ISSN: 2348-8948
Vol: 2; Issue: 5

RESEARCH ARTICLE

Primary evaluation of selected steroid containing herbs for their corticoid potential in rats by Freund's adjuvant arthritis

PATEL NILESH K*., PATEL DHAVAL V., BORKHATARIA CHETAN H., MANEK RAVI A., PATEL KALPESH A.

B.K.Mody Government Pharmacy College, Rajkot-360003

Date Received:	Date of Accepted:	Date Published:
25th Apr2014	4th May2014	12th May2014

Abstract:
Many pharmacologic activities of steroid containing plants like Wildyam, Kanthkari, Agave spices, Indian Sarsaparilla and Fenugreek have been reported. In order to find a safer alternate steroid treatment for management of various ailments, we have resolved to investigate corticoid potential of herbal steroids by freund's adjuvant arthritis. Anti-inflammatory compounds do not inhibit secondary lesions, which are prevented or diminished by immunosuppressive agents and both anti-inflammatory and Immunomodulatory action suggest test drug's corticoid potential. In present study Albino Wistar Male rats with an initial body weight of 150 to 200 g was injected into the sub plantar region of the left hind paw with 0.1 ml of heat-killed Mycobacterium tuberculosis (H37Ra) suspended in incomplete freund's adjuvant (4mg/ml). All ethanolic extract of test drugs *Trigonella foenum graecum* (ETF), *Dioscorea alata* (EDA), *Hemidesmus indicus* (EHI), *Agave americana* (EAA) and *Solanum xanthocarpum* (ESX) *300mg*/kg per rat and the standard (Hydrocortisone-Primacort200) 10 mg per rat administered orally for 12 days. Purposely, from day 13 to 21, the animals received neither test compound nor the standard. Paw volumes of both sides and body weight were recorded on the day of injection, day 5, day 12, day 17 and day 21 whereby paw volume is measured plethysmographically. The severity of the secondary lesions on day 12 is evaluated visually and graded according scheme. The %inhibition of paw volume and suppression of arthritic lesion on day 21 for HC, EHI, EAA and ESX found highly significant while ETF and EDA showed significant result ($p<0.05$).

Keywords: *Trigonella foenum graecum, Dioscorea alata, Hemidesmus indicus, Agave Americana, Solanum xanthocarpum,* Freund's adjuvant arthritis

Introduction

Steroids are unique class of chemical compounds that are found throughout the animal and plant kingdom and this class includes sterols such as cholesterol, ergosterol, bile acids, steroid hormones [1]. Since the introduction of cortisone and hydrocortisone anti-inflammatory steroids have remained an important and un-replaced drug class. Although not without adverse effects, these compounds have continued to be the "drug of choice "in the treatment of afflictions ranging from the moderate skin rash to severe acute inflammatory disorders [2]. Glucocorticoids and their metabolites were recognized early as possessing powerful anti-inflammatory and immunomodulatory properties. Herbal medicines are safer than allopathic medicine.

There would be no serious side effects associated with longer use of herbal material. Steroid possesses glucocorticoid as well as mineralocorticoid activity. There is no credible research on the evaluation of herbal steroids. Apart from precursor for allopathic steroids, many activities have been reported for important herbal steroids. Dioscorea Species can be used in preparation of medicaments for preventing mucositis caused by radiotherapy and chemotherapy [3]. The ethanolic extract of Dioscorea membranacea exhibited potent inhibitory activity against β hexosaminidase release as a marker of degranulation in RBL-2H3 cells, which shows their anti-allergic activities [4].

Diosgenin modulates certain aspects of acquired immunity, including the enhancement of antigen-specific IgG2a and IFN-γ expression, which may be mediated through the up-regulation of Th1 differentiation [5]. Plant steroid, diosgenin, causes an inhibition of the growth of fibroblast-like synoviocytes (FLS) from human rheumatoid arthritis (RA), with apoptosis induction associated with cyclooxygenase-2 (COX-2) up-regulation [6]. The anti-inflammatory effect of solasodine and of methanol extract of Solanum trilobatum was evaluated and it showed significant anti-inflammatory activity [7]. Solasodine and preparation of solasodine organic acid salts useful as antitumor, anti-asthmatic or anti-inflammatory agent [8]. Solasodine hydrochloride from the fruit juice of Solanum nigrum or Solanum aviculare can be prepared and it can be used for treatment of neoplasm, asthma, and inflammation as oral or injection medication [8]. A formulation of Smilax china which considered as good source for Yamogenin has been reported for treatment of gynecological inflammation [9]. Agave genus is the only reported source for hecogenin and many agave plants have been identified as source for hecogenin [10]. The methanolic extract of Agave intermixta may be mediated by its constituent hecogenin and might contribute to the anti-inflammatory activity shown by this species. Anti-inflammatory activity also reported for Lyophilized aqueous extract obtained from Agave Americana [11].

Inflammation induced by Viper venom and Propiono bacterium acne are reported to be treated by root extract [5]. A lep of root powder applied topically is used to treat swellings, inflammation and chronic rheumatism [12] [13]. Many other reports suggest the activity against inflammation induced due to pathogenesis for example, asthma, leprosy, swelling of joints, inflamed eyes and in chronic cough, etc.Many pharmacologic activities of steroid containing plants and herbal steroids viz. diosgenine, solasodine, yamogenin, sarsepogenin and hecogenin have been reported [14]. In present protocol albino Wistar rat were treated with complete Freund's adjuvant to evaluate steroidal potential of ETF, EDA, EHI, EAA and ESX. The result of this study gives important direction for preparation of glucocorticoid and mineralocorticoid comparative index of selected herbs. Many well established medicines originate from plants. For example, the painkiller morphine comes from poppies, aspirin comes from the bark of willow trees and digoxin (a drug used to treat heart failure) derives from foxglove. This may be a clue for investigating use of herbal steroids as therapeutic agents.

MATERIALS & METHODS

Preparation of Extracts

The plant materials herbariums (No. SU/DPS/407-411, dated 14/10/2011) were prepared as per the guideline given by centre for advanced studies in plant biotechnology and genetic engineering, Saurashtra University, Rajkot and perform identification and authentification. The powdered plant materials (100 gm) were soaked with 20 ml distilled water for 12 hrs before extraction with ethanol. The drug was defatted with petroleum ether (60-80°C) and extracted with ethanol (95%) using soxhlet apparatus continuously for 48 hrs. All ethanolic extracts were individually filtered, through Whatmann filter paper # 42 and evaporated to dryness at 50°C in oven. The ratio of powder to solvent was 1:5 W/V. The extracts were weighed for and yield were calculated and stored in desiccators till further use [15].

Animal

Male albino wistar rats weighing 150–210 gm were used in this study. Animals were housed in groups of six rats on 12-hour light and 12-hour dark cycle; and were maintained in an air-conditioned animal quarter at a temperature of 22±2 °C and a relative humidity of 60±10 %. They were offered water and food *ad libitum*. The animals were acclimatized to the facilities for 5 days. Experiments reported in this study were carried out in accordance with current guidelines for the care of laboratory animals and the ethical guidelines for investigation of experimental pain in conscious animals. The Institutional Animal Ethical committee (IAEC) has approved the protocol of the study. All efforts were made to minimize the number of animals used and their suffering.

Adjuvant arthritis induced by freund's adjuvant

Albino Wistar Male rats with an initial body weight of 150 to 200 g was used. On day 1, they were injected with ketamine (40mg/kg) intraperitoneally [16] and anesthetized. Each animal injected into the sub plantar region of the left hind paw with 0.1 ml of heat-killed Mycobacterium tuberculosis (H37Ra) suspended in incomplete freund's adjuvant (4mg/ml). Vehicle administered orally and treated as control group, All animal were grouped (n=6) according to following,

Group-I	Control Group
Group-II	Standard Drug- Hydrocortisone
Group-III	Ethanolic extract of *Trigonella foenum graecum* (ETF)
Group-IV	Ethanolic extract of *Dioscorea alata* (EDA)
Group-V	Ethanolic extract of *Hemidesmus indicus* (EHI)
Group-VI	Ethanolic extract of *Agave americana* (EAA)
Group-VII	Ethanolic extract of *Solanum xanthocarpum* (ESX)

All test drugs ETF [17], EDA [18], EHI [19], EAA [20], ESX [21] *300mg*/kg per rat and the standard (Hydrocortisone-Primacort200) 10 mg per rat administered orally for 12 days. Purposely, from day 13 to 21, the animals received neither test compound nor the standard. Paw volumes (Figure 1) of both sides and body weight were recorded on the day of injection, day 5, day 12, day 17 and day 21 whereby paw volume is measured plethysmographically [22]. The severity of the secondary lesions on day 12 is evaluated visually and graded according Table-1

RESULTS & DISCUSSION

The choice of the animal strain has been found to be very important for the performance of this test. Wistar rats have been proven to be very suitable in contrast to other sub strains. In present study albino Wistar rat were used as test animal and reference [23-24] available for adjuvant arthritis animal model. All animal were grouped (n=6) as control, HC, ETF, EDA, EHI, EAA and ESX. Dose 300 mg/kg per rat kept common for all other treatment group for comparative evaluation of relative potencies. Paw volumes of both sides and body weight were recorded on the day of injection, day 5, day 12, day 17 and day 21 whereby paw volume is measured plethysmographically.

Decrease of weight of normally observed with catabolic steroids in rat. On day 21, the increase in body weight in gram for control, HC, ETF, EDA, EHI, EAA and ESX were 36.33±1.358, 2.0 ±0.894***, 31.0 ±2.955, 28.83±2.428, 25.83±3.270*, 23.67±3.721* and 22.17±2.868** when compared with weight on day 1 (*p<0.05, **p<0.01 and ***p<0.001) The result for change in weight shown in Table 2 while its graphical representation on day 21 given in figure 2

The change in paw volume in ml measure for left injected paw as shown in Table 3and right non injected paw as shown in Table 4, compare with control. Change in paw volume expressed as change in ml and % inhibition by treatment group.

$$\% \text{ Inhibition of paw volume} = \frac{V_{Control} - V_{Test}}{V_{Control}} \times 100$$

Where, $V_{Control}$ = Difference in Volume of paw of control (ml) compare to day 1 and V_{Test} = Difference in Volume of paw of test group (ml) compare to day 1

The percentage inhibition (left paw) on day 21 for HC, ETF, EDA, EHI, EAA and ESX were 65.54±2.482, 13.91±4.187, 15.34±1.997, 28.53±3.663, 29.14±4.300 and 25.26±3.939 respectively (figure 3).

The reduction of edema for HC EAA, EHI and ESX were extremely significant and the reduction of edema for EDA was highly significant (**p<0.01 and ***p<0.001). The reduction of edema for HC EAA, EHI and ESX were highly significant and the reduction of edema for EDA was significant (p<0.05). The change in right (non-injected) paw volume (ml) on day 21 for control, HC, ETF, EDA, EHI, EAA and ESX were calculated and The reduction in paw volume for HC, ETF, EDA, EHI and ESX found highly significant while EAA significantly reduced paw edema (p<0.05). The percentage inhibition (right paw) on day 21 for HC, ETF, EDA, EHI, EAA and ESX were 42.68±3.720, 27.34±4.022, 25.40±3.683, 28.22±5.343, 22.22±4.863 and 30.86±4.003 respectively (figure 4). The reduction in paw volume for HC, ETF, EDA, EHI and ESX found extremely significant while EAA highly significantly reduced paw edema (**p<0.01 and ***p<0.001).

The immunosuppressive activity can also express by inhibition of lesion compare to control on day 21. The arthritic lesion on day 21 for control, HC, ETF, EDA, EHI, EAA and ESX were 6.167±0.402, 1.167±0.167***, 4.667±0.422*, 3.167±0.477**, 2.333±0.333***, 2.167±0.307*** and 2.000±0.258*** respectively. The percentage inhibition of arthritic lesion (Table 5) on day 21 for HC, ETF, EDA, EHI, EAA and ESX were 81.08±2.703, 29.72±3.419, 48.65±4.983, 62.16±5.405, 64.86±4.983 and 67.56±4.187 respectively. The suppression of arthritic lesion on day 21 for HC, EHI, EAA and ESX found extremely significant. ETF and EDA showed significant and highly significant inhibition of arthritic lesion (*p<0.05, **p<0.01 and ***p<0.001).

The total percentage change (table 6) calculated by addition of percentage inhibition of left paw on day 5, percentage inhibition of right paw on day 12 and percentage inhibition of arthritic score on day 21. The total percentage change for HC, ETF, EDA, EHI, EAA and ESX were 124.77, 45.71, 63.76, 86.88, 90.33 and 96.8 respectively. Adjuvent arthritis in rat (n=6) used as animal model to evaluate steroidal potential of selected herbs. The total percentage change was calculated for all extracts and top three results against control group were 96.8, 90.33 and 86.88 for ESX, EAA and EHI respectively.

CONCLUSION

Ethanolic extract of *Hemidesmus indicus* (EHI) *Agave americana* (EAA) *Solanum xanthocarpum* (ESX) found with significant antiimflamatory and immunomodulatory action implies its significant corticoid like action and Ethanolic extract of *Trigonella foenum graecum* (ETF) *Dioscorea alata* (EDA) found only with significant antiinflamatory action.

Table 1. Grading for Evaluation of Secondary lesions

Body Part	Effect	Score
Ears	Absence of nodules and redness	0
	Presence of nodules and redness	1
Nose	No swelling of connective tissue	0
	Intensive swelling of connective tissue	1
Tail	Absence of nodules	0
	Presence of nodules	1
Forepaws	Absence of inflammation	0
	Inflammation of at least 1 joint	1
Hind paws	Absence of inflammation	0
	Slight inflammation	1
	Moderate inflammation	2
	Marked inflammation	3

Table 2. Effect of HC, ETF, EDA, EHI, EAA and ESX on body weight during Adjuvant arthritis study

Group	Increase in Body Weight (gram)			
	At Day 5	At day 12	At day 17	At day 21
Control	12.00± 0.516	22.50±0.991	28.17±1.424	36.33±1.358
HC	8.50 ±0.341*	16.67±0.666*	9.66 ±0.802***	2.0 ±0.894***
ETF	9.83 ±0.601	18.17±1.249	22.83±2.040	31.0 ±2.955
EDA	10.50±0.763	18.33±1.542	23.00±2.324	28.83±2.428
EHI	10.00 ±0.816	18.50±1.522	22.33±2.431	25.83±3.270*
EAA	10.00 ±0.632	17.33±1.820	20.50±3.233	23.67±3.721*
ESX	11.17 ±1.641	17.00±1.983	19.33±2.171*	22.17±2.868**
Six rats were used in each group. The increase in body weight in gram was calculated by subtracting weight at day 1 from that after FCA injection. The value expressed mean±S.E.M. *p<0.05, **p<0.01 and ***p<0.001 vs control group.				

Table 3 Percentage inhibition of left (injected) paw volume during adjuvant arthritis study by HC, ETF, EDA, EHI, EAA and ESX

Group	% inhibition of paw volume of the injected left paw			
	At Day 5	At day 12	At day 17	At day 21
HC	14.51±5.166	29.25±2.960	54.81±2.484	65.54±2.482
ETF	9.07±5.254	17.99±3.455	22.45±2.934	13.91±4.187
EDA	1.41±5.855	10.31±4.767	13.55±3.246	15.34±1.997
EHI	5.72±7.874	11.37±4.895	20.37±4.135	28.53±3.663
EAA	7.96±7.041	14.41±6.348	21.98±4.952	29.14±4.300
ESX	9.39±6.193	14.02±4.071	21.98±4.274	25.26±3.939
Six rats were used in each group. The change in right paw volume in ml was calculated by subtracting weight at day 1 from that after FCA injection. The value expressed mean±S.E.M. *p<0.05, **p<0.01 and ***p<0.001 vs control group.				

Table 4 Percentage inhibition of right (non-injected) paw volume during adjuvant arthritis study by HC, ETF, EDA, EHI, EAA and ESX

Group	% inhibition of paw volume of the non-injected right paw			
	At Day 5	At day 12	At day 17	At day 21
HC	16.31±4.380	29.18±4.354	29.70±6.117	42.68±3.720
ETF	6.86±6.619	6.92±6.815	8.89±6.340	27.34±4.022
EDA	5.67±8.293	13.70±5.452	9.9±3.880	25.40±3.683
EHI	11.58±4.882	19.00±3.937	14.34±4.835	28.22±5.343
EAA	12.06±3.513	17.51±3.649	8.889±4.883	22.22±4.863
ESX	13.24±6.934	19.85±5.793	16.16±5.833	30.86±4.003

Table 5. Percentage inhibition of arthritic index during adjuvant arthritis study by HC, ETF, EDA, EHI, EAA and ESX

Group	Arthritic index on day 21	% inhibition of arthritic lesion
Control	6.167±0.402	------
HC	1.167±0.167***	81.08±2.703
ETF	4.667±0.422*	29.72±3.419
EDA	3.167±0.477**	48.65±4.983
EHI	2.333±0.333***	62.16±5.405
EAA	2.167±0.307***	64.86±4.983
ESX	2.000±0.258***	67.56±4.187
Six rats were used in each group. The arthritic index was graded on the scale of 0-4. The value were expressed mean±S.E.M. *p<0.05, **p<0.01 and ***p<0.001 vs. control group		

Table 6. Total percentage change during adjuvant arthritis study by HC, ETF, EDA, EHI, EAA and ESX

Group	Total percentage change
HC	124.77
ETF	45.71
EDA	63.76
EHI	86.88
EAA	90.33
ESX	96.8

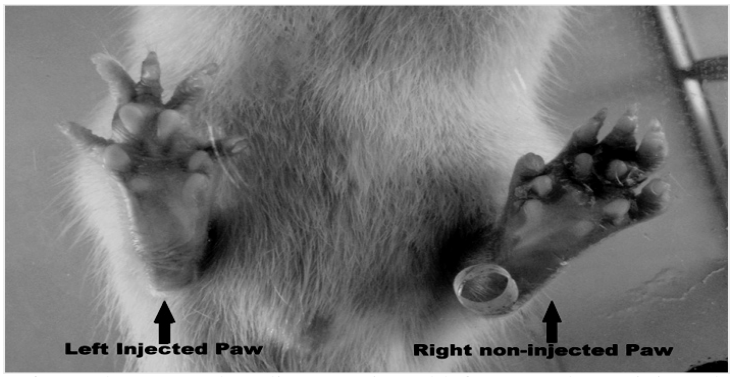

Figure 1. Effect of complete Freund's adjuvant Injected left paw and Non-injected right paw of rat

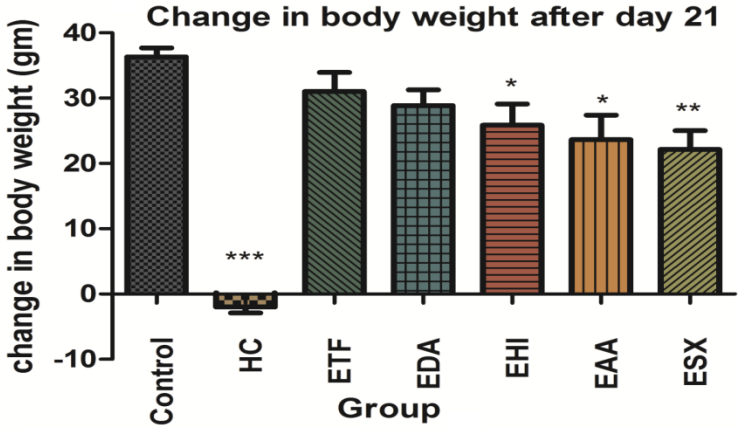

Figure 2 Effect of HC, ETF, EDA, EHI, EAA and ESX on body weight on day 21 during adjuvant arthritis study. Each column represents the mean±S.E.M. of six rats. *p<0.05, **p<0.01 and ***p<0.001 vs. control group.

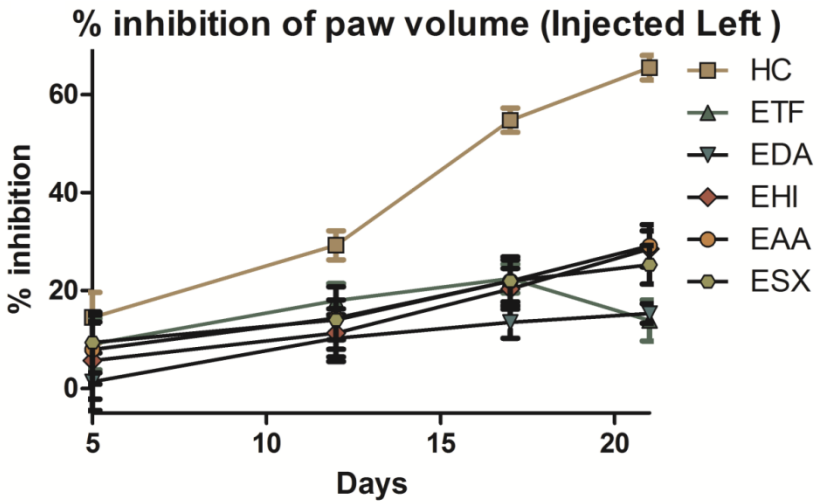

Figure 3 Percentage inhibition of left (injected) paw volume during adjuvant arthritis study by HC, ETF, EDA, EHI, EAA and ESX. Each value represents the mean±S.E.M. of six rats.

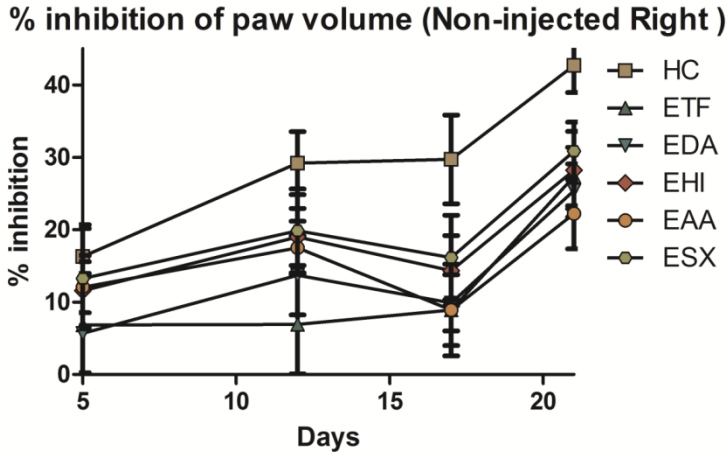

Figure 4 Percentage inhibition of right (non-injected) paw volume during adjuvant arthritis study by HC, ETF, EDA, EHI, EAA and ESX. Each value represents the mean±S.E.M. of six rats.

REFERENCES

1. Petrow ED, A history of *steroid* chemistry: some contributions from European industry. Steroids, 1996;61:473-475.
2. Baxter JD, Rousseau GG. et al. Glucocorticoid Hormone Action: An Overview. Monogr Endocrinol. 1997;12:1-24
3. Feng P. Pharmacological screening of some west Indian medicinal plants. J. of Pharmacy and Pharmacology. 1964;16:115–119.
4. Tewtrakul S, Itharat A. et al. Anti-allergic substances from the rhizomes of Dioscorea membranacea. Bioorganic Medi Chem. 2006;14(24):87-89.
5. Jain A, Basal E. et al. Inhibition of Propioni bacterium acnes-induced mediators of inflammation by Indian herbs. Phytomedicine . 2003;10:34–38.
6. Liagre B, Vergne S, Pascale C, Cecile C, Jean L. et al. Diosgenin, a plant steroid, induces apoptosis in human rheumatoid arthritis synoviocytes with cyclooxygenase-2 overexpression. Arthritis Research & Therapy, 2004;6(4):373-374
7. Emmanuel S, Ignacimuthu S, Perumalsamy R Amalraj T. et al. Anti-inflammatory activity of Solanum trilobatum. Fitoterapia. 2006;77(8):611-620
8. Liu L, Liu X, Cui S. et al. Optically Active beta-Aryl Substitution-2(5*H*)-ketofuran and Its Synthesis Process. Faming Zhuanli Shenqing, Gongkai Shuomingshu. 2001;8-9
9. Kawai T, Tomono N, Wakamatsu K . et al. Antiallergics and antihistaminics containing extract of sisal, Agave, or Forsythia suspensa and cosmetics containing the agents.. JP2000136142 assign to Jpn. Kokai Tokkyo Koho 2000
10. Marston A, Hostetimann K. et al. Plant Molluscicides- Review article. Phytochem. 1985;24 (4):639-643.
11. Quilez AM, Saenz MT, Garcia MD. et al. J. Pharmacy and Pharmacol. 2004;56(9):1185-1190.
12. Alam MI, Auddy B, Gomes A. et al. Isolation purification and partial characterization of viper venom inhibiting factor from the root extract of the Indian medicinal plant sarsaparilla (Hemidesmus indiccus R. Br.).1994; 32:1551-1557

13. Alam MI, Gomes A. et al. Adjuvant and antiserum action potentiation by a (herbal) compound 2-hydroxy-4methoxy benzoic acid isolated from the root extract of the Indian medicinal plant sarsaparilla (Hemidesmus indiccus R. Br.). Toxicon. 1998;36(10):1423-1431
14. Applezwegi N steroids drugs. J Pharmacy and Pharmaco. 1962;16(9):569–595
15. Kotadia KP. Development of validated HPTLC method for estimation of diosgenin in germinated seeds of Trigonella foenum" M.Pharm Thesis. 2005;96-97
16. Fahim I, Ismail M, Osman OH. et al. Role of 5-hydroxytryptamine in ketamine-induced hypothermia in the rat. Br. J. Pharmac., 1973;48:570-576.
17. Asmena M, Alauddin M, Md Atiar R, Kabir A. et al. Antihyperglycemic Effect of Trigonella Foenum-Graecum (Fenugreek) Seed Extract in Alloxan-Induced Diabetic Rats and Its Use in Diabetes Mellitus: A Brief Qualitative Phytochemical and Acute Toxicity Test on the Extract. Afr J Tradit Complement Altern Med. 2009;6(3): 255–261.
18. Dhanabal SP, Mohan Marugaraja MK, Suresh B. et al. Antidiabetic Activity of Clerodendron phlomoidis leaf extract in alloxan-induced diabetic rats. Indian J Pharma Sci, 2008;70(6):841-844
19. Anoop A. et al. A review on Indian sarsaparilla, Hemidesmus indicus (L.) R. Br. J Biological Sci. 8 2008;(1):1-12
20. Dana AH, Nicole G, John SM, Adam M, Steven GO, Chris T, Wendy W, Shannon W, Catherine U. et al. Agave (Agave americana). J Herbal Pharmacotherapy. 200;6(2) :101-122
21. Rahman MT, Ahmed M, Alimuzzaman M, Shilpi JA. et al. Antinociceptive activity of the aerial parts of Solanum xanthocarpum. Fitoterapia 2003;74:119–121
22. Vogel HG. Endocrinology. Drug Discovery and Evaluation. 2002;2: 1131-1143.
23. Rekha R, Ekambaram K. et al. Anti-Arthritic Activity of Premna serratifolia Linn., Wood against Adjuvant Induced Arthritis. Avicenna J Medi Biotech. 2010;2(2):2-3
24. David ET, Joseph MW, Pia S, John RD. et al. Autoimmunity to Collagen in Adjuvant Arthritis of Rats. J. Clin. Invest. 1980;66:1109-1117