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ORIGINAL ARTICLE **OPEN ACCESS**

Evaluation of Analgesics and Anti-inflammatory Activity of Polyherbal Formulation Containing Some Indigenous Medicinal Plants

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ABSTRACT
Rheumatoid arthritis is a chronic multi-systemic disease of unknown cause. It affects the people in their prime of life, predominantly between the ages of 20-50 years with unpredictable course. Various poly-herbal formulations are used in the ayurvedic system of medicine for the treatment of inflammation and pain associated with rheumatoid arthritis, osteo-arthritis, frozen shoulder, ankylosing spondylitis and chronic backache. Our study was aimed to evaluate efficacy of poly-herbal formulation using different animal models such as hot plate reaction time, acetic acid induce writhing in mice, carrageen (1% v/v)-induced paw edema. The results indicated that the poly-herbal formulation possesses good analgesic and anti-inflammatory activities in the experimental animal models.
KEYWORDS: *Analgesic; anti-inflammatory; poly-herbal formulation.*

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease which is characterized by a series of pathological processes of the joints, such as leukocyte infiltration, pannus formation and extensive destruction of the articular cartilage and bone [1]. RA affects ~1 % of the adult population worldwide [2]. Although there are drugs that have been shown to improve signs and symptoms, alter the natural history of the disease and improve quality of life, but there is still no cure. In addition, these available therapies are associated with potential risks of death or irreversible organ damage [3]. The challenge for society is to balance these known potential risks of therapy with acknowledged benefits despite the fact that these drugs do not lead to a cure. The most commonly prescribed medication for RA treatment is steroidal, non-steroidal anti-inflammatory, disease modifying antirheumatic and immunosuppressant drugs. Though the goal of these drugs have been to relieve pain and to decrease joint inflammation, to prevent joint destruction and to restore function of disabled joints, these drugs are known to produce various side effects including gastrointestinal disorders, immunodeficiency and humoral disturbances [4-9]. Accordingly, reducing side effects should be considered while designing improved therapeutics for RA, besides enhancing medicinal effectiveness. The Siddha and Ayurvedic systems of treatment are being increasingly recognized as an alternate approach to arthritic treatment

MATERIAL AND METHODS

I) Plant material

Leafs of *Aerva lanata* Plant collected from Urlugonda (village), Suryapet District, and Telangana. Leafs of Bauhinia *variegata* collected from Herbal Garden Chilkur Balaji College of Pharmacy, *Acmella uliginosa* obtained from Herbal garden, Himayath Sagar, Ranga Reddy district , Hyderabad, all the three plants ere authenticated and Botanical identification of the plants was done by Botanical Survey of India, Deccan Regional Centre , Hyderabad, Specimens of *Aerva lanata, Bauhinia variegata, Acmella uliginosa* (Voucher number: BSI / DRC / 16-17 / Tech/1005).On 22/03/2017.

Preparation of extracts:

Accurately about 5 g of the coarsely powdered air dried powder of *Aerva lanata, Bauhinia variegate and Spilanthes acmella* were macerated with 100 ml of petroleum ether in a closed flask for 24 hours, shaking frequently during every six hours and allowing it standing for about eighteen hours. Filtered it rapidly and about 25 ml of the filtrate been evaporated to dryness in a tarred shallow and flat bottomed dish, and dried at 105°C to abtain constant weight and weighed it. The entitlement of Pet.ether-soluble extractive of plant material was calculated with reference to the air-dried powder of *Aerva lanata, Bauhinia variegate and Spilanthes acmella.*

Animals:

Swiss albino mice weighing 20-25 g and Wistar rats weighing 150–180 g of either sex were used for the study. The animals were housed in solid- bottomed polypropelene cages and acclimatized to animal house conditions. The rats were fed with commercial rat's diet and water ad libitium. The experiments were designed and conducted in accordance with ethical norms approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPSCEA) and Institutional Animal Ethical Committee (IAEC).

Pharmacological screening

***In vivo* Pharmacological screening of plant extracts**

Analgesic activity of *Aerva lanata, Bauhinia variegate and Spilanthes acmella methanolic*

Analgesic activity of *Aerva lanata, Bauhinia variegate and Spilanthes acmella* ethanolic extrtacts was screened by "Hot plate", "Tail immersion" and "Acetic acid induced writhing" models.

i) Hot plate method [31-32]

the consortium of animals was done consequently. The animals were assessed for weight and marked appropriately. The test and standard drugs were given by oral route. The paws of rats are very delicate to warmth at temperatures which does not destructive the skin. The responses are jumping, withdrawal of the paws and hammering of the paws. The hot plate, which is commercially available, comprises of an electrically heated exterior. The temperature is controlled for 55° to 56 °C. This can be a copper plate or a impassioned glass surface. The animals from all groups were positioned on the hot plate and the time until either licking or jumping transpires is known by a stop-watch at the time intermission of 0, 60, 120, 180 and 240 minutes.

ii) Acetic acid induced writhing model [32, 33]

In this method, acetic acid is administered intraperitoneally to the experimental animals to create pain sensation. *Aerva lanata, Bauhinia variegate and Spilanthes acmella* ethanolic vehicle were administered orally 60 minutes prior to intraperitoneal administration of 0.75% v/v acetic acid solution (0.1mL/10g) but Pentazocine (30 mg/kg) was administered 15 minutes preceding to acetic acid injection. Then the animals were located on an observation table. Each mouse of all groups was observed exclusively for counting the number of writhing they made in 15 minutes origination just 5 minutes afterwards the intraperitoneal administration of acetic acid solution. Complete writhing was not always dexterous by the animal, since intermittently the animals ongoing to give writhing but they did not wide-ranging it. This unfinished writhing was measured as half-writhing. subsequently, two half-writhing were recorded as one full writhing. The number of writhes in each treated group was related with the standard group received pentazocine 30mg/kg.

ii) Tail immersion method [34, 35]

For tail immersion method, Swiss albino rats weighing between 100-150g were divided into four group comprising of six animals each as shown in table. Prior to analgesic experiments, the animals were screened for the sensitivity test by immersing the tail of the mice moderately in hot water continued at 55° C – 55.5° C. The animal immersing the tail from hot water with in 5 second was designated for the study. After administration of the drugs, the reaction time was recorded at time intervals of 0, 60, 120, 180 and 240 minutes.

Groping of animals for analgesic activity of *Aerva lanata, Bauhinia variegate and Spilanthes acmella* extracts by hot plate and tail immersion methods

Group	Name of Group	Treatment
I	Control	2% w/v of Gum acacia 2 ml/kg
II	Standard	Pentazocine 30 mg/kg
III	Test I	250 mg/kg (AL + BV)
IV	Test II	500 mg/kg (AL + BV)
V	Test III	250 mg/kg (BV+ SA)
VI	Test IV	500 mg/kg (BV+ SA)
VII	Test V	250 mg/kg (AL+SA)
VIII	Test VI	500 mg/kg (AL+SA)

Anti-inflammatory activity of *Aerva lanata, Bauhinia variegate and Spilanthes acmella* extracts

Anti-inflammatory activity *Aerva lanata, Bauhinia variegate and Spilanthes acmella* extracts was evaluated by carrageenan and Cottoin pelate models.

i) Carrageenan induced inflammation model [36, 37]

For carrageenan induced inflammatory model, assemblage of animals was done as revealed in table. The acute hind paw oedema in animals was induced by Carrageenan, where 0.1 ml of carrageenan (made as 1% w/v suspension in NS) locally injected into sub plantar section of the leftward hind paw of rats. Rats from all the groups were administered with the treatment orally 1 hour former to carrageenan injection.

The rat paw volume up to the ankle joint was measured at 0 (30 min prior to carrageenan injection), 60, 120, 180 and 240 minutes afterwards the injection of carrageenan with digital plethysmometer.

Percent inhibition of paw volume amongst test and control groups were measured as follows:

$$Percentage\ Inhibition = \frac{V_0 - V_t}{V_0} \times 100$$

Where, V0 = volume of the paw of control,

Vt = volume of the paw of test

Cotton Pellet Induced Granuloma [39-44]

The cotton pellet-induced granuloma in rats was evaluated as specified by winter and Porter. The cotton pellets of which weighing about 50 mg were sterilized in an autoclave for 30 min at 120^0 C under 15 Ib pressure. One pellet of cotton was implanted subcutaneously (s.c.), on lumbar region of each rat under light ether anesthesia and sterile technique after shaving the area and disinfecting it with 70% ethanol.

The cotton pellets were removed surgically from extraneous tissues. The dry cotton weight was recorded after dried at 60°C for 24 h. The weight difference between dry cotton and the cotton pellet before implantation is considered as weight of granuloma formed.

Inhibition% was calculated using the following equation:

(Weight of pellet in control group - Weight of pellet in test group)/Weight of pellet in control group×100.

Grouping of animals for anti-inflammatory activity of *Aerva lanata, Bauhinia variegate and Spilanthes acmella* by Carrageenan and Cotton pelate models

S. No.	Name of Group	Treatment
Group I	Control	2% w/v of Gum acacia 2 ml/kg
Group II	Standard	Ibuprofen (10 mg/kg)
Group III	Test I	250 mg/kg (AL + BV)
Group IV	Test II	500 mg/kg (AL + BV)
Group V	Test III	250 mg/kg (BV+ SA)
Group VI	Test IV	500 mg/kg (BV+ SA)
Group VII	Test V	250 mg/kg (AL+SA)
Group VIII	Test VI	500 mg/kg (AL+SA)

Statistical significance

All the research outcomes attained were recorded as the Mean ± S.E.M. The information was evaluated for statistical significance using one-way Analysis of Variance (ANOVA) followed by Bonferroni's test using computerized Graph Pad Prism, version 4.5 software (Graph Pad Software Inc.). Statistical significance was established consequently.

Analgesic activity of *Aerva lanata, Bauhinia variegate and Spilanthes acmella in fixed dose combinations*

Animals treated with the standard drug exhibited a significant ($p<0.05$) upsurge in mean reaction time that was found to be augmented highly at time interval of 120, 180 and 240 minutes of study while the *Aerva lanata* showed a trivial increase in the mean reaction time at its lower dose level that is 250 mg/kg which was found to be significant ($p<0.05$) at the dismissing time of study where as it was significant ($p<0.05$) at its higher test dose 500 mg/kg at the time interval of 180 and 240 minutes of study when related to control group. *Bauhinia variegate withAerva lanata* extract at 500 mg/kg exhibited a significant analgesic activity in hot plate and tail immersion methods. *Spilanthes acmella with Bauhinia variegate* plant extract at 500 mg/kg exhibited a significant analgesic activity in hot plate and tail immersion methods which is very near to the activity of *Spilanthes acmella with Bauhinia variega.*

Acetic acid induced writhing method

In case of writhing model with ***Aerva lanata +Bauhinia variegate*** at 500 mg/kg test dose, number of writhes were condensed and found to be statistically noteworthy and percentage inhibition of writhing test was found to be 50.09 which are nearly comparable with standard drug value that is 62.73. *Bauhinia*

variegate + Spilanthes acmella at 500 mg/kg and *Aerva lanata + Spilanthes acmella* 500 mg/kg showed 41.73% and 49.84% inhibition of the acetic acid induced writhing respectively.

Table:1 Analgesic activity of *Aerva lanata, Bauhinia variegate and Spilanthes acmella* *in combination* by hot plate method

Group (treatment)	Mean Reaction time in Sec				
	30 min	**60 min**	**120 min**	**180 min**	**240 min**
Control	2.80+ 0.29	2.68+ 0.30	3.29+ 0.30	3.21+0.32	3.20+ 0.21
Pentazocine (30 mg/kg)	2.72+ 0.20	4.68+ 0.79	5.95+ 0.85	6.10+0.89*	6.59+ 0.96*
Aerva lanata +Bauhinia variegate (250 mg/kg)	2.49+ 0.22	3.14+ 0.65	4.32+ 0.84	4.35+ 0.81	5.07+ 0.78*
Aerva lanata +Bauhinia variegate (500 mg/kg)	2.63+ 0.29	3.70+ 0.91	5.32+ 0.22	5.38+0.28*	5.79+ 0.39*
Bauhinia variegate + Spilanthes acmella(250 mg/kg)	2.18+0.70	2.60+0.03	3.19+ 0.58	3.18+ 0.68	4.07+ 0.59
Bauhinia variegate + Spilanthes acmella(500 mg/kg)	1.90+ 0.06	2.59+ 0.91	3.42+ 0.68	3.49+ 0.71*	4.02+ 0.89*
Aerva lanata + Spilanthes acmella(250 mg/kg)	2.52+0.28	2.59+0.12	3.25+ 0.41	3.52+ 0.82	4.11+ 0.56
Aerva lanata + Spilanthes acmella(500 mg/kg)	1.52+ 0.02	2.32+ 0.12	3.56+ 0.71	3.59+ 0.32*	4.01+ 0.67*

All values were expresses as Mean+ SEM, one way ANOVA followed by Bonferroni's test, *p<0.05 when compared to control group

Table:2 Analgesic activity of *Aerva lanata, Bauhinia variegate and Spilanthes acmella in fixed dose combinations* by hot plate method

Group (treatment)	Mean Reaction time in Sec				
	30 min	**60 min**	**120 min**	**180 min**	**240 min**
Control	2.32+0.812	2.31+ 0.078	2.49+ 0.178	2.62+ 0.116	2.45+ 0.091
Pentazocine (30 mg/kg)	2.27+0.916	3.42+0.571	4.76+ 0.98	5.24+ 0.08**	6.40+ 0.41**
Aerva lanata +Bauhinia variegate (250 mg/kg)	2.38+0.821	3.20+0.312	3.58+ 0.512	3.89+ 0.62*	4.51+ 0.06*
Aerva lanata +Bauhinia variegate (500 mg/kg)	2.45+0.042	3.88+0.616	3.68+ 0.678	5.20+ 0.07**	5.13+ 0.7**
Bauhinia variegate + Spilanthes acmella(250 mg/kg)	1.92+0.59	2.12+0.32	2.65+0.89	3.19+0.69*	3.49+0.51*
Bauhinia variegate + Spilanthes acmella(500 mg/kg)	1.97+0.51	2.49+0.98	3.12+0.72	4.12+0.96**	4.90+0.49**
Aerva lanata + Spilanthes acmella(250 mg/kg)	2.21+0.85	3.19+0.29	3.47+ 0.42	3.72+ 0.58*	4.67+ 0.04*
Aerva lanata + Spilanthes acmella(500 mg/kg)	1.99+0.54	2.48+0.95	3.33+0.75	4.15+0.99**	4.85+0.22**

All values were expresses as Mean+ SEM, *p<0.05, **p<0.01 when compared to control

Table:3 Analgesic activity of *Aerva lanata, Bauhinia variegate and Spilanthes acmella* extract by writhing method

Group	No. of writhes	% of Inhibition
Control	35.67+1.32	--------
Pentazocine 30 mg/kg	13.69+0.59*	62.73
Aerva lanata +Bauhinia variegate (250 mg/kg)	23.39+0.32	41.81
Aerva lanata +Bauhinia variegate (500 mg/kg)	18.9+0.421*	50.09
Bauhinia variegate + Spilanthes acmella(250 mg/kg)	25.01+ 0.31	33.36
Bauhinia variegate + Spilanthes acmella(500 mg/kg)	21.19+ 0.24*	41.73
Aerva lanata + Spilanthes acmella(250 mg/kg)	23.46 + 0.42	35.47
Aerva lanata + Spilanthes acmella(500 mg/kg)	19.24 +0.23	49.84

All values were expresses as Mean+ SEM, one way ANOVA followed by Bonferroni's test, *p<0.05 when compared to control group

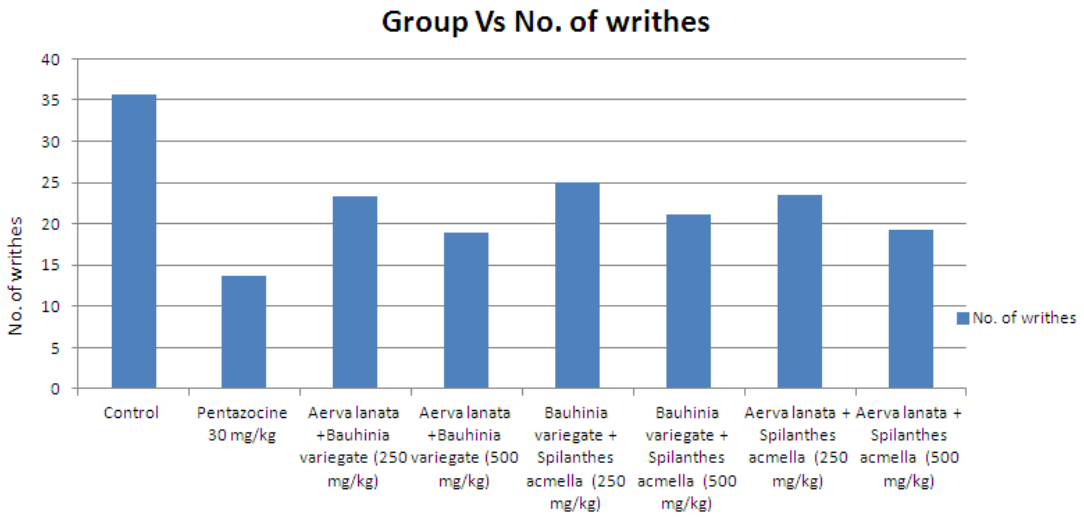

Figure 1. Analgesic activity of *Aerva lanata*, Bauhinia variegate and *Spilanthes acmella* extract by writhing method

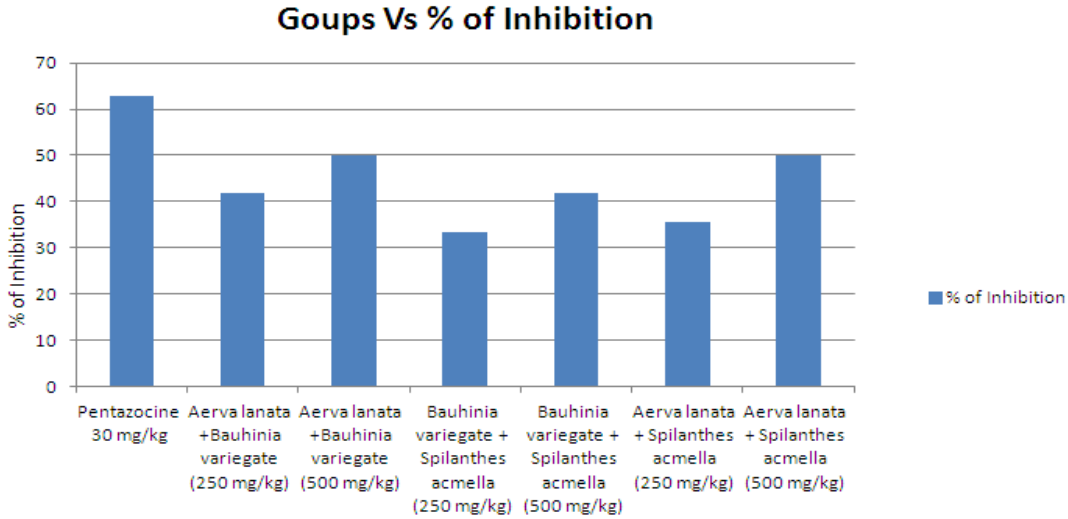

Figure 2. Analgesic activity of *Aerva lanata*, Bauhinia variegate and *Spilanthes acmella* extract by writhing method

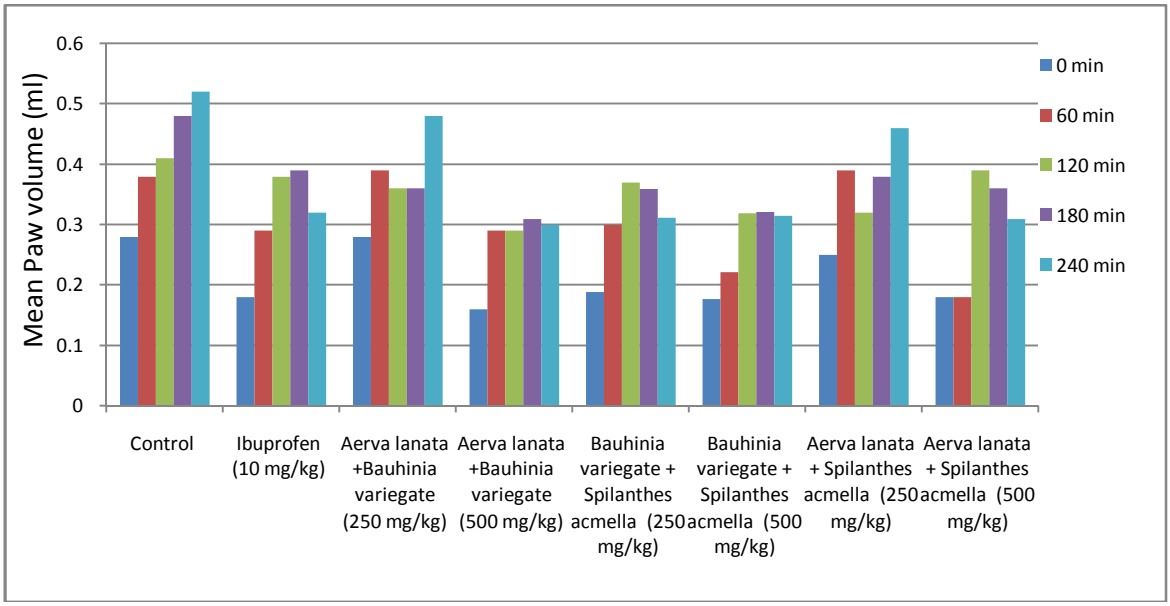

Figure 3. Anti-inflammatory activity *Aerva lanata*, Bauhinia variegate and *Spilanthes acmella* in fixed dose combinations by cotton pellate model

Table:4 Anti-inflammatory activity *Aerva lanata, Bauhinia variegate and Spilanthes acmella in fixed dose combinations* by Carrageenan induced model

Group (treatment)	Mean Paw volume (ml)					% Inhibition at the end of 4th Hr.
	0 min	60 min	120 min	180 min	240 min	
Control	0.28+0.001	0.38+0.009	0.41+0.031	0.48+0.041	0.52+0.021	------
Ibuprofen (10 mg/kg)	0.18+0.028 (25)	0.29+0.021 (43.58)	0.38+0.021 (33.33)	0.39+0.019* (24.44)	0.32+0.018* (48.27)	48.27
Aerva lanata +Bauhinia variegate (250 mg/kg)	0.28+0.041 (12.5)	0.39+0.029 (17.94)	0.36+0.020 (29.16)	0.36+0.031* (22.22)	0.48+0.021 (25.86)	25.86
Aerva lanata +Bauhinia variegate (500 mg/kg)	0.16+0.021 (20.83)	0.29+0.030 (28.20)	0.29+0.021 (37.5)	0.31+0.019* (27.08)	0.30+0.031* (43.10)	43.10
Bauhinia variegate + Spilanthes acmella(250 mg/kg)	0.189+ 0.041 (18.75)	0.300+ 0.026 (23.33)	0.370+ 0.041 (35.41)	0.359+ 0.036 (27.11)	0.312+ 0.029 (44.90)	44.90
Bauhinia variegate + Spilanthes acmella(500 mg/kg)	0.177+ 0.029 (28.33)	0.222+0.041 (43.07)	0.319+ 0.059 (34.37)	0.321+ 0.041* (27.11)	0.315+0.031* (40.00)	40.00
Aerva lanata + Spilanthes acmella(250 mg/kg)	0.25+0.038 (14.5)	0.39+0.022 (15.94)	0.32+0.014 (28.16)	0.38+0.024* (24.22)	0.46+0.024 (23.86)	29.86
Aerva lanata + Spilanthes acmella(500 mg/kg)	0.18+0.008 (21.43)	0.18+0.032 (22.10)	0.39+0.027 (34.5)	0.36+0.017* (87.08)	0.31+0.031* (44.10)	40.10

All values were expresses as Mean+ SEM, one way ANOVA followed by Bonferroni's test, *p<0.05 when compared to control group; Figures in the parentheses shows percentage inhibition

Table:5 Anti-inflammatory activity *Aerva lanata, Bauhinia variegate and Spilanthes acmella* in fixed dose combinations by cotton pellate model

Groups	Mean Exudate Weight(mg)	Exudate inhibition(%)
Disease control	108.7±1.312	
Standard [Aceclofenac -50 mg/kg, b.wt]	59.01±1.562***	46.41
Aerva lanata +Bauhinia variegate (250 mg/kg)	96.01±1.024**	12.52
Aerva lanata +Bauhinia variegate (500 mg/kg)	86.13±3.630***	21.38
Bauhinia variegate + Spilanthes acmella(250 mg/kg)	99.41±1.21**	13.82
Bauhinia variegate + Spilanthes acmella(500 mg/kg)	81.16±3.58***	18.42
Aerva lanata + Spilanthes acmella(250 mg/kg)	91.42±1.01**	16.42
Aerva lanata + Spilanthes acmella(500 mg/kg)	88.63±1.56***	19.48

All values were expresses as Mean+ SEM, one way ANOVA followed by Bonferroni's test, *p<0.05 when compared to control group; Figures in the parentheses shows percentage inhibition

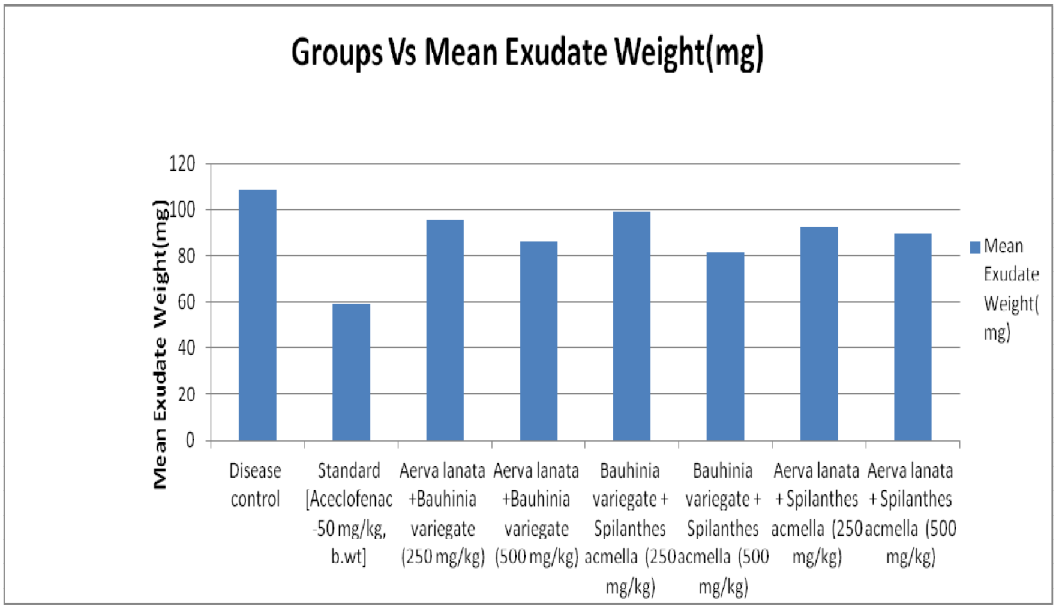

Figure 4.Anti-inflammatory activity *Aerva lanata*, Bauhinia variegate and *Spilanthes acmella* in fixed dose combinations by cotton pellate model

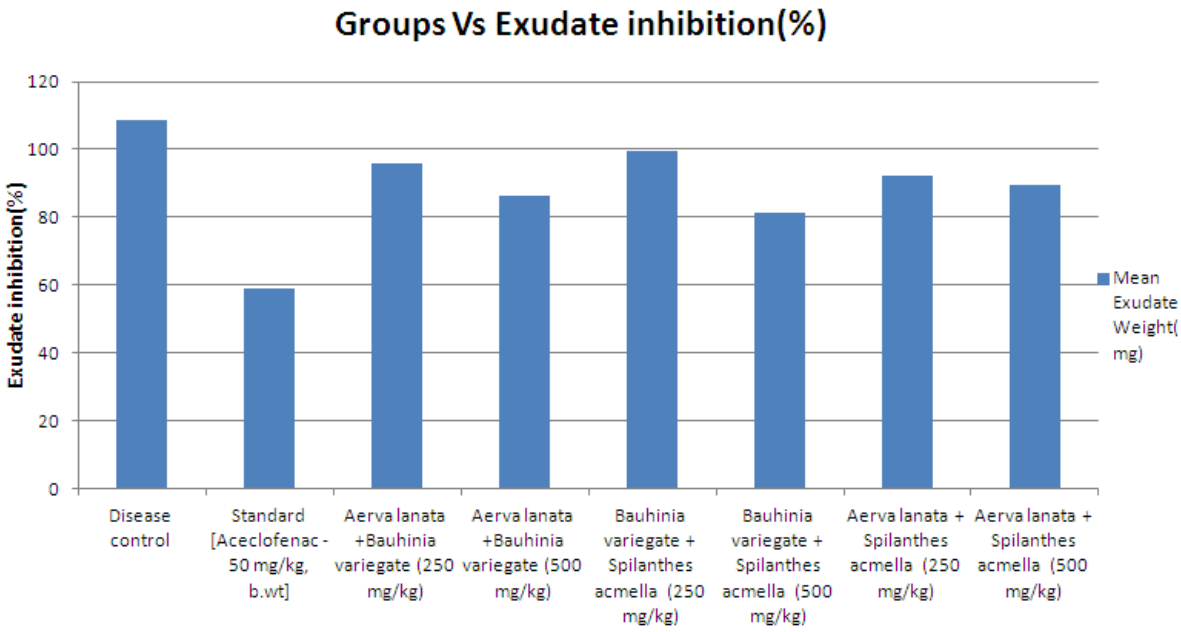

Figure 5. Anti-inflammatory activity *Aerva lanata*, Bauhinia variegate and *Spilanthes acmella* in fixed dose combinations by cotton pellate model.

DISCUSSION

In the present-day rich targeted & poor clue molecule situation, ethno-pharmacology and drug finding from vegetative sources persist imperative matter for instance several contemporary drugs devour their beginning in ethnopharmacology. At the moment, there is a constructive inclination with respect to traditional and integrated health disciplines in research and practice joined together. The communal tactics to drug discovery embraces the application of chemical biology, preparation by chemical methods, combinatorial chemical knowledge and genomic information with state-of-the-art slants concerning ethnopharmacology, reverse pharmacology, holistic approaches, systematic biological sciences and adapted medicine. The conventional stream in therapeutic exploration is passing away from solitary molecule or solitary target style to amalgamations and numerous target lines [1, 15].

Protection and safety remind as the greatest imperative opening point and the efficiency turn into a staple of endorsement in this line. Old-style Acquaintance, Contemporary Treatment and Modern Knowledge with systematic positioning will establish a golden threesome that will congregate to form a state-of-the-

art unearthing tool for innovative, safe, economically affordable and of course, active rehabilitations. Drugs established amongst 1981 and 2002 disclosed that medicines of natural origin shared about 28 % of all the innovative chemical molecules tossed into marketplace and 24 % of them were phytochemicals instituting 52% of all first-hand biochemical molecules, recommends that phytoconstituents are domineering foundations for innovative drug molecules & virtuous prime composites fit for supplementary amendment thru remedy development [45, 16].

Basic pharmacognostic surveys are beneficial in evolving standardization parameters of plants and their features, by which authenticated models from the impure samples can be differentiated. It aids in development of crude drugs profiles. The occurrence of noticeable amount of ash in plants under investigation influence be owing to existence of rich in-organic compounds such as Sodium (Na+) and Calcium (Ca2+) salt which are not detrimenta [17].Fluorescence analysis of the powdered whole plant of *C* revealed their behavior in various solvents and reagents. Preliminary phytochemical screening of the *Aerva lanata* revealed presence of alkaloids, carbohydrates, saponins, phenolic compounds, tannins and flavonoids [11, 12, 10].

Preliminary phytochemical screening of the *Bauhinia variegate* revealed presence of alkaloid, carbohydrate, saponin, phenolic-compounds, tannin, flavonoids, proteins and amino-acids.

Preliminary phytochemical screening of the *Spilanthes acmella* revealed presence of alkaloid, carbohydrate, saponin, phenolic-compounds, flavonoids, proteins and amino-acids.

Total flavonoids content was found to be 59.56, and 49.57 for *Aerva lanata, Bauhinia variegate and Spilanthes acmella* mg/ml of standard compound quercetin and total phenols was found to be 33.47, and 39.05 mg/g of gallic acid.

Aerva lanata, Bauhinia variegate and Spilanthes acmella extracts were subjected to thin layer chromatographic studied in which they revealed for the presence of various phytoconstituents which were calculated for their Rf values.

In chromatography, *Aerva lanata, Bauhinia variegate and Spilanthes acmella extracts* were underwent for the isolation of pure phytoconstituents in which two flavonoids (EEAL1- Kaempferol, EEAL2- Quercetin) from whole plant of *Aerva lanata*, two phytosterols (EEBV1- Stigmasterol, EEBV2- β-Sitosterol) from whole plant of *Bauhinia variegate* and one flavanoid (EESA1- Kaempferol), and one phytosterol (EESA2- β-Sitosterol)were isolated, purified by preparative thin layer chromatography and characterized by IR, NMR and Mass spectroscopic methods.

The *Aerva lanata, Bauhinia variegate and Spilanthes acmella* extracts did not show any injuriousness or impermanence up to 4000 mg/kg b.w. by conventional route, so the two extracts were reflected the category no. 5. The behavioral signs like change in skin, hair, eye moment, mucous membranes secretions, breathing, autonomic, CNS, communicating array, symptoms of shakes, diarrhea, lassitude, and snooze taken into consideration for observation. The onset and symbols of injuriousness are absent in animals till three days of toxicity surveillance dated. These sign represents harmlessness of both plant extract. So, 250 and 500 mg per kg were designated to animal screening.

Plants took a chief role in the overview of novel anti-diabetic agents and the advantageous manifold happenings like, manipulating carbohydrate metabolism by several mechanisms, avoiding, reestablishing compactness and role of cells, insulin emancipating activity, enlightening glucose uptake and usage and antioxidant and free radicle scavenging activities in herbs, suggest exhilarating prospect to advance them into innovative therapeutics [18, 20].

Thus, the area of medicinal plants research is in acquisition of momentous reputation and the call to employ phytoconstituents in management of diabetes in the growing so the weight is wavering back and the worth of herbs in the treatment of diabetes is getting consideration. The multi factorial pathogenesis of diabetes demands multi model therapeutic approach [19-24].

In evaluation of analgesic activity of *Aerva lanata, Bauhinia variegate and Spilanthes acmella in fixed dose combinations* , the test extracts demonstrated inhibition of pain in two pain models employed in a dose dependent manner. Centrally acting analgesics uplift the pain threshold of tested animals with respect to heat and pressure induced. The tested extracts demonstrated noteworthy and statistically significant analgesic activity in three pain models that is hot plate and tail immersion model, portentous that *Aerva lanata, Bauhinia variegate and Spilanthes acmellain fixed dose combination w*are showing their action on pain through these simulations due to central intervened nociception.

Acetic acid induced writh response concerning resident upper front nerve endings made by CH_3COOH is a delicate procedure of investigating tangentially performing analgesic agents. Injecting CH_3COOH i.p. founded a rise in of Prostaglandins E2 & F2α and resulted pain by encouraging duct absorptivity, redeeming internal ingredients approximating H2, H3, H4, 5HT, PG's, & BKN which presage ache wind-ups. So, the hypothetical action may perhaps be owed to barricading the creation or proclamation of internal compounds or senseless ness of pathway which transports soreness perception.

The outcomes advocate that *Aerva lanata, Bauhinia variegate and Spilanthes acmella* i fixed dose combination retain substantial tangentially umpired pain-relieving influence. Results performed by three models advocate that the *Aerva lanata, Bauhinia variegate and Spilanthes acmella* in combination hold palliative assets, intermediated thru bordering and principal inhibition contrivances. Proceeding to forward, further work is essential to diagnose molecular mechanisms through which *Aerva lanata, Bauhinia variegate and Spilanthes acmella* in combination are demonstrating the palliative action, inflexibility of Cyclooxygenase- I, II opposing nature of *Aerva lanata, Bauhinia variegate and Spilanthes acmella* [27-30].

Effect on carrageenan inflammatory module in animals was solitary of the supreme appropriate laboratory protocol for screening anti-inflammatory agents from plant origin as described by several researchers. The sub planter administration of carrageenan induced inflammation of significant force and continued for 3 h afterward administration 104-105. The anti-inflammatory action of *Aerva lanata, Bauhinia variegate and Spilanthes acmella* in the form of formulation on carrageenan induced inflammation in animals might be facilitated via mediators either alone or in amalgamation.

Henceforth, *Aerva lanata, Bauhinia variegate and Spilanthes acmella* in combination auxiliary scrutinized against paw oedema convinced by Histamine and disclosed a significant anti-inflammatory action at the dose of 500 mg/kg. Outcomes recommend *Aerva lanata, Bauhinia variegate and Spilanthes acmella in combination* at 250 and 500 mg of kg administered orally ominously condensed the oedema created by both chemicals employed & the results were akin with that of reference drug employed.

It's remained described by voluminous scholars that flavonoids prevent local hormones amalgamation by impeding COX and LOX- activities and also hinder the non- enzymatic peroxidation of polyunsaturated fatty acids essential for the instigation of these enzymes. The naturally occurring flavonoids like quercetin impede production of leukotrienes and also proclamation of H2, H3, H4, 5HT, PG's and correspondingly works as O2- scavengers [19-26].

CONCLUSION

Animals treated with the standard drug showed a significant ($p<0.05$) increase in mean reaction time that was found to be increased highly at time interval of 120, 180 and 240 minutes of study whereas the *Aerva lanata* with *Bauhinia variegate* showed a slight increase in the mean reaction time at its lower dose level that is 250 mg/kg which was found to be significant ($p<0.05$) at the terminating time of study where as it was significant ($p<0.05$) at its higher test dose 500 mg/kg at the time interval of 180 and 240 minutes of study when compared to control group. *Bauhinia variegate with Spilanthes acmella* extract 500 mg/kg exhibited a significant analgesic activity in hot plate and tail immersion methods. *Spilanthes acmella with Aerva lanata* plant extract at500 mg/kg exhibited a significant analgesic activity in hot plate and tail immersion methods.

In case of writhing model, *Aerva lanata* with *Bauhinia variegate at* 500 mg/kg test dose number of writhes were reduced and found to be statistically significant and percentage inhibition of writhing test was found to be 50.09 which are nearly comparable with standard drug value that is 62.73. *Bauhinia variegate with Spilanthes acmella*and *Spilanthes acmella with Aerva lanata* at 500 mg/kg showed 41.73% inhibition of the acetic acid induced writhing.

In carrageenan induced inflammatory model, *Aerva lanata* with *Bauhinia variegate* 250 mg/kg exhibited significant ($p<0.05$) mean paw volume at 180 and 240 minutes of study with a percentage inhibition value of 22.22 and 25.86 respectively where as significant ($p<0.05$) mean paw volume at 180 and 240 minutes of study with a percentage inhibition value of 27.08 and 43.10 respectively in its 500 mg/kg dose when compared to the control group. *Bauhinia variegate with Spilanthes acmella*and *Spilanthes acmella with Aerva lanata* 500 mg/kg showed 43.88% inhibition in carrageenan model at its 60 minutes of study.

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