Protective Role of *Evolvulus Alsinoides* Linn. on Chemical Induced Prostatitis in Male Albino Rats

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Abstract

"Evolvulus alsinoides Linn. (Vishnu Kiranti) plant is widely used as antibacterial and anthelmintic, anti-stress and anti-amnesic and a good memory tonic".The present this study aimed to investigate the protective role of Evolvulus alsinoides L.on the inflammatory effects of AlCl3 induced prostatitis in rat model.Prostatitis was induced in male Wistar rats (n=24) treated with AlCl3 for 12 weeks. Following prostatitis induction, the rats were randomly assigned to one of four treatment groups: normal control (NC-group), prostatitis (AlCl3-group), Evolvulusalsinoides methanolic extract (EAME group), and AlCl3+EAME group. The animals were giventheir routinediets throughout the study period. The parameters including Reproductive hormones, inflammatory markers and Prostate Specific Antigen (PSA), were evaluated.The histopathological changes in the prostate were also studied. The results showed that there was a significant ($p<0.05$) reduction in PSA levels and a significant ($p<0.05$) increase in reproductive hormones, including LH, FSH, Progesterone, Testosterone, and Estradiol levels, in groups fed with EAME. The study concluded that the EAMEhad aneffective anti-inflammatory property, and histological examinations revealed a significant improvement in the prostatic histoarchitecture of groups fed EAME. Our research findings suggest that EAME is beneficial in the prevention and treatment of nonbacterial prostatitis.

Keywords: Prostatitis, Aluminum Chloride, Evlovulusalsinoides. L.PSA, Cytokines, LH, FSH, Progesterone, Testosterone

Introduction

"Prostatitis is a very common clinical illness that causes irritative urine symptoms, pelvic pain, and sexual dysfunction. According to previous research, prostatitis accounts for 5% of ambulatory visits by adult men and 8% of urological visits"(1)."Prostatitis is

associated with significant decreases in both quality of life and patient costs (2). Furthermore, some investigations have found that chronic inflammation and elevated oxidative stress may play essential roles in the development of prostatitis" (3). "Serum PSA levels tend to rise in prostate illnesses such as BPH, prostatitis, and even prostate cancer, and they are used as a clinical indicator of disease prognosis" (4). "Aluminium has the potential to cause hazardous consequences in humans or laboratory animals exposed via inhalation, ingestion, or superficial exposure. It absorbs and accumulates in humans through drinking water; however, intake of fruit juices or citric acid induces a significant increase in both GI absorption and urine excretion of aluminium in healthy persons. Apart from that, excessive aluminium ingestion causes build-up in target organs and has been linked to testicular tissue damage in both humans and animals". High aluminium levels in human testes, Leydig cells, spermatozoa, seminal plasma, blood, and urine were linked to poor sperm quality and viability (5,6,7). Aluminium exposure has been linked to reduced spermatogenesis and sperm quality, as well as disruptions in sex hormone secretion (8). Besides from its involvement in inducing oxidative stress, AlCl3's toxic effect may be due to its capacity to bind to cellular DNA, RNA, and proteins, causing their levels to decrease in various tissues (9). It raises permeability and causes oedema in inflammatory reactions due to the release of inflammatory mediators from degenerative and necrotic parenchymatous cells (10), which may explain inflammatory cell infiltration into the prostate parenchyma. Based on this, we chose AlCl3 as an induction agent. Traditional/complementary and alternative medicine is becoming more popular in many affluent nations as a result of widespread concern about the side effects of modern pharmaceuticals (11). Several kinds of phytomedicines were previously used to prevent and treat prostate problems. "Evolvulus alsinoides L. is a medicinal plant utilised in the Indian traditional system of medicine to make 'Shankhpushpi', an important and popular ayurvedic treatment that greatly improves memory (12). E. alsinoides has been shown to have a variety of therapeutic effects, including neuroprotective (13), antioxidant (14) anti-diabetic (15), and immunomodulatory (16) capacities".

Evolvulus alsinoides may be a good source of phytochemicals, vitamins and minerals (17). Previous research using GC-MS analysis revealed the presence of various compounds like Caryophyllene, octodeconoic acids, hexadecanoic acid, ascorbic acid and squalene in the methanolic extract of Evolvulus alsinoides (18). As a result, the existence of secondary metabolites in E. alsinoides suggests that it may have anticancer properties. Though E. alsinoides has been shown to be effective in the treatment of a variety of disorders, we investigated its anti-inflammatory efficacy in male albino rats.

Materials and Methods

Collection of the Plants

"The single herb (Evalvulusalsinoides Linn.)from the matured whole plants were collected from Thanjavur District, Tamil Nadu, India. The plantidentified and authenticated for identification by a Botanist Dr. S.John Britto S.J, The Director, The Rapinat Herbarium and Center for molecular systematic, St. Joseph's College, Tiruchirappalli, Tamil Nadu". The collected plants were sliced into little pieces, shade dried,powdered thoroughly and stored in air tight container for extraction.

Preparation of the Plant Extract

"The Evolvulus alsinoides leaves were washed multiple times with distilled water to remove any dust and impurities. The leaves were dried at room temperature and then coarsely pulverised. The powder was extracted using 75 % methanol for 48 hours. A semi-solid extract was refrigerated until for further use."

Preparation of Aluminum Chloride (AlCl3)

About two grams of aluminum chloride was taken and dissolved in 100 ml of distilled water for preparing 20 mg /ml of stock solution. The solution was prepared every week and stored in an aircraft bottle at the temperature of 4ºC. Rats were given 0.1ml (2mg)/100gm of AlCl3 orally once a day".

Preparation of Animals

The male Wister albino rats weighing between 150 and 200g were used in this study. "The animals were kept in a spacious polypropylene cages bedded with rice husk. The animal room was well-ventilated and kept under standard experimental conditions (27 ± 2ºC and 12-hour light/dark cycle) during the study period.All of the animals were fed a regular pellet diet and had unlimited access to water. The animals were fed with standard pellet diet with an adequate amount of water. They were acclimatized to the environment for one week prior to experimental use". The experiment was conducted in accordance with the guidelines of the committee - Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Experimental Design

"The body weights of the animals were recorded, and they were separated into two groups of six each. The first group was a normal control group that received 3 mL of distilled water orally once day. The second group received aluminium chloride (20 mg/kg BW); the LD50 of $AlCl_3$ when administered orally to rats was reported to be (380 - 400 mg/kg BW (14). The third group received 75 mg/kg BW of Evolvulusalsinoides methanolic extract (EAME) which is dissolved in 3ml of distilled water orally once daily as suggested by Lekshmi and Reddy (18). The fourth group received $AlCl_3$ and EAME in the same doses as the second and third groups".

Collection of Blood and Prostate Tissues

"After the experimental feeding period, the rats were fasted overnight and sacrificed using pentobarbital under mild euthanasia. Blood was obtained in plain, heparinized, and EDTA vials for pro-inflammatory analysis. The blood in plain bottles was allowed to coagulate, and the serum was separated at 3500 rpm for 15 minutes to determine PSA, hormones, and inflammatory markers. Prostate tissues were immediately removed and preserved in 10% formyl saline".

Determination of Serum Prostate Specific Antigen (PSA)

"The serum Prostate Specific Antigen (PSA) levels were measured using a PSA ELISA kit as directed by the manufacturer (Rapid Labs. Ltd, Colchester, Essex, UK) (19). The absorbance was measured at 450 nm with a microplate ELISA reader (Bio-Rad Laboratories, Inc.). The results were presented as ng protein mLG1".

Determination of Hormones

"Hormone levels in serum were measured using a Hormones ELISA kit (Rapid Labs. Ltd, Colchester, Essex, UK) as instructed by the manufacturer (19). The absorbance was measured at 450 nm with a microplate ELISA reader (Bio-Rad Laboratories, Inc.). The results were presented as ng protein mLG1".

Inflammatory Marker Analyses

Serum levels of N-acetyl-β-glycosaminidase (20), β-glucuronidase (21) and NO concentration, as well as CRP, homocysteine, TNF-α, and IL-6 (22) were tested using specific procedures.

"The cytokine concentration was determined using a spectrophotometer at 450 nm and animmunoassay ELISA kit (R&D Systems, Minneapolis, MN, USA), following the manufacturer's procedure".

Histological Analysis

At the end of the experiment, the animals were slaughtered, and the prostate was removed and kept in 10 % formaldehyde for four days. Tissue sections (7 μm thick) were decalcified in 5 % formic acid and paraffin embedded before being stained with hematoxylin and eosin (23).

Statistical Analysis

"The statistical values were expressed as mean ± standard deviation for six rats in each group. Significant differences between mean values were assessed by one-way analysis of variance followed by Tukey's test for multiple comparisons (24). SPSS (Statistical Packages for Social Studies) version 20 was used to analyse the results, with a significance level of $p<0.05$".

Results

Effect of Evolvulus Alsinoides Leaves on PSA and Hormones in Experimental Rats

In the present study, we investigated the effect of Evolvulus alsinoidesleaveson Testosterone, luteinizing hormone, Follicle stimulating Hormone, Estradiol, Progesterone and Prostate Specific antigen (PSA) in animal model. Results showed that there was a highly significant ($p < 0.05$) reduction in serum Testosterone, LH, FSH, Estradiol and Progesterone concentrationand substantial improvement in PSA levels in AlCl3 group compared to control group. The EAME treatment group had significantly increased serum testosterone concentration ($p < 0.05$), alleviated the negative effects of AlCl3when compared to the untreated AlCl3 group.Results were presented in Table 1 & Table 2.

Inflammatory Marker Analyses

Prostatic animals showed much greater levels of inflammatory markers than normal animals. Supplementing with EAME restored levels of NO, CRP, Homocysteine, TNF-α, IL-6, and cortisol. The restoration with EAME therapy was statistically significant in Group IV. Group II demonstrated increased activity of N-acetyl-βglycosaminidase and β-glucuronidase compared to Group I. EAME treatment resulted in restored N-acetyl-βglycosaminidase and β-glucuronidase activity. Results are shown in Table 3.

Histological Analysis

Sections of group I and III rats revealed that the prostatic parenchyma was made up of packed acini of different diameters. "Acini were lined with simple columnar cells with basal nuclei and a few epithelial papillary folds. The acini were separated by a minimal fibromuscular stroma. Some acini had homogeneous acidophilic secretion. Sections from the Group II aluminium chloride-treated group revealed a greater epithelial cell layer and stromal space than controls. Desquamated cells were found within the lumina of many acini".

The acini had several epithelial folds, and the epithelial cells were grouped in multiple unorganised layers. Sections from the central component of the ventral prostate of this group revealed massive dilatation of prostatic acini that fled with secretions. In several parts of the acini, the lining epithelium was noticeably thinned and fattened. Prostatic sections from the Group IV AlCl3 + EAME-treated group showed significantly better glandular morphology. The lining epithelium was reduced in the acini, resulting in a simple columnar shape. Some epithelial cells have tiny, black

nuclei. The lumina of the acini seemed wide, whereas the epithelial folds were reduced. The acini were separated with less stroma than the aluminium chloride-treated group.Results were given in Figure 1(1a,1b,1c, &1d)

Table 1 Effect of Evolvulus Alsinoides Leaves on PSA in Experimental Rats

Parameters	Group- I	Group-II	Group-III	Group-IV
Prostate Specific antigen (PSA) (ng/ml)	0.59 ± 0.04	2.89±0.23a	0.62±0.45b	0.64±0.45b

Values are expressed as mean ± SD for six rats in each group.
[a]Significantly different from group I ($p < 0.05$)
[b]Significantly different from group II ($p < 0.05$)

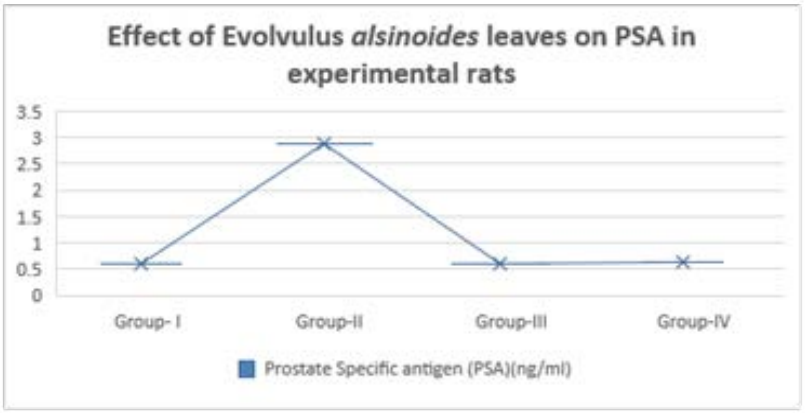

Figure 1 Effect of Evolvulus Alsinoides Leaves on PSA in Experimental Rats

Table 2 Effect of Evolvulus Alsinoides Leaves on Hormones in Experimental Rats

Parameters	Group- I	Group-II	Group-III	Group-IV
Testosterone (ng/ml)	2.58 ±0.17	1.62 ±0.10a	2.78 ±0.20 b	2.51 ±0.21 b
LH (ng/ml)	3.80 ± 0.27	2.04 ±0.14a	3.65 ±0.23 b	3.57 ±0.26 b
FSH (ng/ml)	1.76 ±0.15	1.04 ±0.08a	1.78 ±0.16 b	1.54 ±0.18 b
Estradioal (pg/ml)	7.98 ± 0.56	4.87 ± 0.29a	7.95 ±0.57 b	7.93 ±0.54 b
Progesterone (ng/ml)	12.47 ±0.95	8.75±0.58a	13.86 ±1.23 b	11.54 ±0.07 b

Values are expressed a Mean ± SD for six rats in each group.
[a]Significantly different from group I ($p < 0.05$)
[b]Significantly different from group II ($p < 0.05$)

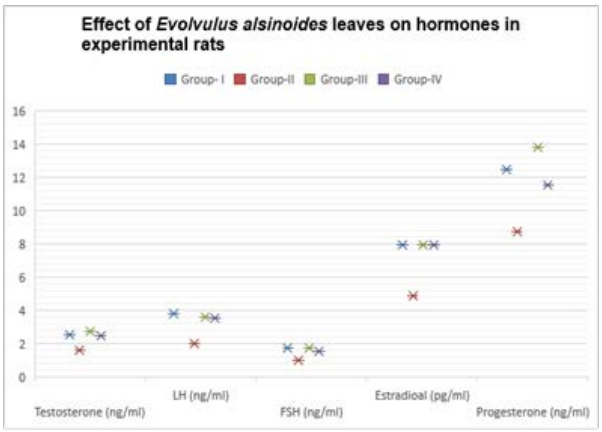

Figure 2 Effect of Evolvulus Alsinoides Leaves on Hormones in Experimental Rats

Table 3 Effect of Evolvulus Alsinoides Leaves on Inflammatory Markers in Experimental Rats

Parameters	Group- I	Group-II	Group-III	Group-IV
CRP (mg/dl)	2.80±0.24	4.75±0.30[a]	3.11±0.40[b]	2.98±0.20[b]
TNF-α(pg. /ml)	09.12±0.62	14.80±1.02[a]	10.40±0.74[b]	9.44±0.52[b]
IL-6(pg. /ml)	30.20±2.24	96.44±6.62[a]	35.42±5.59[b]	31.42±3.21[b]
Homocysteine (µg/ml)	7.12±0.53	13.87±0.92[a]	8.32±0.80[b]	7.80±0.69[b]
NO (µM/L)	25.41±1.87	54.72±3.41[a]	30.74 ±2.25[b]	28.14±2.11[b]
N-acetyl-β-glycosaminidase (U /min/ml)	33.04±2.50	48.74±3.44[a]	34.55.±2.12[b]	30.17±2.46[b]
β-glucuronidase (mU)	1.35±0.08	3.12±0.29[a]	2.16±0.19[b]	1.28±0.15[b]

Values are expressed as mean ± SD for six rats in each group.
[a]Significantly different from group I ($p < 0.05$)
[b]Significantly different from group II ($p < 0.05$)

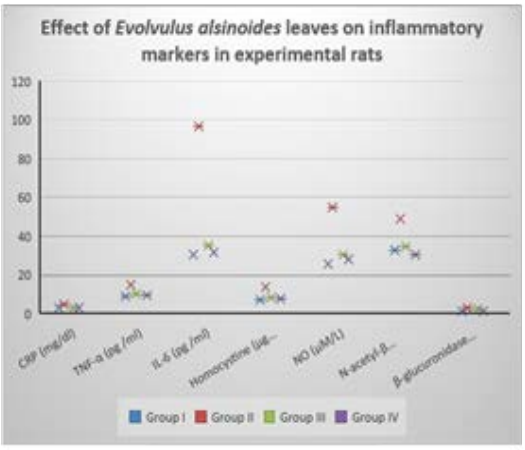

Figure 3 Effect of Evolvulus Alsinoides Leaves on Inflammatory Markers in Experimental Rats

Histopathology of Rat Prostate

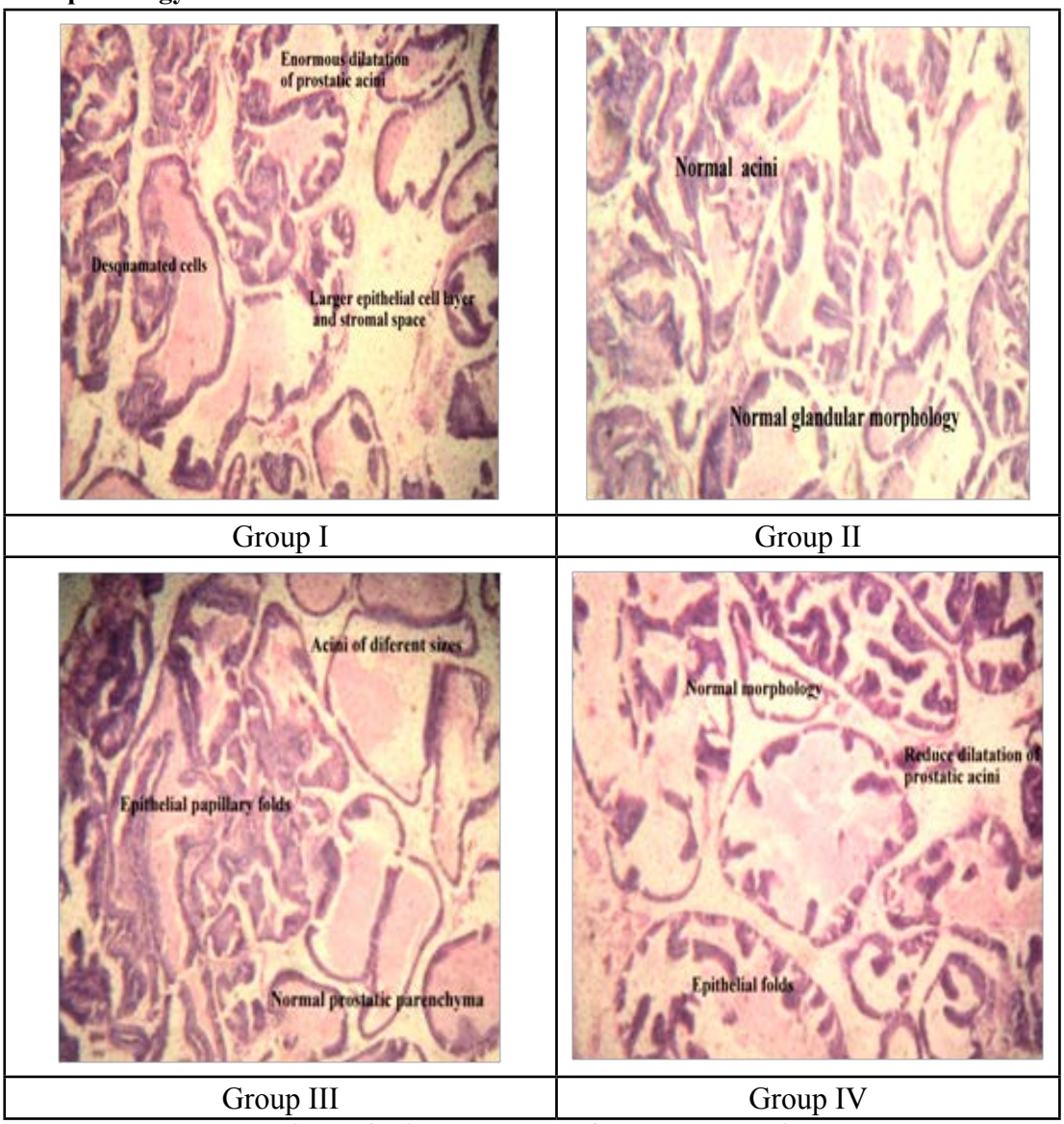

Figure 4 Histopathology of Rat Prostate Tissue

Discussions

In the current study, Group II animals had significantly lower levels of Testosterone, Luteinizing Hormone, Follicle stimulating hormone, Oestradiol, and Progesterone and higher levels of Prostate Specific Antigen (PSA) than Group I (normal control animals). Group IV treated with EAME demonstrated a significant increase in the levels of luteinizing hormone, follicle stimulating hormone, testosterone,oestradiol, progesterone, and a decrease in the level of Prostate Specific Antigen (PSA). When Group III animals were given only the methanolic extract of Evolvulus alsinoides, no negative effects were seen. No deleterious effects were found in Group III animals treated just with Evolvulusalsinoides methanolic extract.

This is consistent with previous studies (25, 26, 27). This could be associated to calcium channel blocking effect of Al,(28) which resulted in reduced gonadotrophin secretion in the hypophysis

(29); (30), resulting in low testosterone levels. It could also be related to high testicular nitric oxide levels and low cAMP associated with Al, which inhibited steroidogenesis. On the other hand, Evolvulus alsinoides therapy increased levels of testosterone, luteinizing hormone, follicle stimulating hormone, oestradiol, and progesterone, most likely via blocking these mechanisms. Our results were agreement with the earlier study (31).

Our study demonstrated significant improvements in serum levels of IL-6, CRP, TNF-α, and homocysteine in rats with prostatitis. The current findings support the discovery of (32) that inflammation is produced by a number of pro-inflammatory chemicals secreted by macrophages. Proinflammatory cytokines, such as IL-1, TNF-α, and IL-6, have been shown (33) to enhance plasma protein synthesis, including CRP. This supports the current findings. Evolvulus alsinoides therapies reduced TNF-α, IL-6, and serum CRP levels compared to the prostatitis group. Inflammation is known to cause increased synthesis of nitric oxide and homocysteine. Evolvulus alsinoides may have anti-inflammatory action by restoring inflammatory markers and enzymes.

The possible means of anti-inflammatory activity ofEvolvulus alsinoides' appears to restore inflammatory indicators and enzyme activity, including N-acetyl-β-glycosaminidase and β-glucuronidase, by regulating inflammatory mediators.

Microscopic examination revealed that the EAME-treated rat group had lower prostate inflammation and less glandular epithelial degradation than the control group. We found that EAME reduces AlCl3-induced nonbacterial prostatitis in rats by regulating pro-inflammatory cytokines while having an anti-inflammatory effect. As a result, the methanolic extract of Evolvulus alsinoides Linn. is likely to be effective in the prevention and treatment of nonbacterial prostatitis in humans.

Conclusions

The following conclusion obtained from the study

- Regulated the reproductive hormones and decreased in the level of Prostate Specific antigen (PSA) on treatment with EAME.
- "The possible mode of anti-inflammatory activity of Evolvulus alsinoides appears to be restored the inflammatory markers like CRP, IL-6, TNF-α,Homocysteine and enzyme activity N-acetyl-β-glycosaminidase and β-glucuronidase activity through regulation of inflammatory mediators".
- Histopathological studies of Prostate Gland further confirmed the restoring effect of Evolvulusalsinoides.L on AlCl3 induced prostatitis.

In conclusion, Evolvulus alsinoides Extract appears to be protective against prostate inflammation and is an appropriate choice for future laboratory and clinical research on prostate-related disorders, including prostate cancer.

References

1. Z.T. Su, J.M. Zenilman, K.S. Sfanos, A.S. Herati(2020) Management of chronic bacterial prostatitis Curr Urol Rep, 21 , p. 29
2. A.M. Duloy, E.A. Calhoun, J.Q. Clemens(2007)Economic impact of chronic prostatitisCurr Urol Rep, 8 , pp. 336-339
3. Sciarra, A.,G. Mariotti, S. Salciccia, A.A. Gomez, S. Monti, V. Toscano and F. Di Silverio, (2008). Prostate growth and inflammation. J. Steroid Biochem. Mol. Biol., 108: 254-260.
4. Takizawa, I., T. Nishiyama, N. Hara, E. Isahaya, T. Hoshii and K. Takahashi, (2010). Serum prostate-specific antigen levels reflect the androgen milieu in patients with localized prostate cancer receiving androgen deprivation therapy: Tumor malignant potential and androgen milieu. Prostate, 70: 1395-1401

5. Dawson, E.B., Evans, D.R., Harris, W.A., Powell, L.C.(2000). Seminal plasma trace metal levels in industrial workers. Biol. Trace elem. Res. 74, 97–105
6. Hovatta, O., venalainen, E.R., kuusimaki, L., heikkila, J., hirvi, T., reima, I.(1998). Aluminum, lead and cadmium concentrations in seminal plasma and spermatozoa, and semen quality in finnish men. Hum. Reprod. 1998; 13, 115–119
7. Reusche, E., lindner, B., arnholdt, H.(1994). Widespread aluminum deposition in Extracerebral organ systems of patients with dialysisassociated encephalopathy. Virchows arch.; 424, 105–112.
8. Guo,C., Lu,Y. and Hsu,G.S.W.(2005). The influence of aluminum exposure on male reproduction and off spring in mice. Environ. Toxicol. Pharmacol. a; 20:135-141.
9. Fyiad, A. A. (2007). Aluminium toxicity and oxidative damage reduction by melatonin in rats. J Appl Sc Res; 3: 1210-1217.
10. Kumar, V., Cortan, R. and Robins, M. D. (1997). Acute and chronic inflammation. Basic pathology. 6th ed. WB Saunders Company. Philadelphia: 25-46.
11. World Health Organization. The World Health Organization report, (2002)
12. Sharma S, Shrivastava N (2016). Internal transcribed spacer guided multiplex PCR for species identification of Convolvulus prostratus and Evolvulusalsinoides. Acta Pharm Sin B.; 6:253–258.
13. Naikawadi VB, Ahire ML, Lahiri A, Nikam TD(2016). In vitro propagation and cell cultures of memory tonic herb Evolvulusalsinoides: a best source for elicited production of scopoletin. Appl MicrobiolBiotechnol. ;100:3463–3476.
14. Gomathi Rajashyamala, L., & Elango, V. (2016). Antioxidant activity of Evolvulus alsinoides Linn. Asian Journal of Innovative Research, 1(2), 39-43.
15. Gomathi D, Ravikumar G, Kalaiselvi M, Devaki K, Uma C.(2013) Efficacy of Evolvulus alsinoides (L) L on insulin and antioxidants activity in pancreas of streptozotocin induced diabetic rats. J Diabetes MetabDisord.;12:39.
16. Gupta P, Akanksha, Siripurapu KB, Ahmad A, Palit G, Arora A, et al.(2007) Anti-stress constituents of Evolvulusalsinoides: an Ayurvedic crude drug. Chem Pharm Bull.; 55:771–775. doi: 10.1248/cpb.55.771.
17. Gomathi Rajashyamala L and Elango V (2015) Analysis of Bioactive compounds in Evolvulusalsinoides. World Journal of Science and Research. 1(1): 50-55.
18. Gomathi Rajashyamala L, Elango V. Identification of bioactive components and its biological activities of Evolvulusalsinoides linn.-- A GC-MS study. Int J Chem Stud 2015;3(1):41-44.
19. Dhana Lekshmi U. M and Neelakanta Reddy P.(2009) Preliminary studies on anti infammatory, antipyretic, antidiarrhoeal properties of Evolvulus alsinoides Bio Organic Chemistry Laboratory, Central Leather Research Institute .
20. Nilsson, O., A. Peter, I. Andersson, K. Nilsson, B. Grundstrom and B. Karlsson, 1997. Antigenic determinants of Prostate-Specific Antigen (PSA) and development of assays specific for different forms of PSA. Br. J. Cancer, 75: 789-797.
21. Walker, P., Mary, E., Woollen and Doreen, (1960). Pugh N-acetyl-β-glucosaminidase activity in serum during pregnancy. J Clin Pathol 13: 353-357.
22. Fishman WH, Springer B and Brunetti R. (1948) Application of an improved glucuronidase assay method to the study of human blood. Jo Biol. Chem, 173: 449-456.
23. Sastry KVH, Moudgal RP, Mohan J, Tyagi JS and Rao GS. (2002). Spectrophotometric determination of serum nitrite and nitrate by coppercadmium alloy; Analytical biochemistry, 306: 79-82.

24. Abudoleh S, Disi A, Qunaibi E, Aburjai T (2011). Anti-arthritic activity of the methanolic leaf extract of Urtica pilulifera L. on albino rats. Am. J.Pharmacol. Toxicol. 6(1):27-32.
25. Harvey J and Paige SM. (1998) The Instat Guide to choosing and interpreting statistical tests: A manual for Graph pad Instat, Version 3. San Diego, CA USA
26. Guo C, Huang C, Chen S, Wang Hsu G. (2001). Serum and testicular testosterone and nitric oxide products in aluminum-treated mice. Environ ToxicolPharmacol. 10:53-60.
27. Mohammad SR, Palan M.J.(2006). Effect of aluminium on testosterone hormones in male rats. J Med Sci. 6:296-9.
28. Yousef MI, Salama AF. (2009). Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. Food and Chem Toxicology. 47:1168-75
29. Platt B, Busselberg D.(1994). Actions of aluminium on voltage-activated calcium channels currents. Cell Mol Neurobiol .14:819-29.
30. Tse A, Tse FW, Almers W, Hill B. (1993). Rhythmic exocytosis stimulated GnRHinduced calcium oscillations in ratsgonadotropes. Science. 260:82-4.
31. Mills LR, Niesen CE, So AP, Carlen PL, Spigelman I, Jones OT. (1994). N-type Ca2+ channels are located on somatadendrites a subpopulation of dendrite spines on live hippocampal pyramidal neurons. J Neurosci. 14:6815-24
32. Ige SF, Akhigbe RE. (2012). The role of Allium cepa on aluminum-induced reproductive dysfunction in experimental male rat models. J Hum Reprod Sci. 5:200-5.
33. Gonzalez-Gay, M.A., Gonzalez-Juanatey, C., Garcia-Porrua, C., Pineiro, A., Testa, A., Llorca, J. (2005). High-grade C-reactive protein elevation correlates with accelerated atherogenesis in patients with rheumatoid arthritis. J. Rheumatol. 32: 1219–1223.
34. Suffredini, A.F., Fantuzzi, G., Badolago, R., Oppenheim, J.J.,O´Grady, N.P., (1999). New insights into the biology of the acute phase response. J. Clin. Immunol. 19: 203–214.