

COMPARATIVE ANTI-INFLAMMATORY ACTIVITY OF POLYHERBAL FORMULATIONS VELFLEX SYRUP, PEEDAANT SYRUP, AND FLEXCUS ORTHOCARE SYRUP IN ALBINO WISTAR RATS

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ABSTRACT

In present research investigation, an attempt to conduct *in-vitro* antioxidant activity, and finally *in-vivo* anti-inflammatory activity of polyherbal formulations Juncus Flexcus Orthocare Syrup (JFOS; Juncus Life Sciences Private Limited), Vritikas Peedaant Syrup (VPS; Swastik natural) and Velflex Syrup (VS; Velnex medicare india private limited) against Carageenan. JFOS contains 120 SPMs from 19 medical plants, VPS has 112 SPMs from 17 natural herbs, and Polyherbal formulation VS has 93 SPMs from 25 medicinal plants. In contrast, JFOS, VPS, and VS are all scientifically established compositions. The polyherbal formulation JFOS was discovered to contain a relatively higher quantity of TPC and TFC than VPS or VS. Additionally, there was a direct correlation between the concentrations of VS, VPS, and JFOS and their antioxidant activity. NO scavenging activity was higher in JFOS. As the dosage of JFOS, VPS, and VS increased, so did their reducing

power (they have anti-oxidant and free radical scavenging properties). JFOS and VPS were discovered to have extremely strong antioxidant activity using the FRAP assay. *In-vivo* anti-inflammatory activity indicated that treatment with the standard, polyherbal formulations JFOS, VPS and VS treated animals showed improved edema inhibition at the 5-hour observation. Further, anti-inflammatory effects of polyherbal formulations JFOS was more

significant than anti-inflammatory effects of polyherbal formulations VPS and VS. Finally, anti-inflammatory effects of polyherbal formulations JFOS, VPS and VS were less significant than standard reference drug i.e. Diclofenac.

KEYWORDS: Anti-inflammatory, antioxidant, free radical scavenging, polyherbal, total phenolic content, total flavonoid content.

INTRODUCTION

According to Campos *et al.*, 2014, the term "inflammers" or "inflammatio" (Latin, inflammatio, to put on fire) refers to a normal part of the immune system's reaction; a series of actions that take place in reaction to trauma, illness, or noxious stimuli.

According to Hawiger and Zienkiewicz (2019), inflammation is a physiological process that has been conserved throughout evolution and affects every area of the body where the immune system detects an injury or infection. Redness, heat, swelling, pain, and loss of function are the five traditional indicators of inflammation, though not all inflammatory responses exhibit all five. The start of an inflammatory reaction is seen in Fig. 1. Inflammation is a temporary reaction that helps the immune system get rid of a pathogen or heal a lesion, but allergic and chronic inflammations are frequent and potentially dangerous.

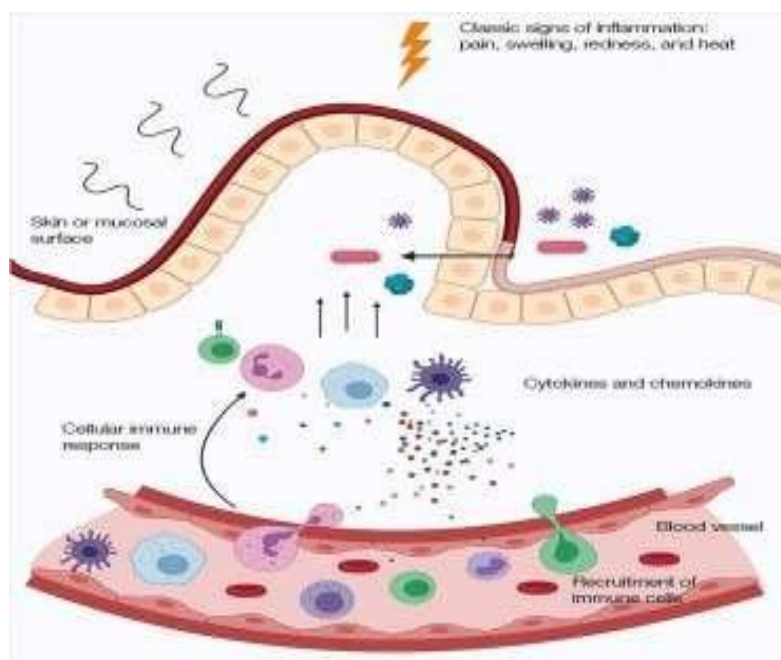


Fig. 1: Initiation of inflammation.

According to Azwanida *et al.*, 2015, the four main signs of swelling are pain, redness, heat or

warmth, and inflammation (the body's severe reaction to any damage; ubiquitous process which induced homeostasis such as damage, exposure to contaminants or infection, and also triggered by innate immune system receptors). When an injury occurs, arterioles in the local tissue dilate, increasing blood flow to the affected area (redness; Bauri *et al.*, 2015).

Kumar, (2013), inflammation is either acute or chronic type. Besides, on the basis of their cause inflammation may be microbial inflammation (abscess), autoimmune inflammation (rheumatoid arthritis), allergic inflammation (atopic dermatitis), metabolic inflammation, (gouty arthritis) and physical inflammation (burns). Acute Inflammation: Increased vascular permeability, infiltration and emigration of leukocytes. Chronic inflammation: Infiltration of mononuclear immune cells, macrophages, monocytes, neutrophils, fibroblast activation, proliferation and fibrosis.

Pilotto *et al.*, 2010, inflammation is either acute or chronic. Acute inflammation is an initial response of the body to harmful stimuli. Parham *et al.*, 2008, in chronic inflammation, the response resulting in damage to the body (out of proportion; rheumatoid arthritis, asthma, colitis, allergies, hepatitis, metabolic syndrome, autoimmune diseases cancer, cardiovascular dysfunctions and neurodegenerative disorders).

Histologically, acute inflammation is defined by neutrophil buildup and occurs over a period of minutes to days. Either resolution (healing) or chronic inflammation may result from acute inflammation. The inflow of monocytes, lymphocytes, and other immune cells is typically linked to chronic inflammation, which lasts more than a few days. A minor acute reaction, a recurring low-level inflammation without a resolution phase, or a progression from acute inflammation with no resolution phase can all result in chronic inflammation.

Azwanida *et al.*, 2015, Inflammation disturb homeostasis and severe cause of morbidity (etiology or pathology) (Fig.2).

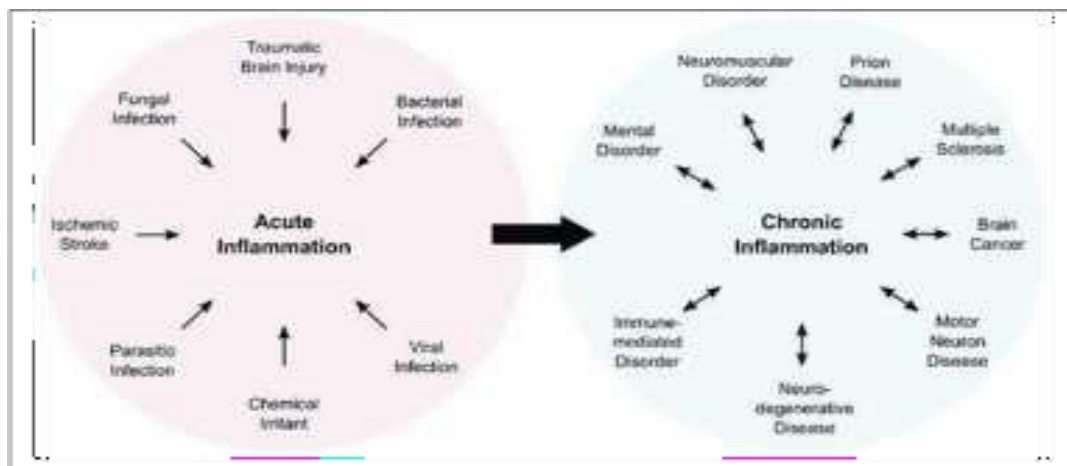


Fig. 2: Cause and Consequences of Inflammation.

Parhnam *et al.*, 2008, signs of inflammation include redness (local), swelling, pain, heat and loss of function.

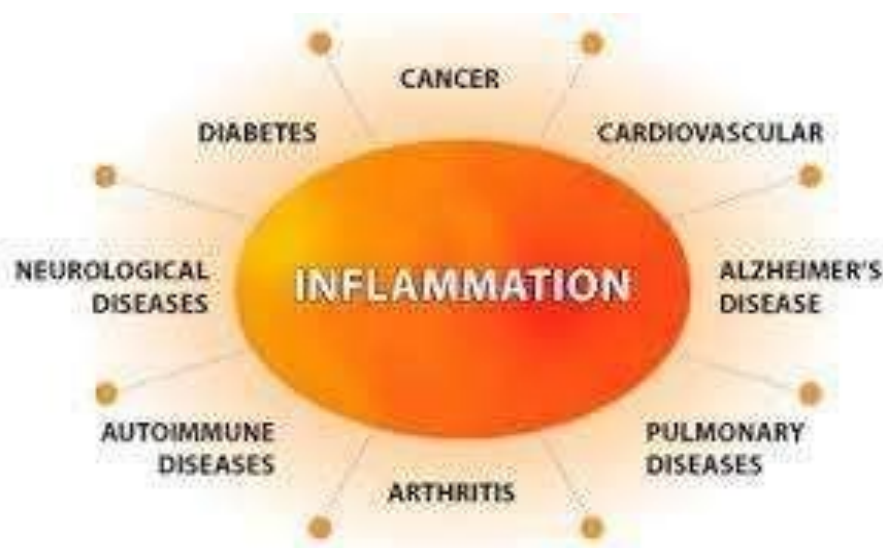


Fig. 3: Diseases associated with Inflammation.

Iwalewa *et al.*, 2007, cyclooxygenase (COX) is a key enzyme that contributes to the synthesis of prostacyclins, prostaglandins, and thromboxanes, which are implicated in pain, inflammation, and platelet aggregation. According to Watson and Hamilton (2012), indicators of inflammation are used to evaluate therapy response and identify acute inflammation that may be a sign of a particular illness. Increased inflammatory marker levels may be a sign of cancer, autoimmune diseases, and infections. Certain conditions can be excluded when levels are normal. Inflammatory indicators are useful for identifying illnesses, but they are not specific enough to diagnose major underlying conditions. Procalcitonin, erythrocyte sedimentation rate, and C-reactive protein are the most prevalent inflammatory

indicators.

Hunnskaar *et al.*, 1986, evaluation of anti-inflammatory activity by formalin test; Koster *et al.*, 1959, acetic acid induced vascular permeability tests; Coura *et al.*, 2015, Oxazolone induced ear edema test; Batista *et al.*, 2016, carrageenan induced paw edema; Da Silva *et al.*, 2018, pleurisy test; Calil *et al.*, 2014, lipopolysaccharide induced edema test.

Non-steroidal anti-inflammatory medicines (NSAIDs) are used to treat inflammation (both acute and chronic pain) by inhibiting COX-1 and COX-2, which prevents prostaglandin and thromboxane buildup. According to Pereira-Leite *et al.*, 2017, NSAIDs are a broad class of drugs whose activities are all associated with COX inhibition in the production of prostaglandins and thromboxanes.

Conversely, COX-2 is activated by inflammation and pro-inflammatory cytokines. According to Wallace (2001), long-term usage of NSAIDs might result in harmful side effects include gastric lesions, cardiovascular, renal, and gastrointestinal damage. According to Percival (1999), the toxicity of NSAIDs and the recurrence of symptoms after stopping them are drawbacks. Therefore, attempts are made to locate anti-inflammatory medications from natural sources, and screening and development of new anti-inflammatory drugs are urgently needed.

Newman (2000), more than 80% of medicines have been developed from natural products (NP) obtained from natural source (NPs or pharmacophore from a NP). Srinivasan *et al.*, 2001, biologically active compounds for many centuries and use extensively as crude material or as pure compounds for treating various disease conditions. Vashishtha *et al.*, 2014, India with its biggest repository of crude drugs or as bioactive compounds in the formulation of pharmaceuticals and cosmetics etc. (Goel *et al.*, 2020).

According to Tripp *et al.*, 2012, adequate preventative and therapeutic evaluation of their anti-inflammatory potential is being studied in vitro and in vivo. There have been positive results when using medicinal herbs to treat inflammatory diseases. As a result, many patents in this subject have been acquired. Before utilising state-of-the-art technologies and study methodologies in clinical investigations. Gessner *et al.*, 2017, by focusing on several pathways and molecules implicated in the inflammatory response, polyherbal medications can offer a holistic strategy for reducing inflammation. The safety and effectiveness of

polyherbal compositions should be assessed through thorough scientific research, but it's crucial to remember that the precise effects and mechanisms of particular herbs may differ.

Phytochemical screening methods used in modern science. Velflex syrup, Vritikas peedaant syrup, and Juncus flexcus orthocare syrup's total phenolic and total flavonoid contents are estimated. Velflex syrup, Vritikas peedaant syrup, and Juncus flexcus orthocare syrup are polyherbal formulations that were tested for antioxidant activity using DPPH/NO analysis. anti-inflammatory research both *in vitro* and *in vivo*.

MATERIALS AND METHODS

Composition of Velflex Syrup (Velnex Medicare India Private Limited)

Table 1: Velflex Syrup (Velnex Medicare India Private Limited) Composition.

S.No.	Botanical / Hindi Name	Quantity (mg)
	Velflex Syrup (Velnex Medicare India Pvt. Ltd.) contains	
1	Maharasnadi Kwath (also called Maharasnadi Kadha or Kashayam) (Maharasnadi Kwath)	125
2	<i>Zingiber officinale</i> (Sunthi; Ginger)	125
3	<i>Pluchea lanceolata</i> (Rasna)	100
4	<i>Ricinus communis</i> (Erand Mool)	100
5	<i>Withania somnifera</i> (Ashwagandha)	100
6	<i>Commiphora mukul</i> (Guggulu)	100
7	<i>Vitex negundo</i> (Nirgundi)	50
8	<i>Cyperus rotundus</i> (Musta)	50
9	Dashmoola : Chhoti kateri- <i>Solanum xanthocarpum</i> ; Badi kateri- <i>Solanum indicum</i> ; Gokhru- <i>Tribulus terrestris</i> ; Arni- <i>Premna integrifolia</i> ; Sonapatha- <i>Oroxylum indicum</i> ; Gambhari- <i>Gmelina arborea</i> ; Padhal- <i>Stereospermum suaveolens</i> ; Shalparni- <i>Desmodium gangeticum</i> ; Prishniparni- <i>Uraria picta</i> ;	250
Total		1000

Composition of Vritikas Peedaant Syrup

Table 2: Composition of Vritikas Peedaant Syrup.

S. No.	Scientific and Vernacular / Hindi Name	Quantity (mg)
10 ml Vritikas Peedaant Syrup contains:		
1.	<i>Saraca indica</i> (Ashoka)	400
2.	<i>Symplocos racemosa</i> (Lodhra)	100
3.	<i>Asparagus racemosus</i> (Satavari)	100
4.	Indian Gooseberry (Amla)	40
5.	<i>Withania somnifera</i> (Ashwagandha)	20
6.	<i>Berberis aristata</i> (Daru Haridra)	50
7.	<i>Cedrus deodara</i> (Deodar)	40
8.	<i>Cyperus rotundus</i> (Cyperaceae; Musta)	40

9.	<i>Piper cubeba</i> (Piperaceae; Kabab Chini)		10
10.	<i>Syzygium aromaticum</i> (Myrtaceae; Clove)		10
11.	<i>Embelia ribes</i> (Primulaceae; Vidanga)		10
12.	<i>Hemidesmus Anantmul</i>	<i>indicus</i> (Apocynaceae ;	15
13.	<i>Swertia chirata</i> (Gentianaceae; Chireta)		10
14.	<i>Valeriana Valerianaceae)</i>	<i>jatamansi</i> (Jatamansi;	10
15.	<i>Syzygium cumini</i> (Myrtaceae; Jamun)		20
16.	<i>Cinnamomum tamala</i> (Lauraceae; Tejpat)		20
17.	<i>Tinospora cordifolia</i> (Guduchi)		05
Grand Total			900

Composition of Juncus Flexcus Orthocare Syrup

Table 3: Juncus Flexcus Orthocare Syrup Composition.

S. No.	Botanical Name / Common name	Quantity (mg)
Each 10 ml. of Juncus Flexcus Orthocare Syrup contains:		
1.	<i>Vitex negundo</i> (Nirgundi)	200
2.	<i>Pluchea lanceolata</i> (Rasna)	50
3.	<i>Boerhavia diffusa</i> (Punarnava)	50
4.	<i>Tribulus terrestris</i> (Gokhru)	50
5.	<i>Commiphora wightii</i> (Shudh gugglu)	50
6.	<i>Asphaltum punjabianum</i> (Shudh Shilajit)	50
7.	<i>Withania somnifera</i> (Ashwagandha)	100
8.	<i>Piper longum</i> (Pippali mool)	50
9.	<i>Celastrus paniculatus</i> (Malkagni)	50
10.	<i>Curcuma longa</i> (Haldi)	150
11.	<i>Boswellia serrata</i> (Shallaki)	150
12.	<i>Suranjan moringa</i> (oleifera)	150
13.	Triphala: <i>Emblica officinalis</i> (Amla/ Indian Gooseberry), <i>Terminalia chebula</i> (Haritaki / Chebulic Myrobalan), and <i>Terminalia bellirica</i> (Bibhitaki / Belleric Myrobalan.	50
14.	<i>Zingiber officinale</i> (Saunth)	100
15.	<i>Piper nigrum</i> (Kali mirch)	50
16.	<i>Mucuna pruriens</i> (Kaunch beej)	50
17.	<i>Cissus quadrangularis</i> (Hadjod)	150
Grand Total		1500

Table 4: Pharmacognostical and Pharmacological Comparison.

Velflex Syrup	Vritikas Peedaant Syrup	Juncus Flexcus Orthocare Syrup
Constituents		
93 SPMs from 25 natural herbs / minerals	112 SPMs from 17 natural herbs / minerals	120 SPMs from 19 natural herbs / minerals
Main Constituents Plants and Minerals		
Maharasnadi Kwath	<i>Saraca indica</i>	<i>Vitex negundo</i>
<i>Zingiber officinale</i>	<i>Symplocos racemosa</i>	<i>Pluchea lanceolata</i>

<i>Pluchea lanceolata</i>	<i>Asparagus racemosus</i>	<i>Boerhavia diffusa</i>
<i>Ricinus communis</i>	Indian Gooseberry	<i>Tribulus terrestris</i>
<i>Withania somnifera</i>	<i>Withania somnifera</i>	<i>Commiphora wightii</i>
<i>Commiphora mukul</i>	<i>Berberis aristata</i>	<i>Asphaltum punjabianum</i>
<i>Vitex negundo</i>	<i>Cedrus deodara</i>	<i>Withania somnifera</i>
<i>Cyperus rotundus</i>	<i>Cyperus rotundus</i>	<i>Piper longum</i>
<i>Dashmoola</i>	<i>Piper cubeba</i>	<i>Celastrus paniculatus</i>
	<i>Syzygium aromaticum</i>	<i>Curcuma longa</i>
	<i>Embelia ribes</i>	<i>Boswellia serrata</i>
	<i>Hemidesmus indicus</i>	<i>Suranjan moringa</i>
	<i>Swertia chirata</i>	<i>Emblica officinalis</i>
	<i>Valeriana jatamansi</i> <i>Syzygium cumini</i> <i>Cinnamomum tamala</i> <i>Tinospora cordifolia</i>	<i>Terminalia chebula</i> <i>Terminalia bellirica</i> <i>Zingiber officinale</i> <i>Piper nigrum</i> <i>Mucuna pruriens</i> <i>Cissus quadrangularis</i>
Pharmacological / Medicinal Uses		
joint and muscular pain (musculoskeletal discomfort), stiffness, arthritis, backaches, muscle soreness, inflammation;	pain relief, anti-inflammatory action, and vitality;	Anti-inflammatory, Pain & Mobility, Detoxifying & Restorative
Dose		
1-2 teaspoonful twice a day	10 ml twice a day;	1-2 teaspoonful twice a day
Toxicity Profile		
Safe dose: 2000 mg/kg bw (Toxicity absent)	Safe dose: 2000 mg (Toxicity absent)	Safe dose: 3000 mg (Toxicity absent)
Side Effects		
Absolutely safe and no side effects.	Free from side effects (safe)	No side effects (safe)

Detection of PPMs / SPMs

Velflex Syrup (VS), Vritikas Peedaant Syrup (VPS), and Juncus Flexcus Orthocare Syrup (JFOS) polyherbal formulations were employed for preliminary phytochemical screening for the detection of different was carried out by employing conventional protocols outlined by Kokate.

Estimation of Total Phenolics Contents (TPC)

The total phenolics content (TPC, possess antioxidant activity) in polyherbal formulations JGOS, VPS and VS were undertaken by method of Jeong *et al.*, 2010.

Estimation of Total Flavonoids Content

The total flavonoids content (antioxidant potential is correlated with TFC content) were determined in polyherbal formulations JGOS, VPS and VS as per method reported by

Kamtekar *et al.* 2014.

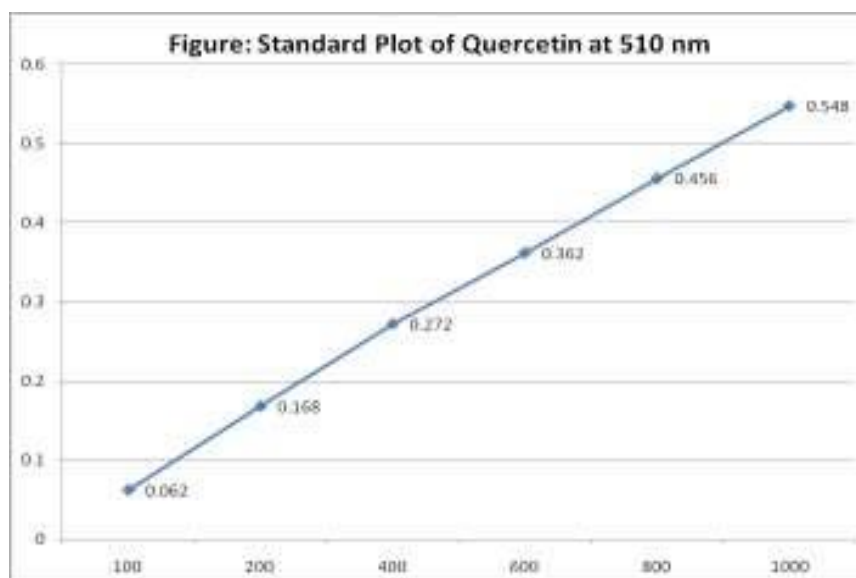


Fig. 4: Standard Plot for TFC.

Table 5: TFC in JFOS, VPS and VS.

Sample	Absorbance (510 nm)	TFC Concentration Eq.to Quercetin (µg/1 ml)	TFC (mg of Quercetin Eq./100mg CE)
Juncus Flexcus Orthocare Syrup	0.518	916	9.16
Vritikas Peedaant Syrup	0.478	860	8.60
Velflex Syrup	0.354	586	5.86

***In-vitro* Antioxidant effects**

In-vitro antioxidant activity of Velflex Syrup, Vritikas Peedaant Syrup, and Juncus Flexcus Orthocare Syrup by DPPH, NO radical scavenging method was performed (Gutteridge, 1995). DPPH : DPPH by method of Blois (1958); NO Scavenging (Larson, 1994); FRAP assay (Benzie and Strain, 1996); Reducing Power Assay (Jayaprakasha *et al.*, 2001) : As per SOP.

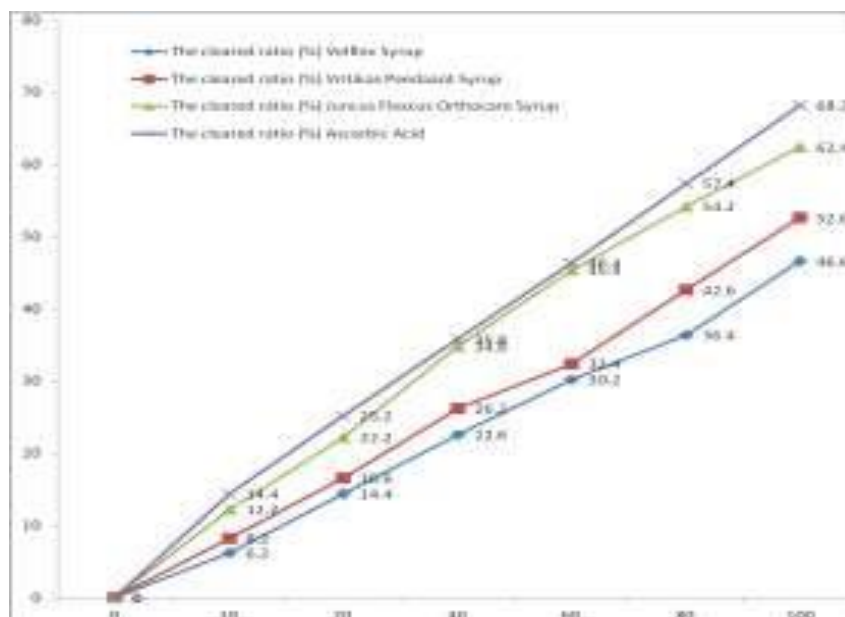


Fig. 5: DPPH regression curve.

***In-vivo* Anti-inflammatory Activity**

Anti-inflammatory studies (IAEC Form B approval : IEC/IAEC/2026/04 dated 20-02- 2026) of Velflex Syrup, Vritikas Peedaant Syrup, and Juncus Flexcus Orthocare Syrup were carried out as *in-vivo* method reported by Winter *et al.*, 1962.

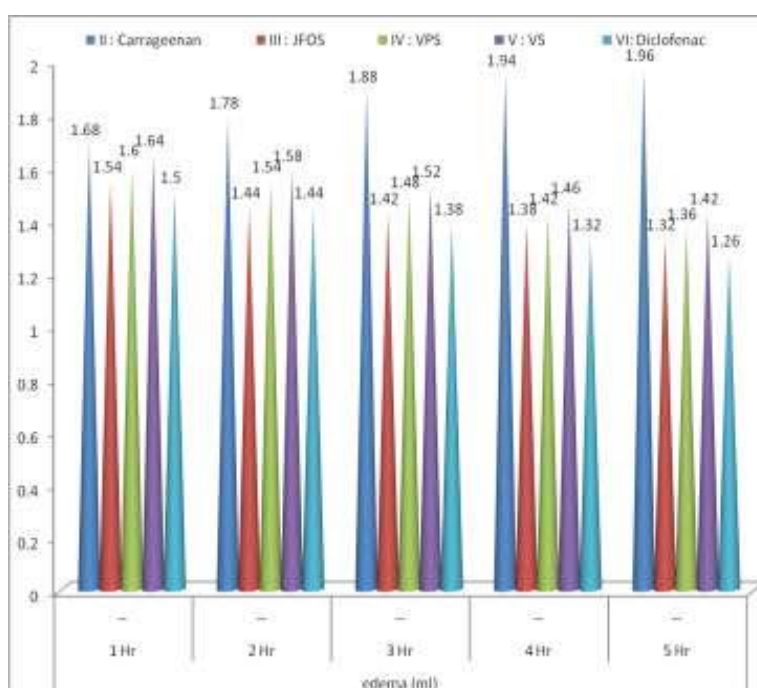
Carrageenan Induced Rat Paw Edema

For the investigation, 36 albino wistar rats in good health (weighing 200 ± 20 g) were chosen. Following acclimation, the animals were split into 06 groups at random (Group I–VI; 06 animals/group). The rats were fed regular rat food (Golden Animal Feeds, Delhi, India) and were allowed unlimited water. Water was given freely to Group I (Normal), also known as the control group. Animals in Group II (Toxic Control) received intraperitoneal injections of $100 \mu\text{L}$ of 1% (1% w/v carrageenan) and left untreated. Groups III, IV, V and VI were given intraperitoneal injections of $100 \mu\text{L}$ of 1% (1% w/v carrageenan) followed by treatment with polyherbal formulations Juncus Flexcus Orthocare Syrup (JFOS), Vritikas Peedaant Syrup (VPS), Velflex Syrup (VS) and Diclofenac (standard reference drug) respectively. Group III received oral administration of JFOS (150 mg/kg/b.w.p.o./day), Group IV received VPS (90 mg/kg/b.w.p.o./day), Groups V animals were given VS ((100 mg/kg/b.w.p.o./day), and finally Group VI animals were treated with Diclofenac (standard reference drug; 10 mg/kg/b.w.p.o./day).

Table 6: Effect of JFOS, VPS and VS in rat edema.

Group	edema (ml)				
	1 Hr	2 Hr	3 Hr	4 Hr	5 Hr
I : Normal	--	--	--	--	--
II : Carrageenan	1.68±0.14	1.78±0.18	1.88±0.22	1.94±0.32	1.96±0.18
III : JFOS	1.54*±0.18	1.44*±0.14	1.42*±0.12	1.38*±0.14	1.32*±0.12
IV : VPS	1.60*±0.24	1.54*±0.18	1.48*±0.12	1.42*±0.16	1.36*±0.16
V : VS	1.64±0.22	1.58±0.18	1.52±0.16	1.46±0.18	1.42±0.14
VI: Standard (Diclofenac; 10 mg/kg)	1.50*±0.14	1.44*±0.16	1.38*±0.14	1.32*±0.22	1.26*±0.14

Note: *significant (P<0.05)

**Fig. 6: Effect of polyherbal formulation JFOS, VPS and VS on paw edema.****Table 7: JFOS, VPS and VS and Diclofenac induced inhibition of edema.**

Group	edema inhibition (%)				
	1 Hr	2 Hr	3 Hr	4 Hr	5 Hr
III : JFOS	12.68	16.42	22.12	28.68	33.62
IV : VPS	10.38	14.92	18.42	22.26	27.18
V : VS	8.92	12.18	16.68	20.14	23.38
VII : Diclofenac	15.42	22.36	29.48	34.84	38.88

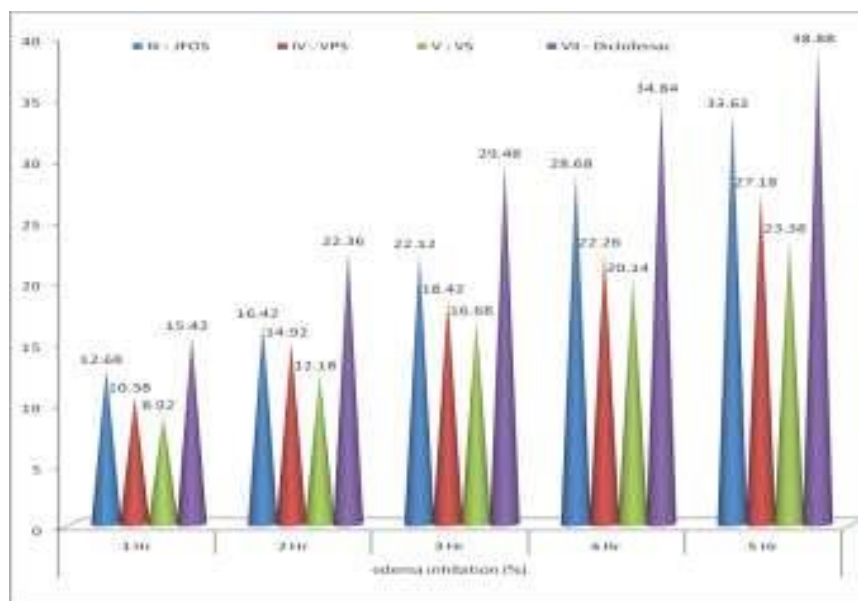


Fig. 7: Effect of JFOS, VPS, VS and standard drug on edema inhibition.

RESULTS AND DISCUSSION

The research began with the procurement of polyherbal formulations JFOS, VPS, and VS from reputable and authentic commercial sources. Their literature was reviewed and compared. While Vritikas Peedaant Syrup (VPS) has 112 SPMs from 17 natural herbs and minerals and Juncus Flexcus Orthocare Syrup (JFOS) has 120 SPMs from 19 natural herbs and minerals (Table 4.6), Velflex Syrup (VS) has 93 SPMs/Phytoconstituents that are derived from 25 natural herbs and medicinal plants. Comparatively, it was found that JFOS, VPS, and VS are polyherbal formulations with scientific anti-inflammatory properties. The following PPMs and SPMs were then found in the polyherbal formulations JFOS, VPS, and VS through phytochemical screening: alkaloids, flavanoids, phenolic compounds, terpenoids, reducing sugars, glycosides, steroids, phytosterols, proteins, carbohydrates, etc.

Further, in TPC analysis high concentration of TPC were found in the polyherbal formulations JFOS Syrup (32.46 ± 2.96 mg GAE/100 gDW in JFOS), followed by VSS (28.24 ± 2.88 mg GAE/100 gDW) and VS (24.46 ± 2.52 mg GAE/100 gDW). In TFC content assessment standard plot was prepared and concentrations of TFC were observed in Juncus JFOS, VPS and VS were analysed and calculated. Comparatively high amount of TFC was found in the polyherbal formulation JFOS than VPS and VS. The amounts of JFOS, VPS, and VS were directly correlated with their antioxidant activity ($p < 0.05$). The greatest NO scavenging activity was produced by JFOS. Reduced power assays of JFOS, VPS, and VS showed directly proportional antioxidant activity (increasing activity::increased dose). It

was discovered that ascorbic acid and the JFOS and VPS extracts had very strong antioxidant properties. VPS and JFOS have demonstrated the ability to scavenge free radicals. As the dosage of JFOS, VPS, and VS grew, so did their reducing power (they have anti-oxidant and free radical scavenging properties).

In-vivo anti-inflammatory activity of polyherbal formulations JFOS, VPS, and VS, 36 albino wistar rats in good health (weighing 200 ± 20 g) were chosen. Water was given freely to Group I (Normal), also known as the control group. Animals in Group II (Toxic Control) received intraperitoneal injections of 100 μ L of 1% (1% w/v carrageenan) and left untreated. Groups III, IV, V and VI were given intraperitoneal injections of 100 μ L of 1% (1% w/v carrageenan) followed by treatment with polyherbal formulations JFOS, VPS, VS and Diclofenac (standard reference drug) respectively.

Group III received oral administration of JFOS (150 mg/kg), Group IV received VPS (90 mg/kg), Groups V animals were given VS (100 mg/kg), and finally Group VI animals were treated with Diclofenac (standard drug). Results indicated that treatment with the standard, polyherbal formulations JFOS, VPS and VS treated animals showed improved edema inhibition at the 5-hour observation. At high dose, polyherbal formulations JFOS, VPS and VS produced significant anti-inflammatory effects (edema inhibition). Further, anti-inflammatory effects of formulations JFOS was more significant than anti-inflammatory effects of polyherbal formulations polyherbal formulations VPS and VS. Finally, anti-inflammatory effects of polyherbal formulations JFOS, VPS and VS were less significant than standard reference drug i.e. Diclofenac.

CONCLUSIONS

It was found that high concentrations of TPC & TFC were found in the polyherbal formulations JFOS followed by VPS and VS. The greatest NO scavenging activity was obtained by JFOS. At high dose, polyherbal formulations JFOS, VPS and VS produced significant anti-inflammatory effects. Further, anti-inflammatory effects of polyherbal formulations JFOS was more significant than anti-inflammatory effects of polyherbal formulations polyherbal formulations VPS and VS.

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