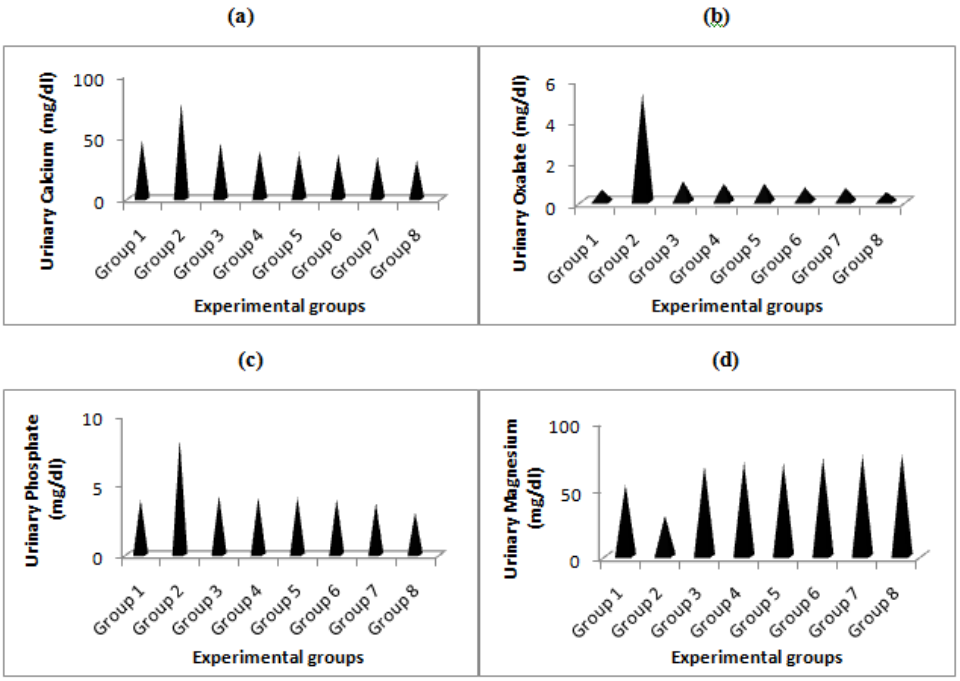

Figure 1- Effect of *S. dulcis L.* and *A. lanata L.* whole plant and fruit part extracts on Urinary parameters of Ethylene glycol induced male albino rats (a) Calcium, (b) Oxalate, (c) Phosphate and (d) Magnesium. Group 1 - Control, Group 2 - EG induced, Group 3 - EG induced + *S. dulcis* - whole plant, Group 4 - EG induced + *S. dulcis* – Fruit, Group 5 - EG induced + *A. lanata* – whole plant, Group 6 - EG induced +*A. lanata* – Fruit, Group 7 - EG induced + *S. dulcis* – Fruit + *A. lanata* – Fruit, Group 8 – *S. dulcis* fruit + *A. lanata* fruit - control

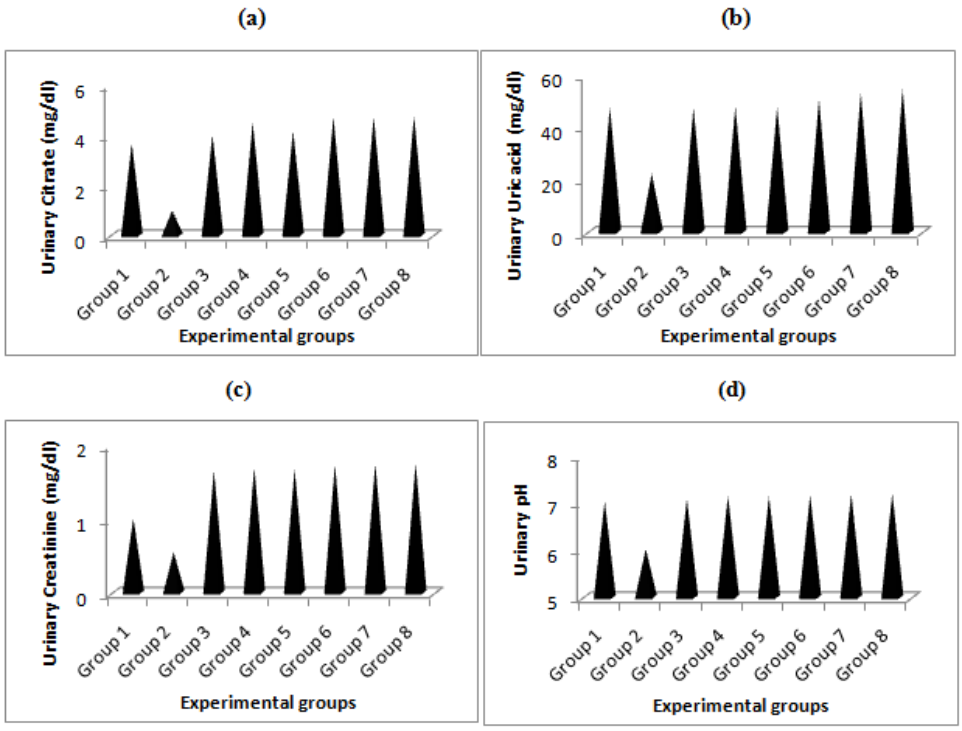

Figure 2- Effect of *S. dulcis L.* and *A. lanata L.* whole plant and fruit part extracts on Urinary parameters of Ethylene glycol induced male albino rats. (a) Citrate, (b) Uric acid, (c) Creatinine and (d) pH. Group 1 - Control, Group 2 - EG induced, Group 3 - EG induced + *S. dulcis* - whole plant, Group 4 - EG induced + *S. dulcis* – Fruit, Group 5 - EG induced + *A. lanata* – whole plant, Group 6 - EG induced + *A. lanata* – Fruit, Group 7 - EG induced + *S. dulcis* – Fruit + *A. lanata* – Fruit, Group 8 – *S. dulcis* fruit + *A. lanata* fruit - control

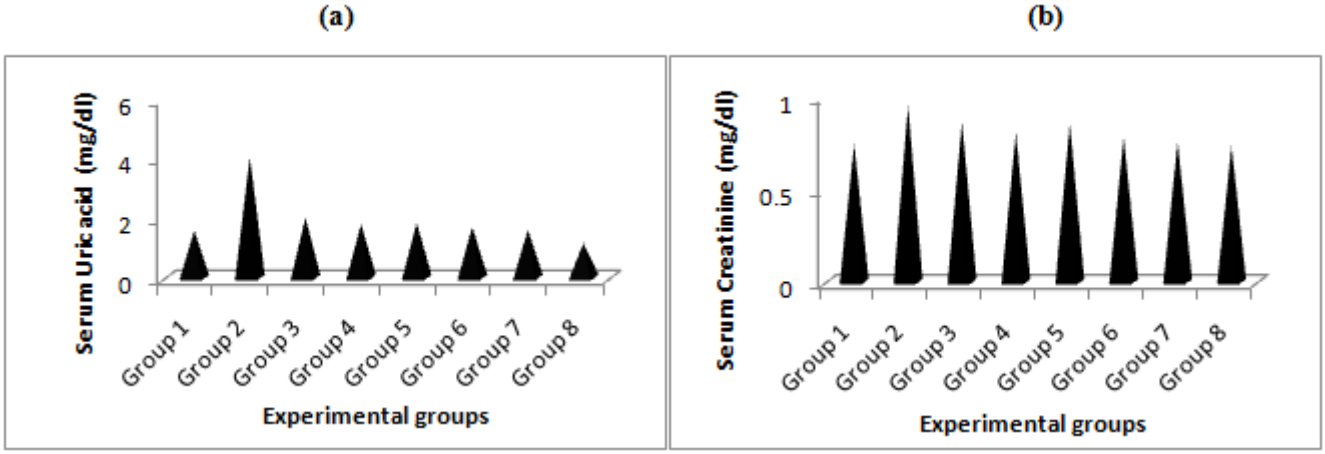

Figure 3- Effect of *S. dulcis L.* and *A. lanata L.* whole plant and fruit part extracts on Serum parameters of Ethylene glycol induced male albino rats. (a) Uric acid and (b) Creatinine. Group 1 - Control, Group 2 - EG induced, Group 3 - EG induced + *S. dulcis* - whole plant, Group 4 - EG induced + *S. dulcis* – Fruit, Group 5 - EG induced + *A. lanata* – whole plant, Group 6 - EG induced + *A. lanata* – Fruit, Group 7 - EG induced + *S. dulcis* – Fruit + *A. lanata* – Fruit, Group 8 – *S. dulcis* fruit + *A. lanata* fruit - control

5. Discussion

Administration of Ethylene glycol induced kidney stones in rats. This finding is in corroboration with many earlier research evidences. Treatment with ethylene glycol (0.75%) for 14 days is reported to develop renal calculi composed mainly of calcium oxalate in young male albino rats (Selvam *et al.,* 2001; Huang *et al.,* 2002; Atmani *et al.,* 2003). Stone formation in ethylene glycol fed animals is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate (Selvam *et al.,* 2001). Similar studies have also been obtained when rats were treated with ethylene glycol and ammonium oxalate (Adhirai and Selvam 1997; Muthukumar and Selvam 1997; Kavadi *et al.,* 2006). Formation of renal calculi depends on the nucleation of stone forming constituents principally calcium oxalate in the urinary tract (Ryall, 2011).

In this study, Calcium, Oxalate and Phosphate excretion were progressively increased in calculi-induced animals (Group II). Increased urinary calcium is a factor favouring the nucleation and precipitation of calcium oxalate or calcium phosphate from urine and subsequent crystal growth (Lemann *et al.,* 1991). Hyperoxaluria is known as a far more significant factor in the pathogenesis of renal stones than hypercalciuria (Tisselius, 1996) and the changes in urinary oxalate levels are relatively much more important than those of calcium (Robertson and Peacock, 1980). Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces calcium oxalate deposition (Roger *et al.,* 1997). Treatment with *S. dulcis* and *A. lanata* whole plant and fruit part lowered the levels of Calcium, Oxalate as well as Phosphate excretion.

Magnesium is suggested to be an important inhibitor of calcium oxalate stone formation. Magnesium may reduce oxalate absorption and urinary excretion nearly as effectively as calcium by binding oxalate in the gut (Liebman and Costa, 2000). A high urinary excretion and concentration of magnesium has been shown to decrease both nucleation and growth rates of calcium oxalate crystals, due to the higher solubility of magnesium oxalate compared with calcium oxalate (Kohri *et al.,* 1988 and Li *et al.,* 1985). Magnesium is a divalent cation, which can complex with oxalate and reduces its urinary station. Calcium oxalate stone formation may thereby be inhibited. Urinary citrate has been identified as a potent inhibitor of Calcium stone formation (Pak, 1994). Evidence over many years has now established hypocitraturia as a key contributor to the formation of CaOx stones (Ryall, 2011). Citrate inhibits stone formation by forming soluble complexes with calcium which inhibit crystal nucleation and growth (Heilberg and Schor, 2006). During the stone formation period urinary pH decreased to a significantly lower level (pH 6.2) (Okada *et al.,* 2007).Our studies also correlates with the above evidences that low urine Magnesium, Citrate and pH may also promote stone formation. However administration of whole plant and fruit part extract of *S. dulcis* and *A. lanata* increased the urinary level of Magnesium, Citrate and pH. Phytochemical studies suggest that *S. dulcis* and *A. lanata* are rich sources of Magnesium (Muthumani *et al.,* 2010; Omoyeni and Adeyeye, 2009).

One of our outstanding findings is that the urinary excretion of Uric acid and Creatinine has been lowered in calculi induced animals due to renal damage. However treatment with whole plant and fruit part extract of *S. dulcis* and *A. lanata* highly increased urinary excretion of Uric acid and Creatinine.

In urolithiasis, the glomerular fitration rate decreases due to the obstruction to the outflow of urine by stone in urinary system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine and uric acid get accumulated in blood (Ghodkar, 1994). Increased lipid peroxidation and decreased levels of antioxidant potential have also been reported in the kidneys of rats supplemented with a calculi producing diet (Sumathi *et al.,* 1993; Saravanan *et al.,* 1995). Oxalate has

been reported to induce lipid peroxidation and to cause renal tissue damage by reacting with poly saturated fatty acids in cell membrane (Ernster and Nordenbrand, 1967). However treatment with whole plant and fruit part extract of *S. dulcis* and *A. lanata* highly reduced the serum levels of Uric acid and Creatinine.

Another important finding in our study is that in Group VIII ((fruit part of *S. dulcis* in combination with *A. lanata* – control), the level of stone forming contituents were highly decreased and stone inhibiting constituents were remarkably increased than that of normal control, this is because even in normal control the stone forming constituents can rise to fairly high levels due to some factors such as drinking water, diet and aging.

In conclusion, the presented data indicate that administration of *S. dulcis* and *A. lanata* whole plant and fruit part extract to rats with ethylene glycol induced lithiasis reduced the levels of stone forming constituents and thereby prevents the formation of kidney stones. However, administration of fruit part of *S. dulcis* in combination with *A. lanata* highly reduces the risk of stone formation than others. The fruit juice and seed extract of the medicinal plants is moderate to good inhibitor of calcium oxalate, calcium carbonate and calcium phosphate mineralization. Sequestering of this insoluble calcium salts by the fruit juice might be due to effective single and mixed ligand chelation by the hydroxyl acids present in them (Mohamed Farook *et al.*, 2009)

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