

Original Research Article

Anti-inflammatory activity and accelerated stability studies of crude extract syrup of *Cannabis sativa*

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Abstract

Purpose: To formulate *Cannabis sativa*-based syrup and investigate its anti-inflammatory potential and the stability of the formulation under stress conditions.

Methods: The syrup was prepared using different combinations of crude *C. sativa* resin, propylene glycol, aspartame, sucrose, sodium metabisulphite (SMBs) and ethylenediaminetetraacetic acid (EDTA). The stability of the formulations was determined under accelerated temperature conditions. The anti-inflammatory activity of the resin and different formulations were evaluated by the egg albumin induced paw edema model in rats. Biochemical assay was determined by Reitman and Frankel colorimetric assay method while hematological assay was evaluated by standard protocols.

Results: EDTA-containing syrup (CE) was the most stable with estimated shelf-life of 2204 days ($K_{25^{\circ}\text{C}}$, $4.78 \times 10^{-5}/\text{day}$). Higher propylene glycol levels significantly improved anti-inflammatory activity compared to those containing a lower amount. All the formulations showed anti-inflammatory activity higher than the crude resin with a dose-dependent inhibition of paw edema compared with the control. There was no significant difference ($p < 0.05$) between the serum glutamate-oxaloacetate transaminase (SGOT, $13.821 \pm 0.190 - 16.008 \pm 1.012$), serum glutamate-pyruvate transaminase (SGPT, $19.241 \pm 1.027 - 22.901 \pm 1.093$) and urea ($9.812 \pm 0.252 - 10.054 \pm 0.252$) levels of the treated and 16.856 ± 1.053 , 24.960 ± 0.101 and 10.654 ± 0.925 units/L of the control animals respectively. With the exception of eosinophil that disappeared from the blood in the third week, all the hematological parameters showed a gradual increase in lymphocytes, neutrophils, monocytes, packed cell volume (PCV), white and red blood cell counts in the third week compared to control.

Conclusion: Formulation of *C. sativa* as syrup using efficient carriers improves the pharmacological activity of the crude extract. SMBs and EDTA significantly enhance the stability of the syrup with no observable biochemical and hematological changes in treated animals

Keywords: *Cannabis sativa*, Syrup, Anti-inflammatory, Stability, Hematological

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INTRODUCTION

Cannabis sativa remains one of the most influential strictly controlled and multi-constituent

plants with multiple medicinal uses [1], yet its place in therapy has remained highly controversial [2] due to strict legislation against its use [3], controversial moral implications on its

use [4] and increased potential for abuse [5]. Notwithstanding, there are established medical benefits attributed to the appropriate use of *Cannabis* and its constituents. It has been established that the seed and its oil is rich in carbohydrate, protein and essential fatty acids and these are implicated in inflammation processes and immuno-modulatory reactions.

Many researchers are of the opinion that purified *Cannabis* and its preparation could find their way into the armamentarium of therapeutic agents while ensuring that illegal use or abuse is completely discouraged [6]. However, to date, smoking remains one of the few ways in which *Cannabis* has been administered with associated low absorption and high risk of toxicity [7]. The traditional use of *Cannabis* for inflammatory disorders has been reported and cannabinoid (CBD) which is one of the components was found to be more effective than aspirin as an anti-inflammatory agent [8].

However, the considerable discrepancy has been reported as to the activity of CBDs in a number of animal tests for anti-inflammatory, mild analgesic and anti-pyretic activity. For instance, Δ^9 -tetrahydrocannabinol (THC) was reportedly 20 times more potent than aspirin and 2 times as potent as hydrocortisone in carrageenan edema test in rats [9] but these findings were not confirmed when Δ^9 -THC was orally administered. In fact, it was inactive in blocking carrageenan-induced edema in the rat paw model [8]. These discrepancies in anti-inflammatory activity of *Cannabis*, however, could be attributed in part to pharmacokinetic-related properties of *Cannabis*, especially the erratic absorption profile from smoking.

Experience has also shown that optimum pharmacological activity may not be resident exclusively in any of the phytoconstituents but in the bulk extract. For example, CBDs were shown to attenuate the signs and symptoms of cerebral damage in animals with autoimmune encephalomyelitis [10] while cannabichromene has also been reported to possess anti-inflammatory activity [11] of less significance compared to the crude extract [8]. Against the backdrop that pharmacological (anti-inflammatory) activity of *Cannabis* could be controlled by complex pharmacodynamic systems as well as pharmacokinetics of the extract, we formulated crude *Cannabis* extract syrup using different vehicles, assessed the anti-inflammatory performances and its biochemical and hematological parameters in experimental animals, and also the stability of the formulations

with a view to improving the anti-inflammatory activity of *Cannabis* extracts.

EXPERIMENTAL

Materials

The fresh *C. sativa* leaves were obtained from the Crude Drug and Research Unit of the National Drug Laws Enforcement Agency (NDLEA) Enugu Zone and identified by Mr. H. Z. Lawal, a taxonomist at the Unit. All the reagents used for this study were obtained from BDH, England or Sigma-Aldrich, Germany and were used without further purification. The UV-VIS spectrophotometer is a U-2900 double beam UV-visible spectrophotometer wavelength range of 190-1100 nm with 1.5 nm spectral bandpass (Hitachi High-Tech Co, Japan).

Preparation of crude *Cannabis* extract

The dry pulverized *C. sativa* whole leaves (500 g) were exhaustively extracted by cold maceration in 5 L (2 x 2.5 L) of methanol (95 % v/v) for 48 h. The extract was filtered, evaporated *in vacuo* and the dried extract stored at 0 - 4 °C.

Preparation of *Cannabis* syrup

Different batches of *Cannabis* extract syrup were constituted from a 100 mg/mL crude *Cannabis* extract (CCR), 67 % w/v sucrose, 0.15 % w/v aspartame, 0.1 % w/v sodium metabisulphite (SMBs), 3.0 % w/v EDTA and 5 % v/v propylene glycol. All the formulations were made up to 50 mL in appropriate diluents as shown in Table 1 to obtain syrup strengths of 5.0 and 10.0 mg/mL. The composition of the batches used for this study was optimized and selected based on solubility, stability, consistency and rheological performance of various trials of different ratios of constituents.

Anti-inflammatory study

A total of 115 adult albino rats (100 – 150 g) of either sex were divided into nine groups (n = 5) as follows: Group A-G received each 5, 10 and 20 mg/kg of CCR, CS-1, CS-2, CB-1, CB-2, CE-1 and CE-2 respectively. Group H received 100 mg/kg of acetylsalicylic acid while Group I received an equivalent volume of blank formulation p.o. Inflammation was induced after 1 h of administration by injecting 0.1 mL undiluted fresh egg albumin into the sub planter region of the right hind paw of the rats. The paw size was measured at 0, 0.5, 1, 2, 3, and 4 h intervals after egg albumin injection using digital vernier caliper [12,13].

Table 1: Composition of crude *Cannabis* resin syrup

Batch	CCR (mL)	Sucrose (mL)	Propylene glycol (mL)	Aspartame (mL)	Diluents (mL)	SMBs (mL)	EDTA (mL)	Strength (mg/mL)
CS-1	2.5	10.0	5.0	10.0	22.5	-	-	5.0
CS-2	5.0	15.0	2.0	15.0	13.0	-	-	10.0
CB-1	2.5	10.0	5.0	10.0	20.5	2.0	-	5.0
CB-2	5.0	15.0	2.0	15.0	9.0	4.0	-	10.0
CE-1	2.5	10.0	5.0	10.0	15.0	2.0	5.5	5.0
CE-2	5.0	15.0	2.0	15.0	5.5	4.0	3.5	10.0

CCR=crude *Cannabis* extract formulated at two different concentration levels 1 (5 mg/mL) and 2 (10 mg/mL) with sodium metabisulphite SMBs (CB) and/or EDTA (CE) or base formula (CS)

Edema formation was assessed in terms of the difference between the zero time linear diameter of the injected paw and its diameter at the various time intervals after egg albumin injection. For each treatment, edema level and percent inhibition of edema were calculated [14].

Determination of biochemical and hematological parameters

Animals were grouped into four (n = 5). Three groups received 10 mg/kg each of formulations CS-1, CB-1 and CE-1 while one group served as control. At predetermined intervals of 0, 7, 14 and 21 days and prior to weekly treatment with the selected formulations, 5 μ L blood samples were collected from the retrobulbar plexus of the medial canthus of the eyes of each animal, pooled together group by group and centrifuged at 10000 x g for 5 min. A 1 mL appropriate buffer substrate solution (DL-aspartate and α -ketoglutarate for serum glutamate oxaloacetate transaminase, SGOT, diacetyl monoxime and thiosemicarbazide for urea and DL-alanine and α -ketoglutarate for serum glutamate pyruvate transaminase (SGPT)) and 0.20 mL of serum from different animal groups treated with the syrup and controls were incubated for 1 h at 37 $^{\circ}$ C. The SGOT and SGPT levels were determined by Reitman and Frankel colorimetric assay method [15] using 2,4-dinitrophenylhydrazine as chromogen which forms an adduct that absorbs at 520 nm and urea by urease Berthelot method [16]. The other portion of the serum was used to determine the WBC (white blood cells), RBC (red blood cells), PCV (packed cell volume), eosinophils, monocyte, lymphocyte and neutrophils by standard methods [17]. In all cases, baseline hematological and biochemical measurements were carried out prior to treatment of the animals to serve as control values.

Stability studies of crude extract syrup

In order to assess the stability of the formulations, all the batches were subjected to controlled temperatures of 30, 40 and 50 $^{\circ}$ C for

90 days. At predetermined time intervals of 10, 20, 30, 40, 50, 60, 70, 80 and 90 days, the *Cannabis* contents were assayed spectrophotometrically and the trends of decline in concentration ascertained using zero, first and second order reaction equations. The rate constants determined were used to derive specific rate constant, $K_{25^{\circ}\text{C}}$ at 298 K using Arrhenius equation and then shelf-life (t_{90}) of selected formulations.

Statistical analysis

The experimental results were analyzed using the Statistical Package for the Social Sciences (SPSS Inc. Chicago), v. 15.0 and GraphPad Prism v. 6.01.2012 (GraphPad Software Inc., San Diego, CA, USA) software. The anti-inflammatory activities (rat paw sizes in mm), biochemical parameters (SGOT, SGPT and urea in units/L) and hematological parameters (WBC in cells/ μ L, RBC, lymphocytes, monocytes, neutrophils, eosinophil in units/ μ L and PCV in %) were expressed as a mean \pm standard deviation from the mean (SD) (n=5), Student's t-test was done to test for the significant difference between the means of samples and control at $p < 0.05$. In all cases, a $p < 0.05$ was considered to be significant.

RESULTS

The crude *Cannabis sativa* resin and its various syrup formulations caused varying degrees of suppression of paw edema in rats compared to control with somewhat dose-dependent effects in most cases. However, this activity was much more evident when the *Cannabis* resin was formulated and delivered in syrups (Table 2).

The stability of syrups is important because their physicochemical properties depend on excipients employed in preparation and was assessed by determining the amount of initial concentration degraded after certain time under stressed conditions.

Table 2: Anti-inflammatory activities of *C. sativa* resin and its formulations

Group	Dose (mg/kg)	Paw size (mm)			Inhibition of edema (%)		
		0.5 h	2 h	4 h	0.5 h	2 h	4h
A	5	0.55±0.09 ^{a,b}	0.52±0.10 ^{a,b}	0.51±0.11 ^{a,b}	40.54	42.30	43.33
	10	0.56±0.05	0.50±0.09	0.49±0.02	40.00	45.05	45.56
	20	0.52±0.10	0.51±0.02	0.49±0.08	43.24	43.95	45.00
B	5	0.42±0.11	0.40±0.08	0.38±0.07	54.05	56.04	57.78
	10	0.41±0.08	0.39±0.06	0.37±0.03	55.68	57.14	58.33
	20	0.40±0.09	0.39±0.08	0.36±0.07*	56.76	57.14	60.00
C	5	0.46±0.10	0.46±0.04	0.41±0.08	50.27	49.45	54.44
	10	0.45±0.09	0.41±0.12	0.40±0.04	51.35	54.95	55.00
	20	0.45±0.07	0.42±0.06	0.40±0.06	51.89	53.30	55.56
D	5	0.32±0.09	0.32±0.06	0.31±0.07	64.86	64.83	65.56
	10	0.32±0.06	0.31±0.00	0.30±0.06	64.86	65.38	66.67
	20	0.31±0.13	0.31±0.04	0.30±0.04*	66.49	65.38	66.67
E	5	0.39±0.08	0.38±0.07	0.36±0.22	57.30	57.69	60.00
	10	0.38±0.04	0.38±0.02	0.37±0.06	58.92	58.24	58.89
	20	0.37±0.04	0.37±0.00	0.36±0.02	59.50	58.79	60.00
F	5	0.30±0.11	0.30±0.08	0.30±0.06*	67.57	67.03	66.67
	10	0.28±0.06	0.28±0.05	0.28±0.03*	69.19	69.23	68.89
	20	0.27±0.09	0.27±0.06	0.26±0.06	70.81	70.33	70.55
G	5	0.35±0.04	0.33±0.04	0.30±0.01*	62.22	63.74	66.67
	10	0.32±0.09	0.32±0.04	0.30±0.02	64.86	64.84	66.67
	20	0.31±0.07	0.32±0.11	0.29±0.41*	66.49	64.29	67.78
H	100	0.26±0.10	0.22±0.08	0.20±0.02	71.35	75.82	77.78
I	-	0.92±0.04	0.91±0.08	0.90±0.10	-	-	-

Values expressed as mean ± SD, *p*-values were calculated using the unpaired Student's *t*-test (*n* = 5); **p* < 0.05 are significant; level of significant compared with controls at 4 h only

The results of accelerated stability studies indicated that the various formulations of crude *C. sativa* syrup formulations may have undergone a first order kinetic degradation (Table 3). It is imperative to point out that only formulations containing equal amount (5.0 mg/ml) of crude Cannabis extract were used for this study, differing only in the composition of EDTA and SMBs.

The biochemical parameters are presented in Table 4. The results indicate that the SGOT,

SPGT and urea levels in the treated animal groups were not significantly (*p* < 0.05) different from the control groups within the 3 weeks of treatment.

The results of hematological parameters presented in Table 5 indicate that the indices were of similar values in all the animals treated with the formulations with fluctuations in the three weeks of administration.

Table 3: Accelerated stability of crude *C. sativa* formulations

Batch	C ₀ (mg/ml)	30 °C		40 °C		50 °C		K _{25°C} (x10 ⁻⁵ /day)	Shelf-Life (day)
		C ₉₀ (mg/ml)	K (x10 ⁻⁵ /day)	C ₉₀ (mg/ml)	K (/day)	C ₉₀ (mg/ml)	K (/day)		
CS-1	5.0	2.98	580	2.56	0.0074	2.41	0.0081	522.7	20
CB-1	5.0	4.65	80	4.47	0.0012	4.28	0.0017	65.5	160
CE-1	5.0	4.96	0.89	4.85	0.0003	4.62	0.00088	4.8	2204

C₀ = initial Cannabis concentration in the syrup, C₉₀ = concentration of Cannabis remaining in the syrup after 90 days, K = specific first order rate constant, K_{25°C} = specific rate constant at 298 K obtained from Arrhenius plots

Table 4: Biochemical and hematological parameters

Treatment	SGOT (units/L) ^a	SGPT (units/L)	Urea (units/L)
CS-1	15.345±0.365	19.241±1.027	9.967±1.067
CB-1	16.008±1.012	22.901±1.093	10.054±1.081
CE-1	13.821±0.190	20.630±0.611	9.812±0.252
Control	16.856±1.053	24.960±0.101	10.654±0.925

Values expressed as mean ± SD, *p*-values were calculated using the unpaired Student's *t*-test (*n* = 5); **p* < 0.05 are significant; control groups were not treated. ^a1 unit = 0.000482 μmoles glutamate formed/min

Table 5: Hematological parameters in experimental animals

Time (week)	WBC (cell/ μ L)	RBC (unit/ μ L)	Lymphocyte (unit/ μ L)	Monocyte (unit/ μ L)	Neutrophil (unit/ μ L)	Eosinophil (unit/ μ L)	PCV (%)
0	9000 \pm 120	(6.9 \pm 0.1) \times 10 ⁶	5500 \pm 31	1900 \pm 14	1400 \pm 23	180 \pm 12	46.50 \pm 2.15
1	10000 \pm 109	(4.0 \pm 0.4) \times 10 ⁶	8000 \pm 28	1250 \pm 28	1400 \pm 21	170 \pm 15	41.20 \pm 1.10
2	9500 \pm 98	(4.5 \pm 0.2) \times 10 ⁶	7000 \pm 80	1200 \pm 21	2650 \pm 54	45 \pm 9	39.60 \pm 0.93
3	15000 \pm 70	(6.5 \pm 0.2) \times 10 ⁶	10000 \pm 98	2500 \pm 34	4100 \pm 45	0.00	47.25 \pm 1.90

Values expressed as mean \pm SD ($n = 5$) for all the *Cannabis* syrup formulations (treated) and untreated animal groups

DISCUSSION

Cannabis extract and its various formulations caused varying degrees of dose and non-dose related suppression of paw-edema in rats. All the administered CCR doses caused an inhibitory effect much lower than that of 100 mg/kg acetylsalicylic acid. Higher propylene glycol containing formulations caused a significant ($p < 0.05$) dose-dependent inhibition of paw edema.

The *Cannabis* syrup containing either EDTA (CE) or sodium metabisulphite (CB) did not produce an inhibitory effect different from other formulations. The potency increased in the order of magnitude CE > CB > CS > CCR. Various preparations of *C. sativa* have been employed for their anti-inflammatory purposes. Extracts of *Cannabis* have been shown to possess anti-inflammatory activity [18] and Δ^9 -THC, the psychoactive component of *Cannabis* has also been shown to possess anti-inflammatory activity in various models [19]. In addition, cannabidiol (CBN) and not cannabidiol (CBD) was shown to exhibit these activities too [20]. The improved anti-inflammatory activities of the formulations could be attributed to the effects of vehicles used and potential of syrup to overcome erratic absorption common with the traditional smoking of *Cannabis*.

Apart from the solubilizing effects of propylene glycol, it has been previously used to improve the bioavailability of orally administered poorly water-soluble drugs by different mechanisms [21,22]. The improved anti-inflammatory activity of the syrups over the crude extract could be partly attributed to the solubilizing power, increased polar pathway for maximum absorption and favourable partitioning of the anti-inflammatory constituent(s) of the extract by propylene glycol, and on the other hand by the increased diffusivity of cannabinoid components of *C. sativa* [8]. Other components of the formulations may not have improved the anti-inflammatory activity of crude *Cannabis* extract extensively but their usefulness in syrup formulations has been reported and validated [23,24].

The stabilization effects of sodium metabisulphite and EDTA contributed to the significant improvement of the shelf lives of the syrup compared to their respective blanks. Degradation of cannabis is complex and has led to the formation of thermodynamically unstable metabolites which are difficult to separate [25]. The reactions are capricious and sensitive to certain conditions resulting in decarboxylation and isomerization reactions [25]. Thus, the stabilizers employed in these formulations could have shielded the THC or other labile components from these reactions thereby prolonging the shelf lives of simple *Cannabis* syrup (CS) from 20 to 160 days in CB and further 2204 days in CE.

The primary metabolic site of *Cannabis* and majority of the syrup components is the liver. In cases of damaged liver, SGOT and SGPT may be elevated due to leakage into the bloodstream especially when excipients are toxic enough to cause liver injury. There was no significant elevation ($p < 0.05$) of all the biochemical parameters measured. SGOT level of 13.821 - 16.008, SGPT 19.241 - 22.901 and urea 9.812 - 10.054 units/L compared to 16.856, 24.960 and 10.654 units/L respectively in the control groups were recorded. The hematological erythrocytic and leucocytic indices were not significantly different in all the treated animal groups.

However, on the third week, results showed a significant increase in PCV, RBC, leucocytes, lymphocytes, monocyte and neutrophils. On the contrary, eosinophils disappeared from the blood of the treated groups in the third week. This could be attributed to the detoxification effect of eosinophils which were consumed by the combined mild toxic effect of the components of the syrup. The general increase observed for other hematological parameters within the three weeks of treatment could be attributed to the haemoconcentration induced by dehydration effect of *Cannabis* [26]. The effect of the formulation on the biochemical and hematological parameters supports the evidence that the components of the syrup (propylene glycol, sucrose, aspartame, SMBs, EDTA and distilled water) belonged to "generally recognized

as safe” chemicals (GRAS) and are relatively safe for orally-targeted formulations [27].

CONCLUSION

The formulation of *C. sativa* resin as a syrup using lipophilic and hydrophilic carriers improves significantly the anti-inflammatory activity of *Cannabis*. Degradation of the resin in such formulations follows first order kinetic model. Furthermore, incorporation of EDTA and/or SMBs into the syrup improves its shelf-life by approximately 99 %. Thus, syrup is an efficient and alternative vehicle to the traditional smoking for oral delivery of *C. sativa* as an anti-inflammatory.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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