World Journal of Pharmaceutical research

Volume 2, Issue 4, 986-1007. Research Article ISSN 2277 – 7105

FORMULATION AND EVALUATION OF ANTIDIABETIC TABLET CONTAINING WHOLE PLANT EXTRACT OF *BIOPHYTUM SENSITIVUM* ON THE BASIS OF TOTAL FLAVONOID CONTENT

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Article Received on
20 April 2013,

Revised on 07 May 2013,
Accepted on 27 June 2013

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ABSTRACT

Biophytum sensitivum D. C. belonging to the family of *Oxalidaceae* and commonly known as 'Nagbeli', and its powdered dry leaves are known traditional remedy for the treatment of 'Madhumeha' (diabetes). After the detailed study of methanolic extract of *Biophytum sensitivum*, an optimized tablet formulation using the dried whole plant methanolic extract was prepared and evaluated for antidiabetic activity in the present study. Three batches of such oral tablet formulations have been comparatively studied for their antioxidative properties *invitro* based on their total flavonoid content (TFC) against a standard flavonoid, Quercetin and also in vivo for their antidiabetic potentialities in Streptozotocin (STZ) induced diabetic rats against Glibenclimide, an antidiabetic drug. The results of preformulation and post compression studies revealed that all the values were within acceptable limit as per the pharmacopoeial standards. The tablets were subjected to physicochemical characterization, *invitro* drug release and pharmacological studies. Different dissolution models were applied to drug release data in order to evaluate release mechanisms and kinetics. The drug release data fit well to the Ficksian mechanism of diffusion. *Invitro* studies revealed that B. sensitivum extract was well tolerated in high doses (200 mg/kg to 500 mg/Kg of B. Wt.) in mild to moderate diabetic cases (till 300 mg/dL of Blood glucose level). Thus the claims made by the traditional Indian systems of medicine regarding the use of this plant in the treatment of diabetes stands confirmed. The final conclusion drawn from the above mentioned data is that the possible use of these economical and relatively non toxic, non-

hazardous natural remedies of plant origin may further be explored as adjuncts to antidiabetic therapy as they are devoid of major side effects associated with synthetic agents.

KEY WORDS: Antioxidant, *Biophytum sensitivum*, Diabetes, Flavonoid, Hypoglycemia, Streptozotocin.

INTRODUCTION

Based on recent advances and involvement of oxidative stress in complicating *Diabetes Mellitus*, efforts are on to find suitable herbal antidiabetic medicines with antioxidant properties. Flavonoid like Naringenin, Rutin, especially Quercetin has been reported to possess antidiabetic activity. Vessal et al. (2003) [1] reported that Quercetin brings about the regeneration of pancreatic islets and probably increases insulin release in streptozotocin-induced diabetic rats. Also in another study, Hii and Howell [2] reported that Quercetin stimulate insulin release and enhanced Ca^{2+} uptake from isolated islets cell which suggest a place for flavonoid in T2DM (Hii and Howell, 1985). Moreover, in a randomized clinical study, Sattanathan and others [3] demonstrated that administration of the flavonoid Rutin, as adjuvant with oral hypoglycemic agents, improves glycemic control and lipid profile in T2DM patients (Sattanathan et al., 2011). It was also observed that some flavonoid possess insulin-mimetic activity based on their anti-hyperglycemic effect [1]. Part of these actions can be explained by their antioxidant properties exerted through direct free radical scavenging [4, 5]. The major flavonoid classes are flavones, flavanonols, flavonols, flavanones and isoflavones. Ethnopharmacological usage and the literature review revealed that the methanolic extract of *Biophytum sensitivum* whole plant have significant antidiabetic activity [6]. *Diabetes Mellitus* is expressed as a metabolic disorder caused primarily by a defect in the production of insulin by the islet cells of the pancreas resulting in an inability to use blood glucose, are characterized by hyperglycemia, glycosuria, polyuria, hyperlipemia, acidosis, ketonuria and a lowered resistance to infection. The diseases which are listed under *Diabetes Mellitus* are many with the most common being Type-1 diabetes and Type-2 diabetes. In Type-1 diabetes, the body produces little or no insulin, With Type-2 diabetes, the body produces plenty of insulin but cells are unable to use it. These are diseases of the metabolic system and involve the body's ability in metabolizing sugar using the hormone insulin [7].

The WHO Expert Committee on diabetes has recommended that traditional medicinal herbs be further investigated as evident from the fact that during the past few years some of the new bioactive drugs isolated from traditional plants showed antidiabetic activity with more

efficacy than oral hypoglycemic agents used in clinical therapy [8]. The large number of plant families, including the species, most studied for their confirmed hypoglycemic effects include: *Leguminoseae* (11 sp), *Lamiaceae* (7 sp), *Liliaceae* (8 sp), *Cucurbitaceae* (7 sp), *Asteraceae* (6 sp), *Moraceae* (6 sp), *Oxalidaceae (2 sp) Rosaceae* (6 sp), *Euphorbiaceae* (5 sp) and *Araliaceae* (5 sp). The most studied species are: *Citrullus colocynthis (Opuntia streptacantha Lem. (Cactaceae), Trigonella foenum greacum L. (Leguminosea), Momordica charantia L. (Cucurbitaceae), Ficus bengalensis L. (Moraceae), Polygala senega L. (Polygalaceae), and Gymnema sylvestre R. (Asclepiadaceae).*

In the traditional system of plant medicine it is conventional to use whole plant formulation and/or combined extracts of whole plant as a drug of choice rather than individual ones or individual parts of the plants (Kumar, 2010) [9], to get the benefit of synergism and to find suitable antidiabetic and antioxidant combination therapy. *Biophytum sensitivum* DC (*Oxalidaceae*) is a small, sensitive annual herb, growing throughout tropical Africa and Asia, especially in Philippines and the hotter parts of India and Nepal. This "little tree plant" is known for its interesting characteristic similar to the touch-me-not plant. It is commonly known as Lajjaluka in Sanskrit, as it can be observed as inward curling of its leaves in response to touch stimuli. The Mukkutti (flowers) are significant for the people of Kerala, both for its medicinal and for its cultural and traditional values. Generally, the whole plant is frequently used for medicinal purpose. Both methanolic extract of *Biophytum sensitivum* (whole plant) and the formulated tablets containing plant material have been experimentally tested at different doses to different batches of rats (both normal and diabetic rats) after an overnight fast. *Biophytum sensitivum* (*Oxalidaceae*) has been reported in treatment of ailments as a traditional folk medicine like hypoglycemic [10], immunomodulatory [11], chemo protective [12], hypocholesterolemic [13], apoptotic [14], anti-inflammatory [15], and cell-mediated immune response [16], antitumor [17], repetitive action potentials [18], effects on prostaglandin biosynthesis [19, 20]. The biochemical properties [21] of the plant showed the presence of amentoflavone [22], 3′, 8″- biapigenin [23], proanthocyanidins [24] and phenolic compounds [25].

The present paper deals with formulation and evaluation of herbal tablets prepared from methanolic extract of the selected whole plant, *Biophytum sensitivum*. Three solid pharmaceutical dosage formulations (Tablets) using dry plant extract has been prepared using various excipients viz., spray dried lactose, MCC pH 102, PVP K30, Cross-Povidone,

Sodium Starch Glycolate, Aerosil-200 and Magnesium stearate by non-aqueous granulation method. The micromeritic properties were determined for all the physical mixtures, the results of angle of repose, Carr's Index and Hausner ratio indicated that the powder mixtures possess good flow properties and good packing ability. The physical properties of the tablets were determined and all the samples of the herbal tablets were found to be complied with the official requirements of uniformity of weight. Finally the best formulation of herbal tablets was evaluated for its TFC content (eqv. to Standard Flavonoid, Quercetin) and *in-vivo* anti-hyperglycemic activity in STZ induced diabetic rats against Glybenclimide, an antidiabetic drug and it was found to be statistically significant. The drug release from all the formulations have also been studied and fitted with Korsmeyer-Peppas model with n value > 0.5 indicating the drug released by non ficksian diffusion mechanism.

MATERIALS AND METHODS

Chemicals

Streptozotocin was purchased from Sigma Aldrich Company (USA). Glibenclamide was obtained from Stadmed Private Ltd, as a gift sample. All other biochemicals and chemicals used in the experiment were of analytical grade were purchased from E. Merck and HIMEDIA (Mumbai) India. The blood glucose level was confirmed by the use of One Touch Glucometer (Prodigy) and compatible blood glucose test strips (Prodigy).

Plant material

The whole plants of *Biophytum sensitivum* were collected locally from the forest of Midnapore, West Bengal in August 2009.The plant material was identified and authenticated taxonomically by an expert taxonomist at the Central National Herbarium, Botanical Survey of India, Botanical Garden, Howrah-711103, West Bengal, India. The voucher specimen of the plant specimen at BSI is 942 (Dt 17.09.68, D.N. Guhabakshi) and the collected sample was taxonomically matched & deposited in the institutional herbarium for future reference.

Preparation of extracts

The collected whole plants of *Biophytum sensitivum* were washed, cleaned, dried under shade and powdered by a mechanical grinder to obtain a coarse powder and then passed through 20# mesh sieve. 5.0 Kg of the pulverized whole plant was extracted with methanol successively in a soxhlet apparatus. Petroleum ether was used in initial step of extraction for defatting the plant materials. The successive methanolic extracts were mixed & concentrated at reduced temperature on a rotary vacuum evaporator and stored at 4°C until further use. A

dark semi-solid (greenish-black) material was obtained and the yield was found to be around 8.26% (W/W). The biologically potent dry extract was further processed for subsequent tablet formulations.

Drug Excipient Compatibility Study

The Extract sample and the pre-compression blends were taken for compatibility study by FTIR spectral analysis at moderate scanning speed between 4000-500 cm^{-1} using FTIR (Bruker). The peak values (wave number) indicating the possibility of functional group is shown in spectra which were compared with standard value. The FT-IR spectra of tablet pre compression blends did not show the presence of any additional peaks for new functional groups. The major peaks of the drug remained unchanged in the mixtures. These results as given in Fig. 2 & Fig. 3 suggest absence of any chemical interaction between the drug (BS Extract) and the excipients used in tablet formulations.

Preparation of herbal tablets from *Biophytum sensitivum* whole plant dry extract

Three batches of tablet formulations, each containing 200 mg of BS extract per tablet were prepared separately by non aqueous wet granulation method using different proportions of excipients as BS 1, BS 2, and BS 3 [Table 1]. All the ingredients were passed through mesh no. 60# and mixed with 1% aerosil (Aerosil-200) and 1% of magnesium stearate. The micromeritic properties were evaluated for all the granules of three batches of Tablets to ensure that pre compression blends possess good flow properties and good compressibility [Table 2]. Tablets were compressed each of 675 mg average weight on a 10-station Mini Press-I rotary tablet compression machine fitted with a set of 16 mm X 8 mm Caplet shaped die-punches. No manufacturing defects were observed in tablets like capping, lamination and chipping. The physical properties of tablet were determined and the results of the uniformity of weight, hardness, drug content and friability of the tablets are given in Tables 3. The drug content was evaluated based on per cent TFC of the extract with respect to a standard Flavonoid, Quercetin.

***Invitro* drug release study of formulated BS tablets by Dissolution test**

In-vitro dissolution studies were conducted on tablets of each of the formulations such as (BS 1, BS 2 & BS 3) using USP Type II dissolution apparatus in 900 mL of 0.1 N Hydrochloric Acid on the basis of estimating per cent cumulative release of TFC from the B.S. Extract at different time interval at 37$\pm$ 0.5 deg C and at 50 rpm. Since, Flavonoid constitutes the major chemical entity in formulation therefore all formulations were evaluated with respect to Total

Flavonoid content (TFC), which was exhibited as the reliable and reproducible parameter for the dissolution studies of the formulation. So with respect to TFC present in the extract, the drug release study was carried out. The mean cumulative percent of TFC released at different time intervals for each formulation is shown in Fig. 4 & Table 6.

Kinetic treatment of dissolution data [26, 27]

In order to describe the kinetics of the release process of drug in the different formulations, zero- order **($Q_t = Q_0 + K_0 t$)**, first order **($\ln Q_t = \ln Q_0 + K_1 t$)**, Higuchi **($Q_t = K_H t^{1/2}$)** and Korsmeyer- Peppas **($Q_t/Q_\alpha = K t^n$)** models were fitted to the dissolution data of optimized formulations BS 1, BS 2 and BS 3 using linear regression analysis. A value of n = 0.5 indicates case I (Ficksian) diffusion or square root of time kinetics, 0.5 < n < 1 anomalous (non- Ficksian) diffusion, n=1 Case – II transport and n >1 Super Case II transport

Three batches of compressed tablets (BS 1, BS 2 & BS 3) were evaluated for their critical quality parameters and an optimized formulation had been selected for further in-vitro TFC content analysis with reference to standard Flavonoid, Quercetin and in-vivo antihyperglycemic activity comparison with an Antidiabetic drug, Glibenclamide in STZ induced Rats. The optimized formulated batch of herbal tablet BS 3 was kept for stability studies at different temperature and relative humidity conditions to ascertain the stability of the drug (for about 3 months at 25^0C/60% RH, 30^0C/60% RH and 40^0C/75% RH).

Estimation of Total Flavonoid Content Aluminum Chloride Colorimetric Method

The basic principle of Aluminum chloride colorimetric method is that Aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition it also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of Flavonoid. [30]

The aluminum chloride colorimetric method was modified from the procedure reported by Woisky and Salatino [31]. 50 milligrams of Quercetin was dissolved in 500 ml methanol and then further diluted to 10, 20, 30, 50, 70 and 100 µg/mL. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm (λ_{max} of Quercetin) with a Shimadzu UV-1800 spectrophotometer. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank.

Preparation of BS Extract stock solution: 100 mg of the BS extract was accurately weighed and transferred to 50 ml volumetric flask and made up the volume of 50 ml with methanol.

Preparation of BS Tablet stock solution: 500 mg of powdered tablet sample (Each Tablet containing 200 mg Plant extract) was accurately weighed and transferred to 50 ml volumetric flask and made up the volume of 50 ml with methanol.

From each stock solution of BS Extract and powdered BS tablet, 0.5ml of each aliquot was taken and to it 1.5 ml methanol, 0.1 ml 10% aluminum chloride, 0.1 ml of 1M potassium acetate solution and 2.8 ml distilled water were added and mixed well. Sample blank was prepared in similar way by replacing aluminum chloride with distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. All prepared solutions were filtered through whatmann filter paper before measuring. A yellow color indicated the presence of Flavonoid.

Determination of Total Flavonoid content

To perform the calculations of Total Flavonoid Content, a standard curve is needed which is obtained from a series of different Quercetin concentrations.[Table 4] Concentration values of all other extracts & test solutions from the Powdered Tablets were obtained from corresponding absorbances of Quercetin standard curve, by interpolating to the X- axis.

Experimental animals

Healthy young Albino rats weighing between 120 g to 200 g were procured from Indian Institute of Cultivation of science, Kolkata. The animals were individually housed in polypropylene cage and the room condition was maintained at temperature of 25±5 deg C and humidity 45±5 per cent with 12 hr day and night cycle. The animals were fed with Pellet chew feed standard diet and water *ad libitum*. All the studies were conducted in accordance with the Institutional Animal Ethical committee of NSHM College of Pharmaceutical Technology.

Acute toxicity studies [27]

Acute toxicity studies for dried methanolic extract of *Biophytum sensitivum* was carried out in different groups of mice (10 mice/group) with one group served as control group, at different graded doses (Oral) of the dried whole plant extract of B. sensitivum (200 - 5000

mg/kg, orally), diluted with 1% w/v of SCMC suspension, showed no gross evidence of any toxicity and abnormalities in the mice up to 72 hr of the observation period. As no mortality signs had been observed even after administration of dose up to 5000 mg/kg of body wt, the extract might have LD_{50} value beyond 5000 mg/kg. Hence, further pharmacological investigation was carried at dose levels equivalent to $1/10^{th}$ of LD_{50} (here maximum therapeutically safe dose) eqv. to 500 mg/kg and below. Acute toxicity study was done as per OECD Guidelines 423.

Induction of diabetes

Rats were allowed to fast for overnight and a single dose of STZ (dissolved in ice cold phosphate buffer of pH 6.8) at a dose of 60 mg/ kg bw i.p. has been administered to individual fasted rats. The blood glucose level was checked before and 72 h after STZ injection. The Glucometer was initially calibrated with maximum 660 mg/dL glucose concentration according to specification. The Blood is taken out of Rat's tail vein and immediately spread on the marked end of the Gluco-strip, which is the inserted in the Glucometer through the electrodes. After few seconds the glucometer displays the blood glucose level. Hyperglycemia was confirmed by the elevated glucose levels, determined at 72 h. The animals with blood glucose concentration more than 250 mg/dL were considered to be diabetics and used for the experiment

Experimental protocol for anti-diabetic activity [29, 30]

Optimized formulation of BS tablets and standard Glibenclimide samples were finely powdered and suspended in 0.1% w/v sodium carboxy methyl cellulose (SCMC). A suitable dose of each of BS tablet (eqv. to 200 mg/kg of B. Wt. & 500 mg/kg of B. Wt.) and standard dose of Glibenclamide (eqv to 5 mg/kg of B.Wt.) were daily administered orally by intragastric (i.g) route by using an intragastric tube to the STZ induced hyperglycemic rats. The stock suspensions were prepared in such a way that that daily dosage to be fed to the rats using the intragastric tube should not exceed beyond 1.0 /1.5 ml at a time.

Thirty rats were divided into five groups, (n=6). Rats were divided into following groups.

Group I: **Normal control**; Received only 0.1% SCMC 2ml/kg per oral.

Group II: **Disease control**; Received STZ 60 mg/kg (i.p) + 0.1% SCMC 2ml/kg per oral.

Group III: **Standard.** STZ 60 mg/kg (i.p) + Glibenclamide 10 mg/kg per oral.

Group IV: **Test 1**. STZ 60 mg/kg (i.p) + B.S. Tablets suspension (eqv 200 mg/kg of extract) pod.

Group V: **Test 2**. STZ 60 mg/kg (i.p) + B.S. Tablets suspension (eqv 500 mg/kg of extract) pod.

As expected, administration of STZ led to the elevation of Blood sugar level, which was maintained in the Disease (Diabetes) control Group II during the entire period of study and Group III animals are daily fed with standard Glibenclimide (suspended in 1% SCMC suspension) at a dose of 10 mg/kg of B. Wt. in each. Similarly STZ induced diabetic rats in Group IV & V received a daily dose of powdered tablets of B.S. (suspended in 1% w/v SCMC suspension) in dose of 200 mg / kg of B.W. (Group IV) & 500 mg / kg of B.W. (Group V) in each rat. The blood samples from the tail vein were collected and analyzed for blood glucose content at 0^{th}, 1^{st}, 3^{rd}, & 22^{nd} hr on the First day (single dose, short term study) and subsequently on 7^{th},14^{th} & 21^{st} day for comparative anti-hyperglycemic activity in multi-dose long term study.

RESULTS & DISCUSSION

Table 1: Composition of different Formulations:

Sl no.	Ingredient	Formulation Qty per Tab (mg)		
		BS 1	**BS 2**	**BS 3**
1	Dry whole plant Extract of B.S.	200	200	200
2	Spray dried Lactose	166	--	--
3	Microcrystalline cellulose pH102	134	134	300
4	Dicalcium phosphate	--	166	--
5	Sodium starch glycolate	50	25	--
6	Sodium bicarbonate	100	100	100
7	Cross-povidone	--	25	50
8	PVP K30	7	7	7
9	BHT	5	5	5
10.	Magnesium stearate	6.75	6.75	6.75
11	Aerosil 200	6.75	6.75	6.75
	Total weight (Theoretical)	675	675	675

Table 2: Micromeretic properties of pre-compression blend:

Batch Code	Bulk Density (g/ml)	Tapped Density (g/ml)	Angle of Repose (deg)	Carr's Index	Flow Properties	Hausner Ratio	Compressibility
BS 1	0.44	0.53	26.30	16.98	Fair/ Passable	1.20	Good/ Compact
BS 2	0.42	0.51	30.96	17.65	Fair/ Passable	1.11	Good/ Compact
BS 3	0.45	0.52	24.47	13.46	Good	1.15	Good/ Compact

Table 3: Evaluation data of the Compressed Tablets

Batch Code	Avg Wt [Mean±SD] (n = 10) (mg)	Hardness [Mean±SD] (n = 10) (Kg/cm2)	Friability [Mean±SD] (n = 10) (%)	Thickness [Mean±SD] (n = 10) (mm)	Disintegration Time [Mean±SD] (n = 10) (min)	Avg. Drug Content/tab (TFC activity eqv to mg of Quarcetin)
BS 1	0.672±0.12	3.72±0.07	0.34±0.02	3.7±0.05	27±0.51	1.816
BS 2	0.674±0.08	3.42±0.22	0.39±0.02	3.8±0.45	28±0.27	1.844
BS 3	0.675±0.05	3.31±0.43	0.41±0.02	3.9±0.25	20±0.34	1.858

Average weight of BS Tablets = 675.7 mg (Optimised Formulation BS 3)

(Each Tablet contains 200 mg of Dry extract of whole plant of *Biophytum sensitivum*)

TFC of each Tablet equivalent to (9.29*200/1000) = 1.86 mg Quarcetin /tablet

Table 4: Data for calibration curve of Quercetin, a standard Flavonoid

Sl no.	Concentration of Quarcetin (mcg/ml)	Absorbance at 415 nm
1	0	0
2	11.04	0.022
3	22.08	0.058
4	33.12	0.077
5	55.2	0.135
6	77.28	0.187
7	110.4	0.279

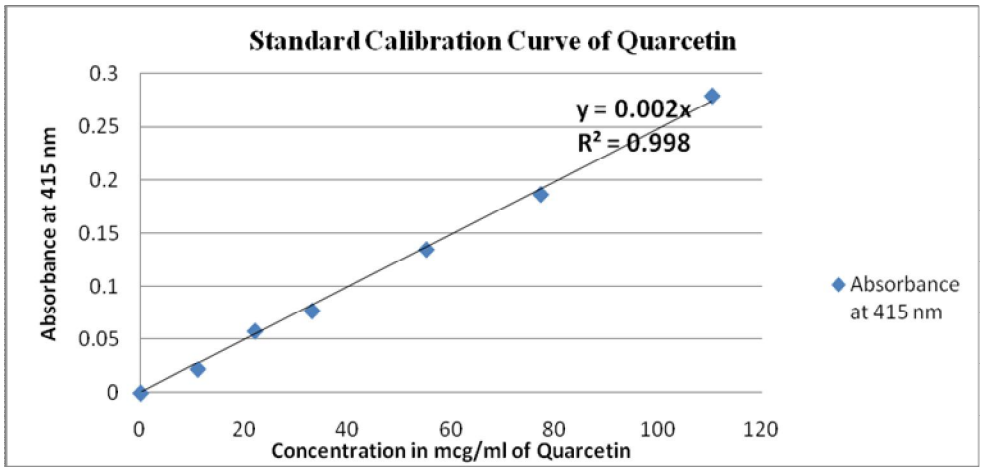

Fig. 1. Calibration Curve of Quercetin, a standard Flavonoid

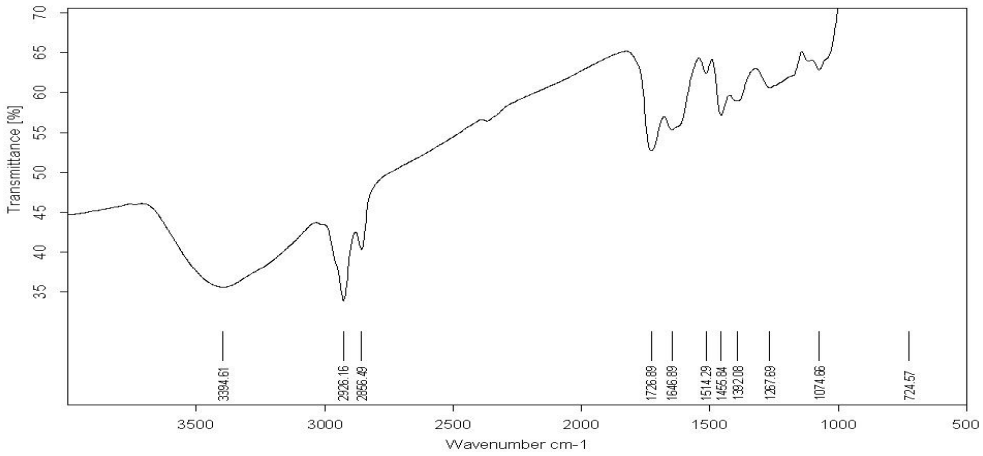

Fig. 2 FTIR spectra of Methanolic Extract of B. sensitivum whole plant

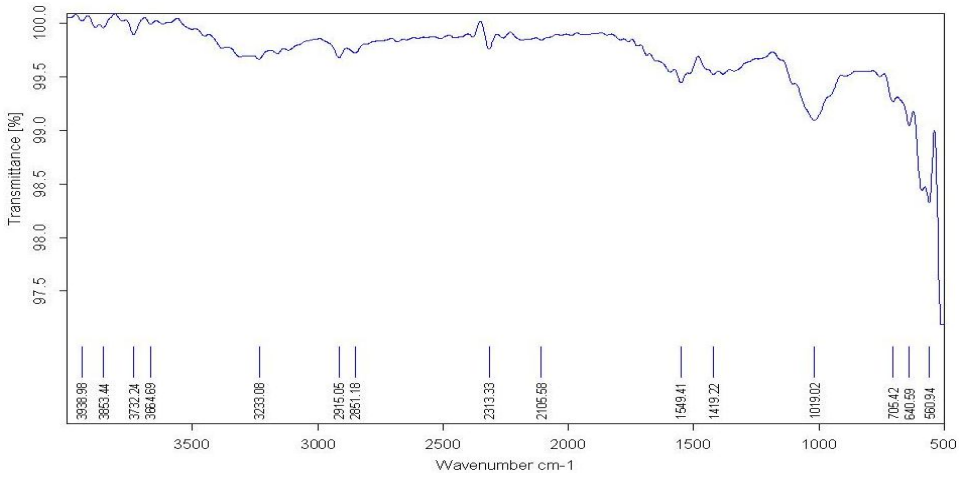

Fig. 3 FTIR spectra of pre-compression blend of BS tablets (batch BS 3)

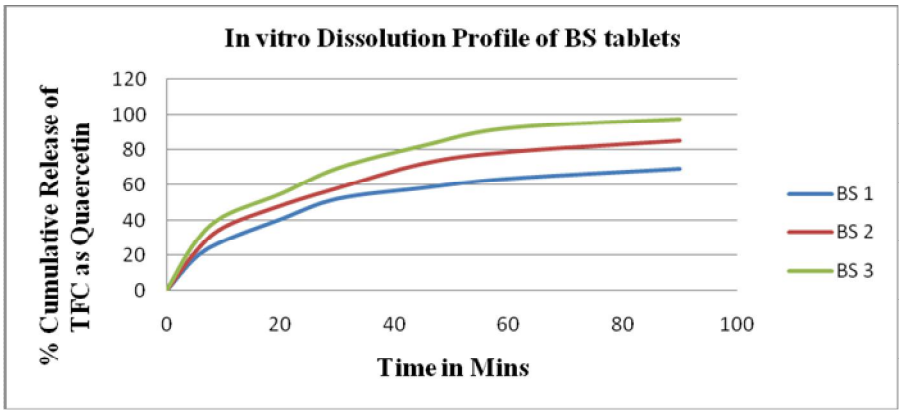

Fig. 4. In Vitro Dissolution Profile of BS tablets on the basis of TFC

Table 5: Data for TFC activity of BS Extract & BS Tablet

Concn of Dry BS Ext in Stock	**Concn of Dry BS Ext in diluted Samples**	**Absorbance at 415 nm**	**TFC activity of BS Ext eqv. to Quarcetin in Stock**	**Avg TFC activity of BS Ext in stock**	**TFC Activity of BS Ext (Eqv to mcg of Quarcetin per mg of Ext)**
(mcg/ml)	**(mcg/ml)**	**(nm)**	**(mcg Q/ ml)**	**(mcg Q / ml E)**	**(mcg Q / mg E)**
2014	0	0	0.00	19.17	**9.52**
	403	0.094	18.80		
	1007	0.243	19.44		
	2014	0.482	19.24		
Concn of BS Ext in Stock soln Powdered Tablets	Concn of BS Ext in diluted Samples	Absorbance at 415 nm	TFC activity of BS Ext eqv. to Quercetin in Stock soln of Powdered tabs	Avg TFC activity of BS Ext in stock soln of Powdered Tabs	TFC Activity of BS Ext (Eqv to mcg of Quarcetin per mg of BS Ext content in Tablet)
(mcg/ml)	(mcg/ml)	(nm)	(mcg Q/ ml)	(mcg Q / ml E)	(mcg Q / mg E)
3013.17	0	0	0.00	27.99	**9.29**
	301	0.0652	26.08		
	904	0.213	28.40		
	1507	0.358	28.64		
	2109	0.494	28.23		
	2411	0.545	27.25		
	3013	0.733	29.32		

Average weight of BS Tablets = 675.7 mg (Optimised Formulation BS 3)

(Each Tablet contains 200 mg of Dry extract of whole plant of *Biophytum sensitivum*)

TFC of each Tablet equivalent to (9.29*200/1000) = 1.86 mg Quarcetin /tablet

Table 6: Data for % Cum. Release of TFC as Quercetin in Dissolution Study

Time in Minutes	% CDR of TFC as Quercetin equivalent		
	BS 1	BS 2	BS 3
0	0	0	0
5	18.39	21.48	27.01
10	28.07	35.85	42.10
20	40.58	48.33	55.01
30	52.38	58.41	69.61
45	58.62	72.32	82.28
60	63.91	78.86	92.22
90	69.55	85.24	97.35

The Improved release pattern in BS 3 tablet Batch formulation, showing more than 85 % CDR within 60 minutes, might be attributed to Superdisintegrant (Crosspovidone) and increased amount of Microcrystalline Cellulose.

Table 7: Data for zero- order Dissolution Model: ($Q_t = Q_0 + K_0 t$)

Time in Minutes	**Initial Qty of TFC**	**Qty of TFC dissolved at time t**	**Zero order Rate Const**	
(t)	**Q_0**	**Q_t**	**K_0**	**R^2**
0	0	0		
5	0	27.01	5.40	
10	0	42.1	4.21	
20	0	55.01	2.75	
30	0	69.61	2.32	**0.5114**
45	0	82.28	1.83	
60	0	92.22	1.54	
90	0	97.35	1.08	

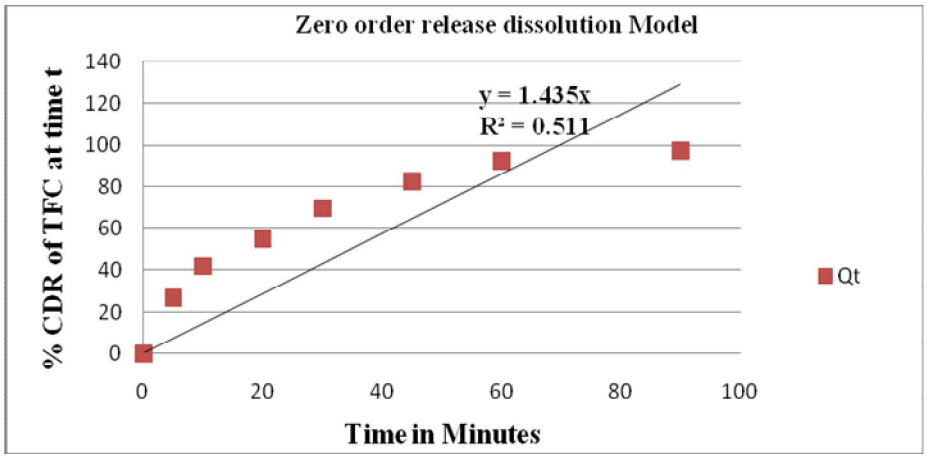

Fig 4. Zero order release Dissolution Model

Table 8: Data for First order Dissolution Model :($\ln Q_t = \ln Q_0 + K_1 t$)

(t)	log Q_0	log (Q_t - Q_0)	K_1	R^2
0				
5		1.43	0.66	
10		1.18	0.27	
20		1.11	0.13	
30		1.16	0.09	**0.8513**
45		1.10	0.06	
60		1.00	0.04	
90		0.71	0.02	

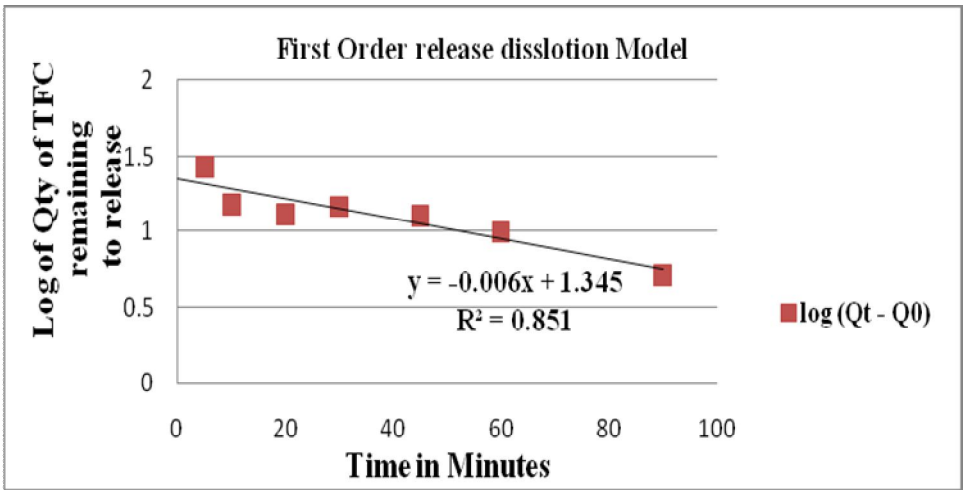

Fig. 5. First Order release Dissolution model:

Table 9: Data for Higuchi's Dissolution Model : (Qt = KH t1/2)

(t)	$t^{1/2}$	Q_t	K_H	R^2
0				
5	2.24	27.01	12.08	
10	3.16	42.1	13.31	
20	4.47	55.01	12.30	
30	5.48	69.61	12.71	**0.9366**
45	6.71	82.28	12.27	
60	7.75	92.22	11.91	
90	9.49	97.35	10.26	

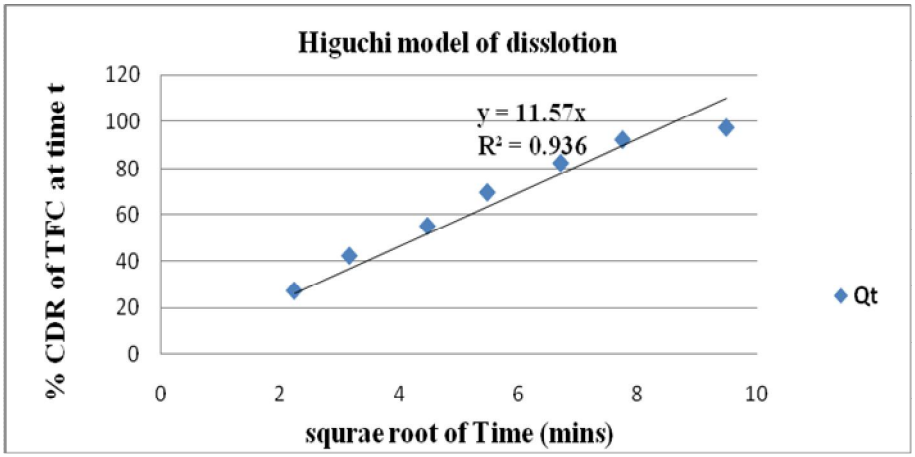

Fig. 6. Higuchi Model of Dissolution

Table 10: Data for Korsmeyer- Peppas Dissolution Model: (Q_t / Q_α = K t^n)

(t)	log t	log (Q_t / Q_α)	K	R^2
0				
5	0.70	-0.568		
10	1.00	-0.376		
20	1.30	-0.260		
30	1.48	-0.157	-7.10	**0.9806**
45	1.65	-0.085		
60	1.78	-0.035		
90	1.95	-0.012		

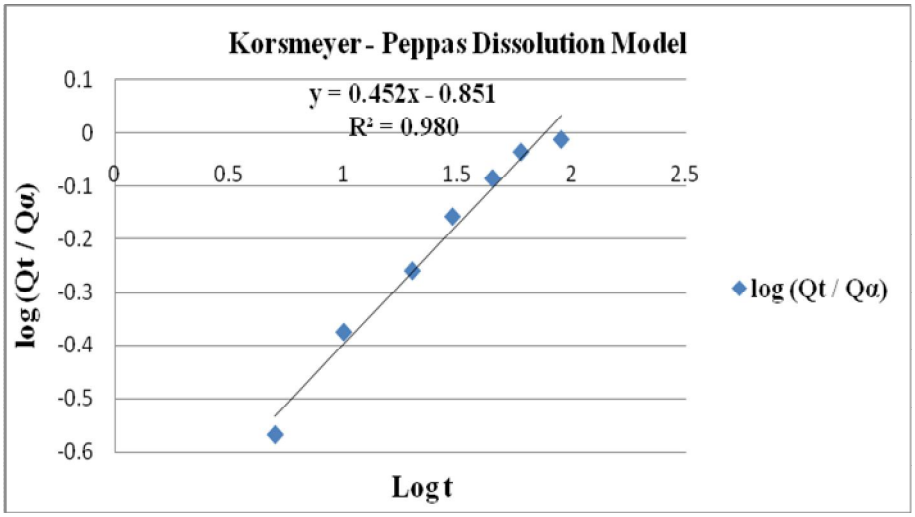

Fig. 7. Korsmeyer - Peppas Dissolution Model

Table 11. Release parameters of BS Tablets (Batch BS 3)

Zero Order		First Order		Higuchi		Korsmeyer-Peppas		
R^2	K_0	R^2	K_1	R^2	K_H	R^2	K	n
0.5114	2.32	0.8513	0.09	0.9366	12.71	0.9806	7.10	**0.45**

DISCUSSION OF DRUG RELEASE KINETICS

As shown in Fig. 3, 4, 5 and 6 - plots drawn, according to various kinetic models, were giving linear relationship. The best linearity was found in Korsmeyer - Peppas equation plot (with the maximum value of R^2 = 0.9806 and n = 0.45) indicating the release of TFC from the herbal matrix Tablets as a time dependent process based on Ficksian diffusion. By incorporating the first 60% of release data mechanism of release can be indicated according to Korsmeyer where n is the release exponent, indicative of mechanism of drug release by the usual molecular diffusion of the drug due to a chemical potential gradient. This has also been well supported by in Higuchi's equation plot (Fig. 5) (where R^2 = 0.9366) indicating the release of TFC from tablet matrix as a square root of time dependent process based on diffusion through pores in the matrix.

Table 12: Effect of Single dose administration of Powdered BS Tablets on Blood Glucose level (mg/dL) in STZ induced Diabetic Rats

(Short Term Single dose study till 22 hours) (Values are mean ± SE from 6 rats in each group, Figures in parenthesis indicates % fall in BGL as compared to Hour 0)

Group Treatment (Dose, mg/ Kg of B.W., PO)		Blood Glucose Level (mg/dL)				
		Hour 0	Hour 1	Hour 3	Hour 15	Hour 22
Group I (Normal Control)	1% w/v SCMC (Oral)	105.3 ± 6.43	104.7 ± 6.66 (0.63)	103.7 ± 7.57 (1.58)	103.3 ± 8.14 (1.90)	1033 ± 8.72 (2.22)
Group II (Diabetic Control)	STZ (IP) + 1% w/v SCMC (Oral)	304 ± 7.94	305.3 ± 8.14 (-0.44)	306.3 ± 8.14 (-0.77)	309.7 ± 6.66 (-1.86)	311.3 ± 3.00 (-2.41)
Group III (Standard)	STZ (IP) + Glib in 1% w/v SCMC (Oral)	289.3 ± 4.51	282.7 ± 3.06 (2.30)	278.7 ± 3.06 (3.69)	276 ± 3.00 (4.61)	274.7 ± 2.52 (5.07)
Group IV (Test 1)	STZ (IP) + Powdered BS Tab in 1% w/v SCMC (Oral Dose 200 mg/kg BW)	286 ± 4.00	285 ± 4.00 (0.35)	283 ± 3.06 (1.05)	280.3 ± 3.00 (1.98)	279 ± 2.52 (2.45)
Group V (Test 2)	STZ (IP) + Powdered BS Tab in 1% w/v SCMC (Oral Dose 500 mg/kg BW)	309.3 ± 3.06	307 ± 3.21 (0.75)	304 ± 2.65 (1.72)	300.7 ± 3.21 (2.80)	299.3 ± 3.79 (3.23)

Table 13: Effect of Multi dose administration of Powdered BS Tablets on Blood Glucose level (mg/dL) in STZ induced Diabetic Rats

(Long Term Single doe study till 21 days with daily once single dose) (Values are mean ± SE from 6 rats in each group, Figures in parenthesis indicates % fall in BGL as compared to Day 3)

Group Treatment (Dose, mg/ Kg of B.W., PO)		Blood Glucose Level (mg/dL)				
		Day 3	Day 5	Day 10	Day 15	Day 21
Group I (Normal Control)	1% w/v SCMC (Oral)	114.7 ± 1.53	114.3 ± 1.53 (0.35)	113.3 ± 1.53 (1.22)	113 ± 2.00 (1.48)	112.3 ± 2.08 (2.09)
Group II (Diabetic Control)	STZ (IP) + 1% w/v SCMC (Oral)	313.3 ± 2.08	305 ± 4.36 (2.65)	301 ± 3.46 (3.93)	272 ± 4.58 (13.18)	268.3 ± 4.51 (18.51)
Group III (Standard)	STZ (IP) + Glib in 1% w/v SCMC (Oral)	277.7 ± 3.21	253 ± 2.00 (8.89)	214.7 ± 4.51 (22.69)	177.7 ± 4.16 (36.01)	146.7 ± 2.08 (47.17)
Group IV (Test 1)	STZ (IP) + Powdered BS Tab in 1% w/v SCMC (Oral Dose 200 mg/kg BW)	290.3 ± 5.13	264.3 ± 4.04 (8.96)	231 ± 3.61 (20.43)	193.7 ± 2.52 (33.28)	158 ± 1.00 (45.57)
Group V (Test 2)	STZ (IP) + Powdered BS Tab in 1% w/v SCMC (Oral Dose 500 mg/kg BW)	279.7 ± 4.04	253.3 ± 4.73 (9.44)	214.3 ± 3.06 (23.38)	171.3 ± 6.51 (38.76)	138 ± 5.86 (50.66)

DISCUSSIONS

From the Table 12 & 13, it is evident that the blood glucose was elevated significantly in diabetic rats as compared with normal control rats after injection of STZ. In-vitro study revealed that the BS tablets prepared with methanolic whole plant extract of *B. sensitivum* was well tolerated in daily doses: 200 mg/kg to 500 mg/kg and was effective in type 2 diabetes as indicated by almost 45 % to 50 % reduction in blood glucose level within 21 days. At the end of experiment (the 21st day) blood glucose level was found (158.0 ± 1.00) and (138.0 ± 5.86) mg/dL at the doses of 200 and 500 mg/kg of BS Tablets respectively. In

diabetic rats, three weeks after treatment with daily dose of Glibenclamide (10 mg/kg of B. Wt) and BS Tablets (200 mg/kg B.Wt. & 500 mg/kg of B.Wt.) lowered the blood glucose level, by 47.17% (in case of Glibenclamide) and 45.57% and 50.66% respectively (in case of BS Tablets) as compared to the increased level of blood glucose level on the 3rd day after STZ injection, which is quite significant.

The deficiency of Insulin production in Type 2 Diabetes leads to rise in blood glucose level and persistent hyperglycemia is a major contributor to metabolic alterations that lead to pathogenesis of diabetic complications like testicular damage, micro vascular disease, and nephropathy. The Insulinotropic activity of B. sensitivum extract might be explained by the hypothesis that the activity of gluconeogenic enzymes present in glucose homeostasis are reduced by the polyphenols especially Flavonoids present in B. sensitivum extract, which by turn stimulates increased synthesis of Insulin from beta cells and enhances the Glycogenesis in STZ-induced diabetic Rats [33]. The other consideration in this respect is whether total whole plant's polyphenol intake is more important in regulating glucose homeostasis, in comparison to the intake of a single polyphenol in large quantities. The Bioactivity of Whole plant methanolic extract of B. sensitivum in the form of Pharmaceutical dosage form can be validated against traditional folk medicinal usage in case of Diabetes [34, 35, 36].

CONCLUSIONS

Despite promising data from *in vitro* and animal studies, the effects of polyphenols, especially Flavonoids on insulinotropic activity in humans have not been consistently established. Currently, there is a major imbalance between the published clinical studies on the benefits of polyphenols to human health and the marketing of pharmaceutical dosage forms containing Flavonoids. Further research in human beings should adopt robust randomized placebo-controlled study designs, and standard techniques to evaluate insulin sensitivity should be utilized where ever possible. Despite the paucity of robust data showing beneficial health outcomes associated with Flavonoid dosage forms in humans, these compounds already have a large commercial value. Further research in this area is urgently needed because Dosage forms formulated with Flavonoid to manage the diabetes pandemic are an exciting prospect.

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