The use of plants in medicine is flourishing speedily. Even in the developed countries also people are returning to nature these days. Use of traditional medicine is the basis of primary healthcare, virtually in all developing countries. The main reasons behind the everyday use of traditional medicine are (a) the age old alliance of community with local flora and their faith on plants as a medicine, (ii) easy availability of local medicinal plants, (iii) comparatively poor or almost no access to other system of drugs and their relatively high cost and (iv) not as good financial profile of the rural people (Upadhyay et al, 2010). Within the context of above information we evaluated the antiurolithitic activity, nephroprotective and other complications of kidney like diabetic nephropathic and antihyperlipidemic activity were investigated by using in vivo techniques in order to assess the validity of the use of *Hordeum vulgare* Linn. seeds.

Results of our investigation have established that *Hordeum vulgare* Seeds possess nephroprotective, antiurolithitic, probably antidiabetic and diabetic nephroprotective, and antihyperlipidemic activities mediated through antioxidant activity. The activity could be attributed to the presence of saponins, flavonoids and tannins in the *Hordeum vulgare* seeds. As traditional to Phytopharmacological work, the dried seeds of *Hordeum vulgare* Linn. were received from commercial supplier of Anand, Gujarat, India. The seeds were authenticated by Dr. G. C. Jadeja, Professor & Head, Department of Agricultural Botany, B. A. College of Agriculture, Anand Agriculture University, Anand. A Voucher specimen (voucher no. IICP/11-JGS/03-HV) was deposited in the herbarium of the Department of Pharmacognosy, Indukaka Ipcoxala College of Pharmacy, New Vallabh vidyanagar, Anand, Gujarat, India. Quantitative limit tests like ash and extractive values are other parameters to standardize the herbal drugs (Bhutani, 2000). *Hordeum vulgare* seeds used in the study showed that the seeds contained 16.26 % of total ash, 6.53 % of acid insoluble ash, 2.67% of water soluble ash, 5.2 % of ethanol soluble extractives, 13.5 % of water soluble extractives, 4.5% of moisture content in the sample.

The authenticated samples of *Hordeum vulgare* seeds, the extract was prepared for carrying out the pharmacological activities. The yield of Ethanolic extract was found
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to be 4.5%. The ethanolic extract of *Hordeum vulgare* seeds was checked for their antiurolithitic activity, antidiabetic and its nephropathic complication, nephroprotective and antihyperlipidemic potential. Phytochemical evaluation was done for presence of various phytoconstituents. Preliminary chemical tests indicated presence of saponins, flavonoids, tannins, some phenolic compounds, carbohydrates, glycosides and alkaloids.

**Urolithiasis** or **Urinary stone** formation in kidney has ever been an annoying urinary disease for human. This disease is mainly the result of supersaturation of urine with certain urinary salts such as CaOx and phosphate, the most common constituent of kidney stones (Daudon et al., 1993). Kidney stone or urolithiasis is a complex process that results from a succession of several physicochemical events including supersaturation, nucleation, growth, aggregation and retention within renal tubules (Atmani et al., 2004). In present study, male rat is the most frequent used animal to induce urolithiasis because the urinary system of male rats resembles that human and also earlier studies have shown that the amount of stone deposition in female rats was significantly less (Bahuguna et al., 2009). So in current study we have also used male wistar rats for experimental purpose.

Different chemicals used to induced urolithiasis in experimental animals includes ethylene glycol, glycolic acid, insertion of calcium oxalate crystals in to urinary bladder, calculi-producing diet model using Gentamicin and 5% ammonium oxalate, insertion of zinc disc in to bladder, sodium oxalate (Perez et al., 1998; Bahuguna et al., 2009; Doddola et al., 2008). In this study we have evaluated the effectiveness of a medicinal plant *Hordeum vulgare* seeds on rats rendered urolithiasis by administration of Ethylene Glycol (EG), glycolic acid (GA) and surgically Calcium oxalate crystals induced urolithiasis.

EG is a high production volume chemical used in a variety of applications ranging from its major uses in the synthesis of polyethylene and polyester resins, fibers and films, to its better known uses as a constituent in antifreeze, deicing fluids, or hydraulic fluids. Over decades of use and testing, two main toxicological issues have emerged as the primary concerns for human exposure - developmental toxicity and renal toxicity. Renal toxicity, however, remains a primary concern for human
exposures as it occurs in all species studied at dose levels below those causing developmental toxicity in rats. EG and GA induced hyperoxaluria models have been employed in numerous studies of calcium oxalate nephrolithiasis and much of our current knowledge base in experimental hyperoxaluria and calcium oxalate kidney stone disease is based upon this model. EG is inexpensive and simple to deliver in drinking water where it is rapidly absorbed and metabolized in the liver via alcohol dehydrogenase/Aldehyde dehydrogenase to glycolic acid. And this Glycolic acid is oxidized to glyoxylic acid which in turn is further oxidized to oxalic acid by glycolate oxidase or lactate dehydrogenase, thus promoting hyperoxaluria (Green et al., 2005; Richardson and Tolbert, 1961). Although quantitative differences exist, the mode of action for kidney toxicity is essentially the same across species and involves three key events: (1) the metabolism of EG to oxalic acid via glycolic and glyoxylic acids; (2) precipitation of oxalic acid with calcium to form calcium oxalate (CaOX) crystals in the kidneys; and (3) degeneration of renal tubule epithelium due to ensuing physical trauma or localized oxidative stress (Corley et al., 2011). In addition to this, the interaction between renal epithelial cells and oxalate or calcium oxalate crystals plays a significant role in the formation, retention and development of calcium oxalate stone disease. In urolithiasis, oxalate or calcium crystals exposure resulted in a significant increase LDH release which is an indicator of cell injury. The cellular injuries potentiate calcium oxalate crystals formation (Thamilselvan et al., 2003). The experiments were performed using oxalate or CaOx monohydrate crystals, since CaOx crystals in the intact organ can not exist in the absence of Oxalate. Our data showed that kidney cells were injured when exposed to Oxalate or CaOx crystals which correlate with result from earlier studies (Scheid et al., 2000; Thamilselvam and Khan, 1998), studies from other laboratories have likewise indicate that oxalate and CaOx crystals are injurious to renal epithelial cell in culture (Verkoelen et al., 1997). As per the findings of our study, it can be suggested that Hordeum vulgare seeds protect against oxalate and CaOx crystals induced cell damage to renal epithelial cell.

Kidney stone is hard, solid pallets that form in the urinary tract that can cause one of the most painful ailments and there is a high incidence and rate of recurrence. Very small size stone can pass out the body without any pain induction. But if the stone may lodged and block the flow of urine, excruciating pain may result and prompt
medical intervention may be needed. Body weight loss observed in untreated group is due to anorexia due to disturbances in carbohydrates, proteins or fat metabolism which is affected by the induction of calcium oxalate stone (Hodgkinsons, 1977). In present study there was significant decrease in body weight in calculi control animals. This condition was improved by test drugs. Stones lodging in the tubules may lead to decrease in the urinary output and their presence in the kidney lead to increase in weight of that organ. Similar results were observed in the present study.

Acidic urine is usually found in humans with idiopathic renal calcium oxalate stone formation, whereas chronic hyperoxaluric rats had alkaline urine (Huang et al., 2000). The mechanism of alkaline urine production after ethylene glycol or glycolic acid treatment and its possible correlation with nephrolithiasis in this rat model remains unclear and needs further study. In experiment there was significant increased in urine pH in calculi control group compared to normal control group while in all other groups who received treatment of standard cystone and Hordeum vulgare seeds shown a significant decrease in pH. Urinary volume is markedly increased in experimental groups. The increased urine volume in all the drug treated groups might be due to diuretic effect of the drug. Similar results are also observed when lupeol is used as an antilithiatic agent (Anand et al., 1991). Diuresis reduced the risk of stone formation by reducing the saturation product of calcium oxalate (Selvam et al., 2001).

Hyperoxaluria is a more significant risk factor in the pathogenesis of renal stone. It has been reported that oxalate play an important role in stone formation and has about 15-fold greater effect than urinary calcium. (Karadi et al., 2006; Soundararajan et al., 2006). Previous studies indicated that, administration of ethylene glycol and glycolic acid to the young albino rats resulted into the formation of renal calculi composed mainly of calcium oxalate (Selvam et al., 2001; Huang et al., 2002; Atmani et al., 2003). And thus, there is an increase in the urinary concentration of oxalate. Stone formation in ethylene glycol and glycolic acid fed is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate (Karadi et al., 2006). Renal calcium oxalate deposition by EG and GA in rats is frequently used to mimic the urinary stone formation in humans. In the present study, urinary oxalate was increased in urolithic rats. The reduction in oxalate excretion was observed on EHV treatment dose dependently.
In the present study, oxalate and calcium excretion are progressively increased in calculi-induced animals. Since it is accepted that hyperoxaluria is a far more significant risk factor in the pathogenesis of renal stones than hypercalciuria (Tisselius, 1996), the changes in urinary oxalate levels are relatively much more important than those of calcium (Robertson and Peacock, 1980). Several studies have shown that, crystal formation results in cell damage and cell detachment from the basement membrane and the released degradation products further promote nucleation of crystals (Hackett et al., 1990; Verkoelen et al., 1998). Increased urinary calcium is a factor favoring this nucleation and precipitation of calcium oxalate or apatite (calcium phosphate) from urine and subsequent crystal growth (Lemann et al., 1991).

Asper (1984) reported that not only calcium and oxalate excretion but excretion of inorganic phosphate is also important in the formation of urinary stone. The available report stated that high urinary phosphate level with calcium forms calcium phosphate crystals, which induces further deposition of calcium oxalate on it (Roger et al., 1997). Decreased renal injury further decreases sites for calcium oxalate deposition. However, Administration of *Hordeum vulgare* seeds to calculi induced rats prevented supersaturation of calcium oxalate and thus decreased their deposition in renal tubules. In the present study, the high urinary phosphate level observed in calculi induced rats as compared to normal control group rats but this was significantly prevented as well as reversed with the treatment of *Hordeum vulgare* seeds.

Normal urine contains many inorganic and organic inhibitors of crystallization; Magnesium is a well-known inhibitor of crystallization in urine. Low levels of magnesium are also encountered in stone formers as well as in stone-forming rats (Selvam et al., 2001; Robertson et al., 1985; Rushton & Spector, 1982). Diets high in magnesium have been found to protect against deposition of calcium oxalate in the kidneys of urolithiatic rats. Magnesium complexes with oxalate and reduce the supersaturation of calcium oxalate by reducing the saturation of calcium oxalate and as a consequence reduces the growth and nucleation rate of calcium oxalate crystals (Soundararajan et al., 2006; Selvam et al., 2001). Our study also revealed a similar observation. Thus, urinary magnesium was significantly diminished in EG and GA induced urolithic rats. The treatment with *Hordeum vulgare* seeds restored the
magnesium excretion and thus reduces the growth of calcium oxalate crystals in calculi induced rats in dose dependent manner.

Low urinary citrate excretion is known risk factor for the development of kidney stone. It is the major metabolic abnormality in patients with renal stones. Urinary citrate plays an important role in reducing the recurrence of CaOx stones, and it has been shown that urinary citrate can inhibit CaOx supersaturation via the formation of complexes with calcium and the direct inhibition of crystal growth via aggregation (Chow et al., 2004). Citrate is a known inhibitor for stone formation, working through variety of mechanism. In the renal tubules citrate complexes with calcium, increasing its solubility and reducing the concentration of free calcium in urine. This calcium-citrate complex limits calcium supersaturation and prevents nucleation of both calcium oxalate and calcium phosphate, at least partly through interaction with protein. Additionally, citrate prevents crystal agglomeration and growth through its ability to bind to the crystal’s surface and may prevent adhesion of calcium oxalate to renal epithelial cell (Zuckerman and Assimos, 2009). Investigations of citrate metabolism in stone formers have shown that tubular citrate reabsorption is the main mechanism regulating urinary citrate excretion (Soundararajan et al., 2006). A decreased level of urinary citrate was observed in calculi induced urolithic rats. The EHV treated rats brought the urinary citrate excretion to normal as compared to calculi control rats. The present study also showed an increase in the urinary output by the EHV at higher dose. The total volume of urine increased in 24 hr in treated group in comparison with the calculi control group. So this effect dilutes the concentration of the urinary electrolyte. As result of this calcium and phosphorus flush out from the urine and there are a lesser chances of precipitation and decreased formation as well as growth of urinary stone.

The increase in urinary uric acid excretion was observed in urolithic rats. Increased excretion of uric acid has been reported in stone formers and hyperoxaluric rats. Uric acid interferes with calcium oxalate solubility and it binds and reduces the inhibitory activity of glycosaminoglycans (Selvam et al., 2001). The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggests its primary role in stone formation (Selvam et al., 2001; Soundararajan et al.,
Treatment of cystone and *Hordeum vulgare* seeds lowered the excretion of uric acid and reduces the risk of stone formation dose dependently.

The glomerular filtration rate decreases in urolithiasis due to the obstruction to the outflow of urine by stones in urinary system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine and uric acid get accumulated in blood. In urolithiatic rats, elevated levels of serum urea, uric acid and creatinin indicate marked damage of kidney. The uric acid crystals adsorb glutamic acid and other organic compounds and promote calcium oxalate crystals growth (Purnima et al., 2010). In calculi control rats, marked renal damage was seen as indicated by the elevated serum levels of creatinine and uric acid which are markers of glomerular and tubular damage. While, calculi animals received treatment of standard and EHV there was significant decreased in the level of uric acid. However, the reversal and prophylactic treatment with *Hordeum vulgare* seeds and cystone had significantly reduced serum levels of urea, uric acid and creatinine.

It was reported that EG induced hyperoxaluria promotes oxidative stress (Touhami et al., 2007). It has been postulated from several *in vitro* and *in vivo* studies that high levels of oxalate may have a detrimental effect on renal architecture by producing superoxide and hydroxyl radicals, leading to redox imbalance and has been manifested as intracellular oxidative stress, followed by changes in membrane structure and cell death (Karadi et al., 2006). These changes facilitate CaOx crystal adherence and retention in renal tubules (Khan, 1985).

Our biochemical analyses were also supported by histopathological examinations. In the urolithiasis control group, structural abnormalities like intratubular crystal depositions inside the tubules and degenerative tubular structures were determined, whereas there were no intratubular crystal depositions and degenerative tubular structures in *Hordeum vulgare* seeds and Cystone treated rats. Our results suggest that *Hordeum vulgare* seeds have both protective effect on tubular structures and preventive effect on intra tubular crystal deposition in urolithiasis model of rats. Also, previous histopathological studies showed EG induced crystal deposition in the renal cells and most of crystal deposition took place in the renal tubules, which corroborates
the results of other studies reporting that crystals deposition mainly occur in tubules are in line with our results (Khan et al., 2006; Kaur et al., 2009).

According to Freitas et al., (2002) in the experimental model of urolithiasis (surgical model), insertion of visceral foreign body i.e. calcium oxalate crystal/seeds in urinary bladder leads to crystal growth after fourteen days in the calculi control animals i.e. urolithiasis with no significant metabolic and systemic alterations. Many factors are involved in the pathogenesis of urolithiasis. The visceral CaOx crystals/seeds acts as a supporting surface, allowing organic and inorganic material to precipitate over the central nidus thereby mimicking a spontaneous calculus growth. Although the presence of a supersaturated milieu is necessary for precipitating CaOx (present in most calculi) acting as a promoter of crystal formation, this is not enough to form a stone, as urine is normally a supersaturated solution and only some individuals are prone to this disease. In fact, increase of diuresis could reduce supersaturation of the urine with precipitating substances which is normally associated with formation of urinary calculi (Fleish et al., 1978; Smith et al., 1992). The growth of the crystals was confirmed by x-ray analysis. With the help of x-rays analysis confirmed the presence of CaOx in main calculi; this suggests that the satellite probably grew on small fragments released from main stone. The treatment with test drug *Hordeum vulgare* seeds and standard drug interfered with crystals deposition and substantially modified stone shape. *Hordeum vulgare* seeds also caused significant diuresis and preventing the growth of stone.

Urinary stones were accompanied by a proportional hypertrophy of the urinary bladder smooth musculature. Such effect indicates increased contraction of the musculature probably to overcome obstruction of the bladder outlet by the formed calculi. Partial obstruction of the urinary bladder outlet leads to a compensatory growth of the detrusor smooth muscle cells, and occurs as a response to the increased intravesical pressure required to empty the bladder (Gabella et al., 1990; Levin et al., 1995). In surgery model similar preventive changes were observed in levels of promoters and inhibitors to that of various treatment groups in ethylene glycol and glycolic acid induced urolithiasis model. Kidney function tests also showed significant prevention in damage with the treatment of test drug and standard drugs.
This finding raises the possibility for an alternative use of *Hordeum vulgare* seeds, to induce changes in calculi that might aid in elimination and/or dissolution of calculi.

Stone inducing treatment caused hypertrophy and extensive CaOx crystal deposition in kidneys of untreated rats accompanied by oxidative damage as reflected from increased levels of markers of oxidative injury: Malondialdehyde (MDA) and diminished level of total protein content and reduced activities of antioxidant enzymes like Superoxide dismutase (SOD) and catalase (CAT) in kidneys. Membrane damage due to lipid peroxidation or the depletion of cellular antioxidant has been suggested to be a predisposing factor for CaOx crystal deposition (Selvam and Bijikurien, 1991). According to Huang et al. (2003) lipid peroxidation has been observed to correlate with hyperoxaluria and renal tubular damage, indicating that hyperoxaluria can induce tubular cell injury and that injury may be caused by the production of free radicals in patients with CaOx stones. The significant increase in the lipid peroxidation contents with CaOx stones and hyperoxaluria in the calculi induced rats agree with the findings of previous report. In addition, another study has reported that free radical damaged cells produce a favorable environment for crystal development and that phytic acid prevents CaOx crystallization by its antioxidant properties (Grases et al., 1998). Therefore, the antioxidant action of herbal extracts could be of importance in explaining their antilithiatic action, particularly if the formations of these calculi are induced by lesions that have been caused by cytotoxic substances with oxidative capacities (Grases et al., 2009).

The significant lowering of serum levels of accumulated waste products is attributed to the enhanced GFR and the anti-lipid peroxidative property. Ethylene glycol treatment may cause the systemic generation of reactive oxygen species. The systemic circulation may damage some part of the kidney, probably the mesangial or glomerular epithelial cells, and cause the kidney to produce more reactive oxygen species than systemic blood. Therefore, the excessive oxidative stress would have been completely compensated for by elevated antioxidant enzymes in the kidney during this period (Huang et al., 2003). Preventive anti-oxidants, such as catalase and superoxide dismutase enzyme is the first line of defense against reactive oxygen species. Catalase is considered an antioxidant enzyme because it regulates H$_2$O$_2$ levels, which can lead to hydroxyl radical excess through the metal catalyzed Fenton
(Fe/Cu) and Haber-Weiss reactions (Pippenger et al., 1998). The liberation of xanthine oxidase during uric acid formation is the key operator for the elevated release of hydrogen peroxides (Farooq et al., 2005). The decreased activities of catalase in the nephrolithiasis in this model may have led to more H$_2$O$_2$ accumulation in the kidney, resulting in more hydroxyl radical formation, because catalase is the only enzyme that regulates the potent hydroxyl radical (Pippenger et al., 1998). As per Karadi et al., (2006) mentioned that the treatment with antioxidants was reported to reduce hyperoxaluria and resultant oxidative stress in rats. Administration of *Hordeum vulgare* seeds results in significant decrease in lipid peroxidation and significant increase in the catalase and superoxide dismutase levels in lithogenic animals. That suggests that *Hordeum vulgare* seeds as excellent antioxidants for the kidneys, which protect the renal cells from oxidative stress induced injury and also prevent oxalate induced free radical damage.

The result showed the anti-nephrolithiatic potential of *Hordeum vulgare* seeds in ethylene glycol, glycolic acid and calcium oxalate induced urolithiatic model. Results indicate that administration of *Hordeum vulgare* seeds reduced and prevented the growth of urinary stones. It seems that the *Hordeum vulgare* seeds effective in prevention as well as reversal of crystal aggregation and which may be result of combination of various effects like diuresis, decrease in promoter’s level, increase in inhibitors level. Therefore, the Ethanolic extract of *H. vulgare* seeds is helpful to prevent the recurrence of the disease as it showed its effect on early stages of stone development.

In the present study, nephrotoxicity induced by Gentamicin was evidenced by depletion of urine volume and increases in kidney weight, serum creatinine, serum urea and blood urea nitrogen concentrations.

Nephrotoxicity occurs as a disturbance in renal function due to various adverse drug interactions, inadequate elimination of radioactive contrast materials and chemicals. It is of great concern in patients with renal failure. Nephrotoxicity may limit the clinical usefulness of many diagnostic and therapeutic agents; recognition of factors associated with higher risk for renal injury is of great importance. However, the end point of nephrotoxicity is always cell death; therefore, it is important to identify the
mechanism in addition to the site of action, in order to formulate a strategy for
damage prevention. In kidney proximal tubular cells are the major site of damage in
patient treated with the Gentamicin (Hell et al., 2009). Gentamicin binds with cell
wall phospholipids, blocking the chain reactions of phosphotidyl inositol which
impairs cell integrity. It results by generation of reactive oxygen species (ROS)
(Keeling et al., 2001). The strategies aimed at ameliorating the nephrotoxicity are of
clinical interest (Bibu et al., 2010). The use of Gentamicin, an aminoglycosides
antibiotic with a wide spectrum of activity against gram-positive and gram-negative
bacterial infections but with high preference for latter is equally associated with
nephrotoxicity as its side effect (Apple, 1982; Barry, 2000). The Gentamicin induced
nephrotoxicity is well established experimental model for drug induced renal injury
(Parlakpinar et al., 2005, Harlalka et al., 2009, Cojocel, 1997). Many animal
experiments have demonstrated overwhelmingly, the positive correlation between
oxidative stress and nephrotoxicity (Devipriya and Shyamaladevim, 1999).
Gentamicin induced nephrotoxicity by causing renal phospholipidosis through
inhibition of lysosomal hydrolases such as sphingomylinase and phospholipases in
addition to causing oxidative stress (Cojocel, 1997; Lindquist, 1986).

Drug induced nephrotoxicity are often associated with marked elevation in blood
urea, serum creatinine and acute tubular necrosis (Giuliano et al., 1984). In the
present study, drug induced nephrotoxicity were established by single daily
intraperitoneal injection of Gentamicin. This toxicity characterized by marked
elevation in circulating levels of urea, serum creatinine and histological features of
tubulo nephritis in model control rats when compared with untreated normal rats.
However these changes were attenuated by pretreatment with single daily graded
dose of *Hordeum vulgare* seeds for 10 days. In renal disease, the serum urea accumulates
because the rate of serum urea production exceeds the rat of clearance. Elevation of
urea, blood urea nitrogen (BUN) and creatinine levels in serum was taken as the index
of nephrotoxicity. The serum urea concentration is often considered a more reliable
function prediction than serum creatinine. The cationic form of aminoglycosides
attaches to the acidic phospholipids in brush border enzymes this results in the
leakage of intracellular ions (K\(^+\), Mg\(^+\) and Ca\(^++\)), proteins and enzymes this results in
the decreased glomerular filtration rate (Yoshiyama et al., 1992). As per Ali (1995)
Gentamicin is actively transported into proximal tubules after glomerular filtration in
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a small proportion where it causes proximal tubular injury and abnormalities in renal circulation that leads to reduction of GFR. The decreased glomerular filtration rate which shown by an increase serum urea, blood urea nitrogen and creatinine (Al-majed et al., 2002). The results of this study show that GM administration to rats produced a typical pattern of nephrotoxicity which was manifested by significant increase in serum urea, blood urea nitrogen and creatinine as compared to normal control. Administration of EHV 100, 250 and 500 has showed significant decrease in serum creatinine whereas EHV 250 and 500 was showed significant decrease serum urea and serum BUN as compared to GM group.

In the pathogenesis of GM nephrotoxicity oxidative stress is probably the most common pathogenic (Stojiljkovic et al., 2008). Recently, ROS were considered to be important mediators of Gentamicin-induced nephrotoxicity (Priyamvada et al., 2008). It has been found that the Gentamicin-treatment increases H$_2$O$_2$ production and it is known that H$_2$O$_2$ and O$_2^-$ induce mesangial cells contraction, altering the filtration surface area and modifying the ultra filtration coefficient factors that decrease the glomerular filtration rate (GFR). O$_2^-$, this radical can react with nitric oxide (NO) to form peroxynitrite, cytotoxic oxidant radical species. The inactivation of NO by O$_2^-$ could also lead to a decrease in the GFR. It has been suggested that the oxidative stress induces tubular damage. It is known that the increase in ROS levels induces cytotoxicity due to a concerted action of oxygen and nitrogen-derived free radicals. The reduction in renal antioxidant enzymatic defense could aggravate the oxidative damage in rats. In the present study Gentamicin has significant increased the malondialdehyde levels while decrease Superoxide dismutase, reduced glutathione and catalase activities as compared to normal control. The administration of *Hordeum vulgare* seeds showed significant decrease in malondialdehyde level whereas increase the superoxide dismutase, reduced glutathione content and catalase activities as compared to Gentamicin control animals. *Hordeum vulgare* seeds attenuate the Gentamicin induced nephrotoxicity probably mediated by its anti-oxidant properties.

In histopathological study of normal group some blood vessels were dilated and congested within the interstitium. Also few scattered mononuclear inflammatory infiltration is seed within the interstitium. GM treated group showed sever proximal tubular necrosis, loss of lining epithelium along with mononuclear cell infiltrations,
diffuse glomerular and blood vessels congestion. In *H. Vulgare* 500 treated group, varying degrees of regeneration and only small foci of mononuclear infiltration could be seen within the interstitium. From the histopathological studies we can conclude confirm the *H. vulgare* seeds showed predominantly regenerative and protective effect on Gentamicin induced nephrotoxicity.

**Diabetes mellitus** is a worldwide problem, with the increasing patients and limited therapeutic options; **diabetic nephropathy (DN)** is a long term complication of diabetic mellitus. The precise mechanism of diabetic nephropathy is not yet fully understood and the effective blockade of the progression of nephropathy remains as a therapeutic challenge. Diabetic nephropathy is one of the most serious complications of diabetes and the most common cause of end-stage renal failure. DN is characterized by specific renal morphological and functional alterations, increase of urine protein excretion and renal dysfunction. Thus, increased urine protein is a key component of DN (Ziyadeh and Sharma, 2003). Many studies have shown that the magnitude of urine protein level is associated with a graded increase in the risk of progression to end-stage renal disease and cardiovascular events (Lea et al., 2005). Reactivated Oxygen Spices (ROS) play an important role in high glucose induced renal injury (Ha & Lee, 2000; Alkami et al., 2001). Oxidative stress induced by hyperglycemia in diabetics is considered a major cause in the development and progression of diabetic microvascular complications, such as nephropathy (Evans et al., 2002; Yorek, 2003).

There is a highly significant association of urolithiasis with type 2 diabetes mellitus. There may be several mechanisms increasing the incidence of urolithiasis in DM. First of all, chronic hyperglycemia may cause a chronic low-grade inflammation in gastrointestinal epithelium by disturbing by distributing the normal balance between intestinal flora and circulatory defence mechanisms. The low grade inflammation induced increase absorption of oxalate, as seen in chronic diarrheal illness, may be the development mechanism of urolithiasis in DM. autonomic neuropathy induced motility disorders may aggravate this instability, since diarrheal fluid losses induced low urinary pH and citrate levels increase urinary CaOx and uric acid supersaturation, because citrate may inhibit calcium crystallization by binding to it. Even gastrointestinal and urinary tract’s epithelial edema, developed secondary to diabetic
nephropathy induced hypoalbuminemia, may aggravate the absorptive and secretory
instabilities. Secondarily, chronic hyperglycemia may disturb both gastrointestinal
and urinary tract’s epithelial functions for absorption and excretions of elements, so
facilitating directly a stone or just a nidus formation for stone. Even glycosuria
induced electrolyte imbalance in urine may facilitate urolithiasis. Thirdly,
immunosuppressant secondarily to DM and chronic glycosuria induced urinary tract
infections may cause urolithiasis, since some type of bacteria can provoke urinary
 supersaturation and modify the environment, thus leading to formation of crystals
deposits that may be factor promoting urolithiasis. Diabetic nephropathy induced
glomerular dysfunction may alter urinary content, facilitating urolithiasis (Davarchi,
2011).

Streptozotocin (STZ) can induce experimental diabetes mellitus (Type 1, IDDM) by
selectively destroying insulin-producing pancreatic endocrine cells. The glucose
 moiety of STZ allows preferential uptake of STZ into β-cells, probably via the
glucose transporter-2 (GLUT-2), which are abundantly expressed in rodent β-cells of
the pancreas (Thulesen et al., 1997). STZ-induced diabetic nephropathy syndrome
characterized by proteinurea and the loss of renal function with elevated blood
glucose level and insulin level (Yu Cai et al., 2010). In Type II (NIDDM) diabetes
Mellitus, it has been reported that administration of nicotinamide, a poly-ADP-ribose
 synthetase inhibitor, protected the islets’ functionality by protecting the decrease in
the levels of NAD and proinsulin thereby partially reversing the inhibition of insulin
secretion to prevent the aggravation of experimental diabetes following the
administration of β-cells toxins, such as, streptozotocin and alloxan (Shima et al.,
1998). This condition contributes a number of features similar with Type II diabetes
mellitus, and is exemplified by stable hyperglycemia, glucose intolerance, and
significantly altered glucose-stimulated insulin secretion both in vivo and in vitro
(Masiello et al., 1998).

In STZ induced and STZ-nicotinamide induced diabetes is characterized by a severe
loss of body weight (Odetola et al., 2006), increased food and water intake, increased
blood glucose concentrations and decreased concentration of insulin (Al-Shamanoy et
al., 1994). Such effect may be due to excessive breakdown or degradation of the
tissue proteins and lipids due to insulin insufficiency. When diabetic rats treated with
Hordeum vulgare seeds, the improvement in body weight was observed in diabetic rats. And, there were normalization of food and water intake in diabetic rats. Blood glucose is an index for the diagnosis of diabetes mellitus. During diabetes, the blood glucose levels are drastically increased which results from reduced glucose utilization by various tissues, which is a typical condition of insulinopenic diabetes (Soling et al., 1976). Oxidative stress induced by hyperglycemia in diabetics is considered a major cause in the development and progression of diabetic micro vascular complications, such as nephropathy (Evans et al., 2002; Yorek, 2003). In the present study, oral administration of standard Protamine zinc insulin (6 unit/kg) and EHV 500 significantly reduced the blood glucose level and increased insulin level in diabetic rats in a dose dependent manner.

Over the experimental period, the levels of urinary protein excretion were significantly elevated in diabetic rats, indicating the changes in the capillary filtration barrier that result in the increased permeability of the glomerular basement membrane (Madianov et al., 2000). Many studies have shown that the magnitude of urine protein level is associated with a graded increase in the risk of progression to end-stage renal disease and cardiovascular events (Lea et al., 2005). The administration of standard Protamine zinc insulin 6 unit/kg, EHV 250 and 500 significantly reduced the levels of urinary protein excretion that shows the improvement of renal functions. Increased serum urea and creatinine level are indications of the development of diabetic nephropathy in diabetic rats (Makino et al., 2002; Breyer et al., 2005). Those are significant markers of renal dysfunction and reflecting a decline in the glomerular filtration in rat (Sun et al., 2006). The urea and creatinine levels are higher in rats with diabetic nephropathy than those in normal control rats. Maintenance of these biochemical variables closer to those in control rats by Hordeum vulgare seeds. Treatment of Hordeum vulgare seeds suggest that the extract plays a role, either directly or indirectly in providing protection from kidney damage in diabetic nephropathy.

The abnormal high concentration of serum lipids is mainly due to increase in the mobilization of free fatty acids from the peripheral fat deposits, because insulin inhibits the hormone sensitive lipase production. Therefore, the elevated level of serum lipids in DM causes the risk of diabetic nephropathy (Vaziri et al., 2006;
Sugano et al., 2006). In our study, STZ treated diabetic rats exhibited clear-cut abnormalities in lipid metabolism as evidenced from the significant elevation of serum total cholesterol, triglycerides, LDL-C, Atherogenic index and reduction of HDL-C levels. Treatment with Standard insulin and *Hordeum vulgare* seeds, significantly reduced level of serum total cholesterol, Triglyceride and LDL-Cholesterol associated with concomitant significant increase in HDL-Cholesterol level and thus, decrease in atherogenic index in diabetic rats indicating its potent anti-hyperlipidemic and anti-Atherogenic activity. The lipid lowering property could indirectly contribute to the overall antihyperglycemic activity through a mechanism of so called glucose–fatty acid cycle According to the Randle’s glucose–fatty acid cycle, increased supply of plasma triglycerides per se could constitute a source of increased free fatty acid (FFA) availability and oxidation that can impair insulin action, glucose metabolism and utilization leading to development of hyperglycemia (Randle et al., 1963).

The mechanism proposes that hyperglycemia, in addition to increasing ROS production, also decreases antioxidant capacity through glycation of scavenger enzymes. According to Ha & Kim, (1999), a causal relationship between oxidative stress and diabetic nephropathy exists Lipid peroxides and 8-hydroxydeoxyguanosin with albuminuria is increased in the kidneys of diabetic rats. High glucose concentrations directly raise oxidative stress in glomerular mesengial cells (which are target cells in diabetic nephropathy). Oxidative stress induces expression of TGFβ2 and fibronectin mRNA genes which have important roles, in diabetic glomerular damage. Inhibition of oxidative stress improves all disorders related to diabetic nephropathy.

Diabetes and **hyperlipidemia** is the world’s largest endocrine disease with deranged carbohydrate, fat and protein metabolism. Diet high in fructose induces insulin resistance (IR) in rats, hamsters and dogs. The general view of insulin action places this hormone at the point of multiple organ adaptations to the ingested nutrients, in particular, dietary carbohydrates (Bessesen, 2001). Investigators have reported The use of 10% fructose in drinking water for a period of 1 week or longer is equivalent to diet containing 48-57% by calories, and has been found to be most suitable for the production of insulin resistance in rats that causes hyperlipidemia, hyperinsulinemia
Discussion

and the development of whole body insulin resistance in rat (Vikrant et al., 2001; Basciano et al., 2005). Insulin resistance in humans has been shown to be present in conditions like NIDDM, obesity and dyslipidaemia. Thus interventions to decrease insulin resistance may postpone the development of NIDDM and its complications (Shalam et al., 2006). Consumption of high dietary fructose might be one of the factors responsible for the development of obesity and the accompanying insulin resistance syndrome. Thus, rats receiving fructose-rich supplementation in drinking water for three weeks could be served as a reliable model for the investigation of insulin resistance (Rajasekar et al., 2006). Obesity is associated with insulin resistance and compensatory hyperinsulinemia, metabolic derangements that may lead to the formation of calcium containing kidney stones. A recent metabolic trial demonstrate that insulin resistance was associated with defects in renal ammonium production (Abate et al., 2004), and an examination of more than 4500 patients with a history of kidney stones showed that urinary pH was inversely related to body weight (Maalouf et al., 2004). Larger body size may also result in increased urinary excretion of uric acid and oxalate, risk factor for calcium oxalate kidney stone (Coe et al., 1974; Coe et al., 1980).

Insulin is the leading hormone that regulates blood glucose and fat metabolism. Insulin resistance is a condition in which the responsiveness to insulin of target tissues, namely skeletal muscle, adipose tissue, and the liver decreases despite higher insulin levels. In type 2 diabetes, the sensitivity of insulin decreases due to β-cell dysfunction, insulin receptor mutation, and obesity factors. Insulin sensitivity may also be affected by many circulating lipids, which include triglycerides and free fatty acids (Boden and Shulman, 2002). Raised plasma free fatty acids level is an important inductor of both peripheral and hepatic insulin resistance because it inhibits insulin signaling. In addition, hypertriglyceridemia is also an important marker of insulin resistance (Steiner, 1994).

The observations obtained in our study are contradictory with others stated that administration of fructose for 21 days significantly increases the glucose, insulin and triglyceride level (Shalam et al., 2006; Reaven et al., 1990; Patrick et al., 1998). Administration of EHV prevented the development of hyperglycemia and hypertriglyceridemia in dose dependent manner. The EHV might have improved
glucose concentration through enhanced glucose sensitivity in peripheral tissue, as was evident from the decreased glucose and increased liver and skeletal muscle glycogen stores. However, treatment with EHV failed to ameliorate insulin resistance to a significant extent.

Free radical-induced oxidative stress has been implicated in the pathogenesis of a variety of clinical disorders resulting usually from deficient natural antioxidant defenses. The development of oxidative stress, an imbalance between oxidative stress and antioxidative defence status, has been shown to play an important role in mediating insulin resistance, and therefore we studied the extent of peroxidation and antioxidant potential. Peroxidative deterioration of lipids in fructose-fed rats is evident from the increased levels of TBARS, and aldehydes, while the increased protein carbonyl content and diminished protein-SH group signify protein damage (Rajasekar et al., 2005). The elevated free radicals and depressed antioxidant defense may lead to cell disruption and oxidative damage to the cell membranes and may hence increase the susceptibility to lipid peroxidation. Therefore, if excessive generation of free radicals like $O_2^-$, $OH^-$ can be checked, protection from free radical-induced damage can probably be accorded. It is believed that plants with antioxidant property can prevent or protect tissues against damaging effect of free radicals (Yazdanparast et al., 2007). Oxidative stress may have implications in insulin signaling and insulin-mediated glucose uptake since free radicals can impair insulin action through membrane structural changes (Rudich et al., 1998). A significant positive correlation between insulin resistance and TBARS formation was observed in fructose-fed rats (Thirunavukkarasu et al., 2004).

Reactive oxygen species (ROS) and Oxidative stress has been shown to play a role in the causation of diabetes 1 and 2 and as such, antioxidants may have a role in the alleviation of diabetes (Baynes, 1991). Oxidative stress is produced under diabetic condition and it is likely involved in progression of pancreatic $\beta$-cell dysfunction (Kajimoto and Kaneto, 2004). Also, because of the relatively low expression of antioxidant enzymes such as catalase and superoxide dismutase, pancreatic $\beta$-cells may be vulnerable to ROS attack when the system is under oxidative stress (Lenzen et al., 1996; Tiedge et al., 1997). Increased peroxidation in fructose-fed rats could be due to hyperglycemia reported in these rats (Rajasekar et al., 2005). Besides this, fructose
feeding itself can induce oxidative stress by a number of mechanisms. The increased catabolism of fructose could be associated with the cellular energy depletion that can increase the susceptibility of cells to lipid peroxidation. Further, down-regulation of HMP shunt enzymes in the presence of fructose (Fields et al., 1992) could lead to decreased generation of reducing equivalents (NADPH). Furthermore, SOD scavenges the superoxide radical by converting it to $\text{H}_2\text{O}_2$ and molecular oxygen (McCrod et al., 1976). The activity of SOD was found to be lower in fructose induced diabetic rats. The observed decrease in SOD activity could result from inactivation by $\text{H}_2\text{O}_2$ or by glycation of the enzyme, which have been reported to occur in diabetes (Sozmen et al., 2001). CAT is a hemeprotein, which catalyzes the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals. Reduced activities of SOD and CAT in liver and kidney tissues have been observed in fructose induced rats, and this activity may result in a number of deleterious effects due to accumulation of superoxide radicals ($\text{O}_2^{•−}$) and hydrogen peroxide ($\text{H}_2\text{O}_2$) (Satheesh et al., 2004). Further, an increase in the SOD activity may protect CAT against enzyme inactivation by superoxide radical as these radicals have been shown to inactivate CAT (Kono and Fridovich, 1982). Thus, the increase in SOD activity may indirectly play an important protective role in preserving the activity of CAT. The reduced activities of SOD and CAT in the liver have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxides. Administration of *H. vulgare* seed extract increases the activities of SOD and CAT in diabetic rats. The result of the SOD and CAT activity clearly shows that *H. vulgare* seeds contains a free radical scavenging activity, which could exert a beneficial action against pathological alteration caused by the presence of $\text{O}_2^{•−}$ and $\text{OH}^{•−}$. This action could involve mechanism related to scavenging activity.

Reduced Glutathione (GSH) is known to protect the cellular system against toxic effects of lipid peroxidation (Nicotera and Orrenius, 1986). It is non enzymatic antioxidant free radicals and other ROS directly and indirectly through enzyme reaction. Hyperglycemia can increase oxidative stress and change the redox potential of glutathione (Rachna and Abdelhaq, 2003). GSH is the most important bimolecular against chemically induced toxicity and can participate in the elimination of reactive intermediates by reducing hydro peroxides in the presence of Glutathione peroxides.
(Meister, 1984; Nicotera and Orrenius, 1986). Decreased level of GSH in liver and kidney of diabetic rats may increase their susceptibility to oxidative injury (Irshad and Chaudhuri, 2002). Reduction of oxidized form of glutathione requires NADPH, as a cofactor and enzyme glutathione reductase. The reduced availability of NADPH, which could be either due to reduced synthesis or increased metabolism of NADPH through some other pathway, could be also responsible for low levels of reduced glutathione in alloxan diabetic rats as compared to control rats (Madhu et al., 1996). In the present study, a significant elevation of GSH level was observed in the *H. vulgare* seeds treated diabetic rats. This indicates that the extract of *H. vulgare* can either increase the biosynthesis of GSH or reduce the oxidative stress leading to less degradation of GSH, or have both effects.

Lipid peroxidation products such as MDA are generated under high levels of unscavenged free radicals (Levy et al., 1999). Lipid peroxidation (LPO) is the process whereby oxygen interact polyunsaturated fatty acids. When this process occurs in biological membrane, gross alteration of structural, organizational and enzyme function may result. Further more, lipid peroxide mediated damage has been observed in the development of type 1 and type 2 diabetes mellitus (Santhakumari et al., 2003). Elevation of Lipid peroxidation is attributed to the enhanced production of reactive oxygen species. In present study we observed a MDA formation, the index of lipid peroxidation, was significantly increased in hyperlipidemic rats. The increased MDA level may have an important role in pancreatic damage associated with diabetes and hyperlipidemia. Under diabetic conditions, the level of lipid peroxidation in the pancreas is enormously higher than nod-diabetic rats (Haluzik and Nedvidkova, 2000). However, in the present study, increase in the level of MDA in diabetic rats and restoration on treatment with EHV at the higher dose supplementation. At lower dose the extract were failed to induce significant effect.

Investigations are required to elucidate the exact mechanism(s) of action for the efficacy of *Hordeum vulgare* in the kidney disorders. Attempts should be made to isolate the active principle of *Hordeum vulgare* responsible for such activities.
References


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