did not reduce duration of immobility and did not improve the antioxidant parameters in rat tail suspension test. Hence, this test formulation may not have antidepressant effect and probably showing window effect. In both models such as FST and TST, at dosage 200mg/kg along with imipramine has shown superior effect to the reference standard imipramine alone.

Imipramine exhibited the characteristic behavioraleffects by noradrenaline and serotonin reuptake inhibition in the modified forced swim test, i.e. a decrease inimmobility coupled with an increase in swimming behaviour. Since antidepressant effects have been observed in severalflavonoids from Hypericum perforatum, it is possible thatthese polyphenolic substances might be responsible, atleast in part, for the antidepressant activity in study. Thus the flavonoids may be responsible for the said antidepressant activity of Lagenariasiceraria (Wankhede S, 2009; Butterweck V, 2000).

Remarkably, TST detects the anti-immobility effects of wide array of antidepressants, including tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors (SSRI), monoamine oxidase inhibitors (MAOI) and even atypical antidepressants. Thus, the activity of ethanolic extract of the fruit of Lagenariasiceraria could involve one of mechanism of established agents as described above. The obtained results of the present study were suggests that the TST is less stressful than FST and has greater pharmacological sensitivity.

Quercetin also protects antioxidative defense mechanism by increasing the absorption of Vitamin C (Vinson and Bose, 1998). Quercetin has been shown to inhibit structural damage to proteins (Salvi et al., 2001), the release and the protection of oxidative products generated by the respiratory burst in phagocytes (Zielinska et al., 2000). Quercetin has recently shown to be an INOS inhibitor, resulting in reduced nitric oxide (NO) and peronitrate generation (Autore et al., 2001). All the study findings were suggested that Lagenariasiceraria may have possible in vivo antioxidant activity in chronic diseases.