

**Research Article****Formulation, characterization and pharmacological evaluation of anti-inflammatory polyphyto matrix tablet as a novel drug delivery system**

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Objective: To deal with the formulation, characterization and pharmacological evaluation of anti-inflammatory polyphyto matrix tablet containing *Carica papaya*, *Vitex negundo*, *Moringa oleifera* and *Boswellia serrata* used as a novel drug delivery system. An attempt has been made to develop polyphyto matrix tablets by using HPMC & ethyl cellulose. **Methods:** Pharmacological screening of polyphyto matrix tablet was evaluated by using the carrageenan-induced paw edema rat model and the study carried out by using various doses (100, 200 & 300 mg/kg body weight) of three different polyphyto matrix formulations. Diclofenac sodium was used as a standard drug, due to its considerable report on anti-inflammatory importance. Data were expressed by mean \pm SD; statistical analysis was performed by using ANOVA and $p < 0.05$ considered as statistically significant. **Results:** Physical and chemical evaluation parameters of polyphyto matrix formulation exhibits potent and dose-dependent anti-inflammatory activity in all the tested animal groups. Formulation F1 significantly ($p < 0.05$) suppresses the inflammation in the rat paw and found to be preeminent and stable in comparison with standard and other two (F2 & F3) formulations. **Conclusion:** Study reveals that the polyphyto matrix formulation could be useful as either an alternative or a complementary therapy in the management of all types of inflammation. It may be due to presence of terpenoids, alkaloids, glycosides and other phytochemical in herbs.

Introduction

Inflammation is a natural response of the mammals in which, the body reacts to infections, irritations and/or injuries, such as redness, warmth, swelling and pain recognized as a nature of nonspecific immune response [1]. At present, inflammation is defined by the presence of five macroscopic pathological phenomenon's, four of them proposed by Celsus as long as 2000 years ago [2]. These are *tumor*–swelling, *calor*–temperature, *rubor*–redness, *dolor*–sensation and *functio laesa*–impaired function. All signs and symptoms have been regarded as enhancement of vascular permeability as a direct consequence related to injury of tissues [3]. In acute inflammatory disorders such as sepsis, the endothelial cells (ECs) are centrally associated with the pathogenesis of organ injury, expressing the cytokines and chemokines, facilitating the trafficking of leukocytes to organs, leading to modulation of vascular barrier functions [4]. Herbal medicines still exert a great deal of importance to the people living in developing countries and also lead to discovery of new drug candidates

[5]. Most of human population worldwide is getting affected by the inflammation associated disorders. It is believed that, the current analgesia inducing drugs such as opiates and NSAIDs are not useful in all cases, because of their side effects like gastrointestinal irritation, ulcers, bleeding, kidney failure, rarely, liver dysfunction etc. [6,7]. Herbal medicines are now considered to be the best healthcare products, offering a massive contribution to the primary healthcare and have shown great potential in modern phyto-medicine against numerous ailments and the complex diseases of the modern world. As per present scenario of the global demand of herbal based medicines, healthcare products, pharmaceuticals, food supplements, cosmeceuticals etc. have been intensified in alarming rate [8]. Conventional drug delivery systems are the primary pharmaceutical products dominating in the prescription and market places of over-the-counter drugs. To achieve and maintain the drug concentration in the body within the therapeutic range; it is required for a medication to

take such type of drug delivery system, several times in a day. Hence by using the sustained release system, the present study may achieve *in-vitro* prolonged release of the drug in a predictable manner, which may be therapeutically effective and non-toxic for an extended period of time [9].

Materials and Methods

Materials

Diclofenac, carrageenan, n-hexane, ethanol, chloroform, HPMC, ethyl cellulose, microcrystalline cellulose, magnesium stearate, talc, glacial acetic acid (all reagents obtained from the local supplier) were used in this study. All the chemicals and drugs used in the present study were of analytical grade.

Diclofenac

Diclofenac sodium was selected as a standard drug for the study because; it is the best nonsteroidal anti-inflammatory drug (NSAID) of this class. This medicine works by reducing substances in the body that cause pain and inflammation. Diclofenac sodium is an odorless, yellowish-white, crystalline powder sparingly soluble in water (Safety review of Diclofenac Version 2.1, October 2014).

Selection of plants

The medicinal plants, Papaya leaves- *Carica papaya* (*Caricaceae*) [10]; Chaste leaves- *Vitex negundo* (*Verbenaceae*) [11]; Drumstick bark- *Moringa oleifera* (*Moringaceae*) [8] & Indian olibanum gum resin- *Boswellia serrata* (*Burseraceae*) [12] have been selected for the anti-inflammatory matrix formulation. As per the market survey it is exposed that no such formulation is available involving above mentioned combination as a novel drug delivery system. The plant parts were purchased from local market and authentication has been done by botanical scientist, at Botanical Survey of India (BSI), the northern regional centre, Dehradun. The morphological study of selected plants was carried out for the observations like, shape, size, colour, odour, taste and microscopic study [13].

Experimental animals

Adult albino Wistar rats (180-230g) of either sex were used for anti-inflammatory pharmacological screening. For this

experiment, all the animals were kept in poly propylene cages (6 rats per cage) at $25\pm 2^{\circ}\text{C}$ temperature with relative humidity 45-55% under 12 h light and dark cycles. All the animals were acclimatized for laboratory condition as per CPCSEA guideline for a week before uses. They were fed with standard animal feed and water *ad libitum*. All experimental protocols were in compliance with the ethics committee on research in animals, department of pharmaceutical sciences, Kumaun University, Nainital (UK) India; as well as nationally accepted principles for use and care of the laboratory animals. Institutional animal ethical committee approval number is PBRI/13/IAEC/PN-291.

Methods

Anti-inflammatory activity

Anti-inflammatory activity was evaluated by using the carrageenan induced-paw edema rat model [14]. After 16 h of fasting, the rats were divided into 12 groups each consisting of six animals. Group I- served as control and received 3ml/kg distilled water. Group II- served as disease control, carrageenan solution (0.1ml of 1% v/v) was injected into the sub-plantar region of the right hind paw of rats. Group III- served as standard, received Diclofenac sodium (10mg/kg b.w.); Group IV to VI- served as test groups, received formulation (F1) of three different doses (100, 200 & 300mg/kg b.w.); Group VII to IX- served as test groups, received formulation (F2) of three different doses (100, 200 & 300mg/kg b.w.) and Group X to XII- also served as test groups, received formulation (F3) of three different doses (100, 200 & 300 mg/kg b.w.). The rats' paw volume was measured using a digital plethysmometer PLM-01 (Orchid Scientifics, India) immediately and after 1, 3, 6, and 12 h of carrageenan injection [15].

Macroscopical evaluation of herbal plants: The macroscopic study of the herbal plants possessing anti-inflammatory potential for matrix formulation carried out in different parts, which were freshly collected, shade dried and prepared by using the standard technique (figure 1) available in the Indian Pharmacopoeia [16].

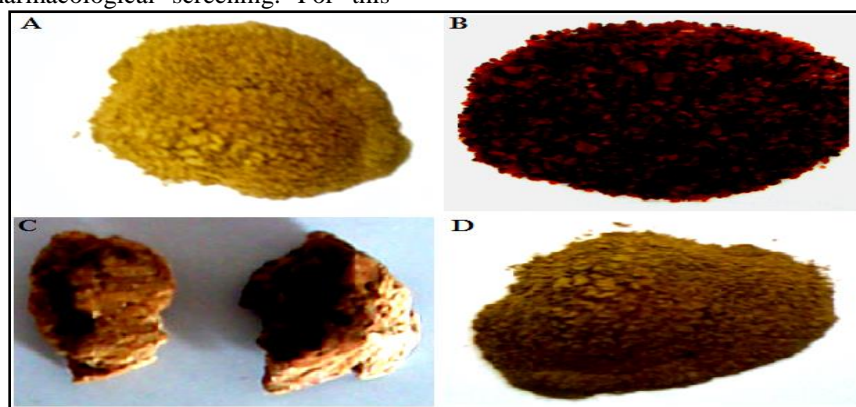


Figure 1: Macroscopic study of plants (A) Leaf powder of *Carica papaya*, (B) Resin of *Boswellia serrata*, (C) Bark of *Moringa oleifera*, (D) Leaf powder of *Vitex negundo*.

Microscopical evaluation of herbal plants: The microscopic study of the plants clearly shows that, the cork cambium consists of a single row of thin-walled, elongated cells, multicellular and glandular trichomes. The phloem consists of thin walled, oval to

polygonal parenchyma; radial vascular bundle is present [17]. All oil globules are scattered in oil region as round to oval in size, starch grains scattered in oil region clearly shown in figure 2.

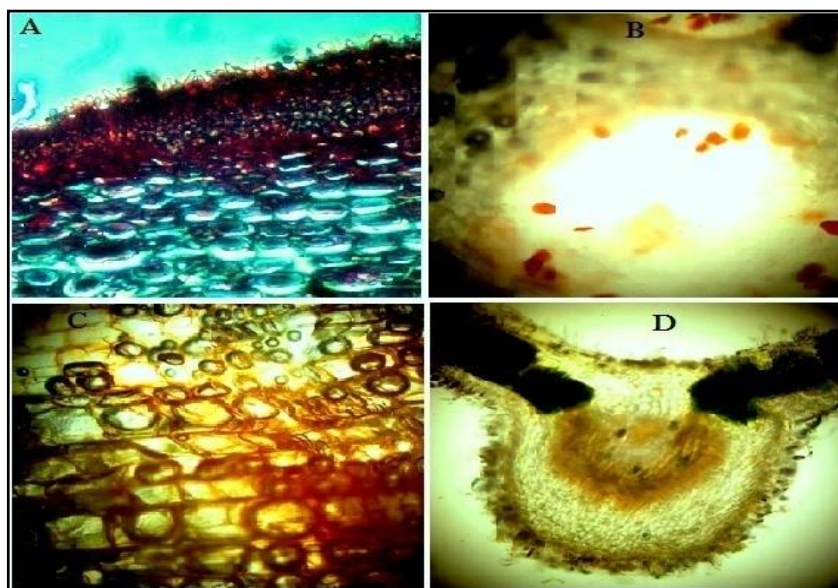


Figure 2: Microscopic study of plants (A) *Carica papaya* leaf, (B) *Boswellia serrata* resin, (C) *Moringa oleifera* bark, (D) *Vitex negundo* leaf was observed under magnification 400x.

Extraction of plant materials

The dried plant material was powdered by using pulverization, followed by passing through 60# sieve. The coarsely powdered drug was stored in tightly packed polyethylene bags in the dark place for protection against sunlight, and also avoiding moisture contact [18]. The powdered drug was exhaustively extracted consecutively in the soxhlet apparatus, using petroleum ether & ethanol solvent respectively. The collected extracts were concentrated under reduced temperature and pressure to obtain its powder form [19].

Statistical analysis

All the observed data in results were expressed as mean±SD. Statistical analysis was performed with one way ANOVA followed by *Tukey kramer* multiple comparison test. All the values were considered to be statistically significant ($P < 0.05$) when compared to standard one.

Results and discussion

Phytochemical screening

Inferences were conferred after the subjecting extracts with phytochemical screening of poly herbal plants (table 1) as per mentioned in the Indian Pharmacopoeia [16].

Table 1: Phytochemical observations of selected herbal plants

S. N.	Chemical Constituents (I.P. standards)	<i>Carica papaya</i>	<i>Boswellia serrata</i>	<i>Moringa oleifera</i>	<i>Vitex negundo</i>
1.	Proteins	–	+	+	–
2.	Amino acids	+	+	+	+
3.	Glycosides	+	+	+	+
4.	Carbohydrates	+	+	+	+
5.	Mucilage	+	–	–	–
6.	Gum	–	+	+	–
7.	Resin	+	+	+	–
8.	Tannins & Phenolics	+	–	+	–
9.	Alkaloids	–	–	+	+
10.	Flavonoids	–	–	–	+
11.	Steroids & Triterpenoids	+	+	+	+

(Note: + sign indicates the presence and – sign indicates the absence)

Physicochemical characterization

Inferences were conferred after the subjecting extracts with physicochemical characterization of poly herbal plants (table 2) as per mentioned in the Indian Pharmacopoeia [16].

Table 2: Physicochemical observations of selected herbal plants

Sr. No.	Physicochemical parameters (I.P.)	<i>Carica papaya</i>	<i>Boswellia serrata</i>	<i>Moringa oleifera</i>	<i>Vitex negundo</i>
1.	Total-Ash	16.36 %	8.45 %	9.90 %	6.90 %
2.	Acid-Insoluble Ash	4.08 %	5.88 %	1.02 %	1.50 %
3.	Water-soluble ash	5.95 %	7.30 %	1.07 %	1.30 %
4.	Loss on drying	4.05 %	4.62 %	3.91 %	5.95 %

Solvent extractive values: The extractive solvent values were conferred after subjecting all plants with alcohol and water individually. The ratio of alcohol and water soluble extractive values were observed as; *Carica papaya* (50.2:25.5%), *Boswellia serrata* (48.3:29.1%), *Moringa oleifera* (3.0:6.5%) and *Vitex negundo* (12.7:21.3%).

Tests for inorganic elements: Inferences were conferred after the subjecting extracts with different inorganic elements tests of poly herbal plants (table 3) as per mentioned in the Indian Pharmacopoeia [16].

Table 3: Inorganic elements declaration of the selected herbal plants

Sr.No.	Inorganic elements (I.P.)	<i>Carica papaya</i>	<i>Boswellia serrata</i>	<i>Moringa oleifera</i>	<i>Vitex negundo</i>
1.	Aluminium	-	-	+	-
2.	Chloride	+	+	-	+
3.	Copper	-	-	-	+
4.	Calcium	+	+	+	+
5.	Bicarbonate	+	+	+	-
6.	Magnesium	+	+	+	+
7.	Nitrate	-	-	+	-
8.	Phosphate	-	-	+	+
9.	Potassium	+	-	+	+
10.	Sodium	+	-	-	-
11.	Zinc	+	-	+	+
12.	Sulphate	+	+	+	-

(Note: + sign indicates the presence and – sign indicates the absence)

Preparation of polyphyto matrix formulation

The wet granulation technique is used for preparation of matrix tablets; in which, individual drug was placed for shade dried followed by cleaning with hand sorting. The required quantity of polymers and other materials were crushed using willing grinder and passed through the 60# sieve. After weighing the individual drugs as per the required quantity were mixed geometrically using a double cone blender. The mixture is then granulated using a weighed quantity of ethyl cellulose in isopropyl alcohol and mix properly to obtain the wet mass, followed by passing through 8# sieve. After that,

the granules were dried in an oven at 50±5°C temperature for one hour; then passed through the 20# sieve followed by retaining on 40# sieve. Subsequently 10% fine powders were taken and then weigh quantity of HPMC added to these granules. Then all the granules were lubricated in poly bag using talc and magnesium stearate (table 4). The weighed quantity of required blend was fed to the hopper of a compression machine to compress tablets, using flat faced punch i.e. upper punch with a break line and plane lower punch with 10mm diameter [20].

Table 4: Materials used for preparation of polyphyto matrix formulation

Ingredients	Formulation-01	Formulation-02	Formulation-03
Extract of <i>Carica papaya</i> in powder form	100 mg	100 mg	100 mg
Extract of <i>Boswellia serrata</i> in powder form	100 mg	100 mg	100 mg
Extract of <i>Moringa oleifera</i> in powder form	100 mg	100 mg	100 mg
Extract of <i>Vitex negundo</i> in powder form	100 mg	100 mg	100 mg
HPMC	25 mg (K-4M)	25 mg (K-15M)	25 mg (K-100M)
Ethyl cellulose	30 mg	30 mg	30 mg
Microcrystalline cellulose	37 mg	37 mg	37 mg
Magnesium stearate	4 mg	4 mg	4 mg
Talc	4 mg	4 mg	4 mg
Total weight (mg.)	400 mg	400 mg	400 mg

Evaluation of polyphyto matrix tablets: Polyphyto combinations of matrix tablets were evaluated concerning following parameters as per the standard specifications of Indian pharmacopoeia [23].

Evaluation parameters of granules: Inferences were conferred after the subjecting extracts with different evaluating parameter of the granules obtained from poly herbal plants (table 5).

Table 5: Evaluating parameters of the granules obtained from herbal plants

Parameters → Formulations ↓	Loose bulk density (LBD)	Tapped bulk density (TBD)	Carr's index (I)	Angle of Repose (Θ)	Total Porosity
F 1	0.424±0.002	0.473±0.002	11.63±0.271	37.45±1.002	38.27±0.452
F 2	0.306±0.015	0.355±0.011	13.18±0.441	37.64±0.114	35.31±1.006
F 3	0.316±0.048	0.356±0.036	11.31±0.097	35.91±0.50	35.05±0.646

All the values are shown as n = 3, and Mean±SD.

Evaluation parameters of tablet formulations: Polyphyto matrix tablets were evaluated by using following parameters as per the mentioned specifications in Indian Pharmacopoeia;

Weight variation: It is desirable that, every individual tablet in a batch should be uniform in weight, but a small variation in the weight of the individual tablet is liable to occur. The percentage deviation in weight variation allowed for the formulation of the tablet is as per I.P. 2010 specifications. The test is performed on 20 tablets with random sampling to determine their average weight. Not more than 2 of the individual tablet weights may deviate from the average weight by more than the percentage deviation given in table and none should deviate by more than twice that percentage [21,23].

Hardness: Hardness for six tablets from each formulation was determined using Monsanto hardness tester. Monsanto Chemicals Co. Ltd. had designed spring pressure device to test the hardness of a tablet. It has a graduated scale which gives the reading in kg/cm². The tablet to be tested is placed between the spindle and the anvil. The desired pressure needed to hold the tablet in position is applied by moving the screw knob in a clockwise direction. The scale is moved so that the indicator is fixed at zero. The pressure is then applied till the tablet breaks. The reading was noted down, which indicates the pressure to break the tablet [16,22]. While the thickness of matrix tablets from each batch was measured using a digital vernier caliper.

Friability test: Friability test is performed to evaluate the ability of the tablet to withstand wear and tear in packing, handling and transporting during whole life-course of medication. The apparatus used to perform this test is known as “Roche friability apparatus” consisting of a plastic chamber, divided into two parts revolving at 25rpm 20 tablets are weighed and placed in the plastic chamber as per specification. The chamber is rotated for 4 min or 100 revolutions. During each revolution, the tablet falls from a distance of 6 inches. The tablets are finally removed from the

chamber after 100 revolutions and weighed. The tablets are considered to be of good quality after observing the loss in weight is less than 1%, as per IP specifications [23].

Bulk density and tapped density: The powder is passed through a standard 20# sieve. The weighed amount of powder is introduced into a 100ml graduated cylinder. The volume occupied by the powder is noted for bulk density. Further, another 50 taps have been continued and the final volume is noted to obtain tapped density [24].

Dissolution profile of prepared formulations

This is due to more rigid complex formed by hydrophobic polymer Ethyl cellulose in presence of HPMC i.e. hydrophilic polymer, which retain the drug in the matrix and impart a hindrance to rapid diffusion of the drug from the matrix [25]. The hydrophobic nature of ethyl cellulose has a major contribution in reduction of penetration of the solvent molecules into the matrix formulation prepared as per mentioned in table 6. Finally it can be concluded that, the presence of Ethyl cellulose as well as HPMC and the total matrix material drastically influenced the release of drugs as shown in figure 3.

Dissolution test: The test is performed to measure the amount of time required for a given percentage of a drug substance in a tablet to go into solution under specified conditions *in-vitro*. The apparatus used for this test is as per IP specification; for such testing 900ml water was placed (free from dissolved air) and previously warmed up to 37°C into the vessel. The specified numbers of tablets were placed in the dry basket. The apparatus was started and adjusts the rotation speed to 100rpm or as directed in the monograph. Withdrawn the stated volume of solution from the vessel after the specified time and same quantity of fresh media was added to maintain sink conditions. This operation is subsequently repeated for 4 times [23].

Table 6: Model parameters and descriptive statistics of regression for dissolution profiles of different matrix formulations

Time in (h)	FORMULATION-01 % Released of drug	FORMULATION-02 % Released of drug	FORMULATION-03 % Released of drug
1	38.89±0.45	37.68±0.32	34.34±0.40
2	48.49±0.64	46.65±0.41	42.47±0.53
3	52.67±0.73	51.93±0.34	47.78±0.62
4	55.52±0.56	54.91±0.51	52.53±0.37
5	59.41±0.77	56.46±0.65	54.81±0.48
6	64.89±0.67	60.45±0.48	57.78±0.65
7	66.19±0.81	65.61±0.76	59.91±0.72
8	74.62±0.48	72.32±0.63	60.42±0.79
9	80.41±0.75	78.86±0.69	62.11±0.83
10	87.68±0.71	83.04±0.92	64.46±0.86
11	95.82±0.84	86.25±0.88	70.70±0.90
12	97.32±0.90	90.12±1.00	73.85±0.89

The percentage drug release was observed by UV spectrometer at a wavelength of 295 nm at pH 6.8 buffer for matrix tablets, 900-mL fill volume, 37°C, rotational speeds of 100 rpm, all the values are shown as n = 3, Mean±SD.

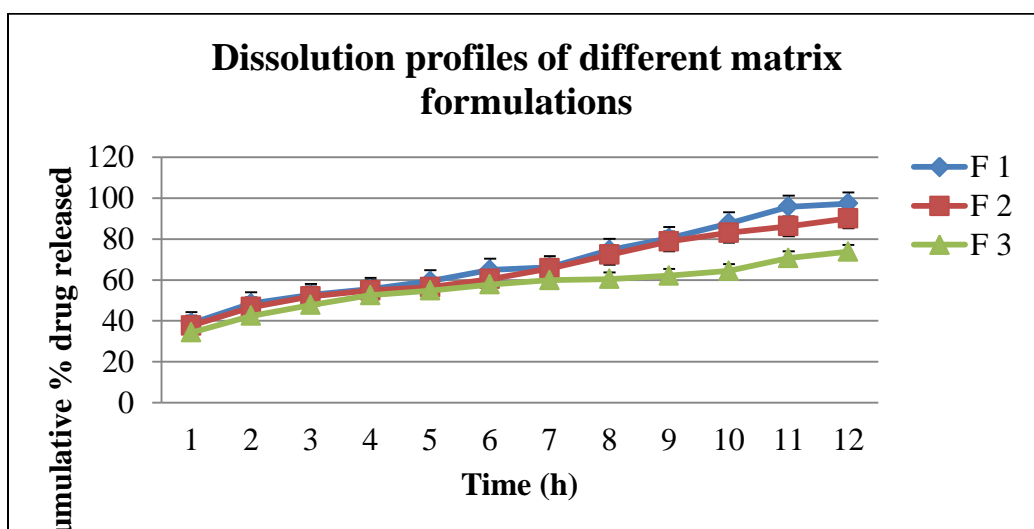


Figure 3: Drug dissolution profiles of three different matrix formulations. Mean values (n = 3) are shown, standard deviations are given by the error bars.

Anti-inflammatory screening

The anti-inflammatory effect of the different (F1, F2, F3) formulations (three different doses) including the reference drug were evaluated by using the carrageenan induced inflammation method [26]. For instance, the 300mg/kg F1 produced its highest effect at 12 h (92.34%) after carrageenan injection, while the 200mg/kg of the same formulation produced its effects at 12 h (79.92%) after injection in comparison to standard treatment. The reference drug, Diclofenac sodium and other two formulations (F2 and F3) produced dose-dependent reduction in suppressing

inflammation which was less pronounced in comparison to F1 formulation (dose 300mg/kg) at 6 h and 12 h of carrageenan administration (table 6). The results of this investigation revealed that the activity of 100mg/kg and 200mg/kg of all the formulations were slightly less effective than that of 300mg/kg (F1 formulation). Carrageenan-induced hind paw edema is the standard experimental model of acute inflammation; it is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects [27].

Table 6: Anti-inflammatory activity of developed formulations using carrageenan-induced paw edema in rats for 12 h measurements

Group(n)	Treatment(mg/kg)	Mean increase in paw volume (ml) at given h					% Inhibition in paw volume at	
		0 h	1 h	3 h	6 h	12 h	6 h	12 h
I	Vehicle Control	0.14±0.03	0.15±0.00	0.14±0.02	0.15±0.00	0.14±0.06	00	00
II	Carrageenan Control	0.28±0.02	0.58±0.05	0.82±0.02	1.02±0.03	0.96±0.02	100	100
III	Diclofenac (10)	0.12±0.02	0.32±0.04	0.48±0.02*	0.27±0.03*	0.15±0.00*	44.7	80.13
IV	F1 (100)	0.12±0.05	0.37±0.08	0.60±0.04*	0.30±0.05*	0.17±0.06*	40.0	70.46
V	F1 (200)	0.11±0.08	0.33±0.06	0.58±0.06*	0.24±0.04*	0.15±0.04*	50.00	79.92
VI	F1 (300)	0.12±0.04	0.30±0.06	0.50±0.05*	0.20±0.04*	0.13±0.02*	59.98	92.34
VII	F2 (100)	0.12±0.06	0.34±0.03	0.68±0.05*	0.26±0.06*	0.18±0.08*	46.12	66.50
VIII	F2 (200)	0.13±0.02	0.33±0.08	0.58±0.04*	0.24±0.06*	0.16±0.03*	49.96	74.98
IX	F2 (300)	0.13±0.00	0.30±0.06	0.54±0.04*	0.22±0.06*	0.15±0.05*	54.48	79.87
X	F3 (100)	0.12±0.08	0.35±0.02	0.60±0.04*	0.25±0.04*	0.18±0.04*	48.00	66.63
XI	F3 (200)	0.13±0.03	0.34±0.06	0.57±0.02*	0.26±0.00*	0.17±0.03*	46.23	70.58
XII	F3 (300)	0.12±0.06	0.31±0.05	0.53±0.04*	0.24±0.03*	0.15±0.04*	50.02	80.00

Values are expressed as mean±SD; $p < 0.05$ considered as statistically significant when compared with standard group (n=6). In the present study, the anti-inflammatory effect of the F1 (300mg/kg) polyphyto matrix formulation was exhibiting a higher anti-inflammatory effect than that of all doses (100, 200 & 300 mg/kg) of F2, F3 polyphyto matrix formulation and standard drug (Diclofenac sodium 10mg/kg). On the basis of results of evaluation parameters, formulation F1 was found out to be best and stable. From the dissolution studies of different formulations, it was found out to be that the formulations were modified release type. This is due to formation of more rigid complex by hydrophobic polymer ethyl cellulose in the presence of hydrophilic polymer i.e. HPMC, which may create a great potential to retain the drug in the matrix and offering a hindrance for rapid diffusion of drug from the total matrix [28]. The hydrophobic nature of ethyl cellulose seems to have contributed towards reduction in the penetration of the solvent molecules into the matrix. Finally it was fulfilled that, the blend of Ethyl cellulose and HPMC as a total matrix material significantly influenced the release rate of drugs. Hence the present matrix formulations can serve as a successful sustained release drug delivery system [29]. From the results of anti-inflammatory activity, it was found out to be that the drug was delivered to the inflammation site at a controlled level over a period of 12 h, offering a great potential to be used for the sustain action of drugs used in formulation and hence in treatment of inflammation [30]. This indicates that the drug was released from the polymer matrix based on the viscosity of the polymer, providing sustained action of the formulation with reference to the viscosity of the polymer used in the matrix tablets formulation. Based on above studies it can be concluded that the present matrix formulations can serve as a successful drug delivery system such as; improvements of patient's compliance, reduction of adverse effects, allowing patients to receive medication as outpatients, possibly a reduction in the overall use of medicinal resources etc.

Conclusion

In the current research work, an attempt was made to formulate matrix tablets of *Carica papaya*, *Boswellia serrata*, *Moringa oleifera*, *Vitex negundo* extract. From the results it can be concluded that, the matrix tablet formulation was found out to be a novel one, comprising a great potential to sustain the action of the drug release hence may be useful in the treatment of inflammation as sustain release drug delivery system. Market and literature survey reveals that, till date no study on Novel formulation of *Carica papaya*, *Boswellia serrata*, *Moringa oleifera*, *Vitex negundo* extract was reported to overcome these problems associated with these conventional dosage forms, an attempt had been done to develop Novel formulations. Novel drug delivery systems are the modifications of conventional dosage forms, and are relatively novel in the market, or they may be relatively new drug delivery systems, or may be used as unique devices before, during or after administration.

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Conflict of interest: We declare that we have no conflict of interest.

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