

Original Article

FORMULATION DEVELOPMENT AND *IN VITRO* EVALUATION OF DENTAL GEL CONTAINING ETHANOL EXTRACT OF *TEPHROSIA PURPUREA* LINN.

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ABSTRACT

Objective: Dental infections are mainly caused by the colonization of anaerobic gram-negative pathogens and a challenge was taken to formulate a dental gel containing ethanol extract of the whole plant of *Tephrosia purpurea* Linn. (EETP) against dental diseases that are mainly caused by *Porphyromonas gingivalis*.

Methods: The defatted and soxhlated ethanol extract of the whole plant of *Tephrosia purpurea* Linn. was estimated for its flavonoid content and the antimicrobial study was performed on clinically isolated *Porphyromonas gingivalis* to determine the dose with a comparison to pure flavonoid to establish the presence of bioactive components. An attempt was made to prepare an herbal bio-compatible dental gel using cold mechanical method containing EETP with varied concentrations of carbopol-934P, HPMC-K₄M and chitosan and various characterization parameters were carried out.

Results: Based on flavonoid content and antimicrobial activity 2%w/v of the extract was used as the dose in all the EETP containing formulations. The pH, viscosity, gelling temperature, spreadability and swelling index of all formulations showed the suitable result. Mucoadhesive strength was found highest for the formulation with 4.0% HPMC-K₄M, which also resulted in the best flavonoid diffusion with highest permeation flux. All the EETP loaded dental gel formulations extruded satisfactorily through needle no-22. The antimicrobial study on clinically isolated *Porphyromonas gingivalis* showed higher antimicrobial inhibition as compared to the marketed metronidazole (1% w/v) gel. Stability study confirmed all the EETP loaded dental gel formulations to be stable after one month.

Conclusion: Therefore, Basing on all aspects of the study, The dental gel containing 4.0%w/w, HPMC-K₄M, 20%w/w PG with 1.0% w/w glycerol was found to be the best.

Keywords: *Tephrosia purpurea* Linn., Dental gel, Carbopol 934 P, HPMC-K₄M, Chitosan

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INTRODUCTION

Human kind's effort to comfort disease dates back to early civilization. From the beginning of advancement of life all cultures used natural sources like herbs as the primary health care management. There has been increasing attention paid in recent years to the oral infectious diseases and oral inflammations, in particular, infectious dental diseases. Dental infections, other wisely known as periodontitis, are the group of infections, predominantly caused by colonization of gram-negative, anaerobic pathogens on sub-gingival areas. Periodontitis can be characterized by; gum inflammation (with redness and bleeding), the formation of deep pocket (in between the teeth and gum) and loosen teeth (erosion of structure of connective tissue and bone).

Periodontitis found to eventuate due to contemporaneous gingivitis that results in connective tissue loss from the teeth [1]. Though the lesions found in both the periodontitis and gingival infections were histopathologically matched but these diseases are clinically different [2, 3]. Clinical symptoms in chronic gingivitis can be distinguished by redness and oedema formation at affected area [4], 1965, bleeding and contour change[5], an increase in gingival crevicular fluid and loosening of connective tissue [6]. According to folklore data, *Tephrosia purpurea* (Linn.) also known as "*Sarwa wran vishapaka*" has been found with all types of wound healing properties [7]. The ethanol extracts of *Tephrosia purpurea* Linn. was also reported to possess potential antibacterial activity [8, 9].

Basing on the above investigations, the investigation has been taken to prepare dental herbal gel formulations containing ethanol extract of *Tephrosia purpurea* Linn. and to evaluate them in terms of their physicochemical characteristics and *in vitro* drug release study.

As per U. S. P. gel is a semisolid or suspension containing small inorganic or large organic particles interpenetrated with liquid [10],

pharmaceutically act as carriers for other drugs for their localized percutaneous absorption [11] and having a good visco-elastic property due to the entrapment of solvent in the network like three-dimensional colloidal structure formed by gelling agent and solvent interaction [12].

Crude drug characteristics differ dramatically to induce the activity of drug at the site of action at sufficient concentration. A drug delivery system (DDS) allied of the carrier and the drug is elevated as a revolutionary to deliver the drug at the preferable site of action, at an accurate concentration at the accurate period of time [13]. Furthermore, obstacles arising from various side effects or toxicity of synthetic drugs can be overcome by using a potent herbal drug in the form of suitable drug delivery systems that is better patient-compatible with least side effect [12]. Substantially, the challenge to formulate a delivery system with increased therapeutic activity and least side effects may be overcome by perfect fabrication of novel drug delivery systems with a proper dose of the herbal extract [14].

MATERIALS AND METHODS

Materials

Plants of *Tephrosia purpurea* Linn. were collected and authenticated by Regional Plant Resource Centre, Bhubaneswar, Odisha. HPMC-K₄M and carbopol-934P was a gift sample from Wockhardt Ltd., Aurangabad, India. Chitosan and quercetin were purchased from Sigma, Poole, UK. Sheep Blood Agar Media was purchased from Hi-Media Laboratories Pvt. Ltd., Mumbai, India.

Methods

Preparation of plant extracts

500g of the drug powder was extracted with 3000 ml of ethanol (90%) for 24 h in soxhlet assembly. The filtered extract was dried by evaporating the solvent till a thick mass was obtained [15].

Analytical method for quantification of flavonoid

An aliquot of given concentrations of stock solution (quercetin) was scanned from 350-400 nm wavelength range in a UV-Visible spectrophotometer (Jasco V-630 Spectrophotometer, Japan, Serial No-A1259611487). From the scanning report, it was evident that the wavelength of maximum absorbance (λ_{max}) of Flavonoid was found at 421 nm (fig. 1).

Aliquots of 0.1, 0.2, 0.3, 0.4, and 0.5 ml from the above stock solution were taken in 5 different 10 ml volumetric flasks. To each flask, 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 was added. The reaction mixture was kept aside at room temperature for 30 min and the volume was made up with water. The absorbance of the resulting different concentration of solutions was measured against reagent blank. The calibration curve was prepared by plotting absorbance vs concentration, and it was found to be linear over this concentration range of 10-50 μ g/ml (fig. 2).

0.1 g of extract was dissolved in 100 ml of ethanol to get 1 mg/ml solution. Required volume of the above solution (1 ml) was transferred into a 10 ml standard flask and colour development was carried out as that for the standard by adding 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. From this 1 ml solution was taken and diluted up to 5.5 ml with distilled water. The absorbance of the test solution was measured at 421 nm against blank. Total flavonoid content was calculated in terms of percentage of quercetin equivalent per 100g [16].

Anti-gingival activity study and dose determination of EETP in comparison with pure flavonoid

The link between oral diseases and the activities of microbial species that form part of the microbiology of the oral cavity is well established and over 750 species of bacteria inhabit the oral cavity and a number of these are implicated in oral disease [17]. Out of various oral micro floras, clinically isolated *Porphyromonas gingivalis* were collected from Institute of Dental Sciences, Siksha'O' Anusandhan University, Bhubaneswar and used for the antimicrobial study. The stains were streak on sheep blood agar media in which columbia agar base (4.25%) was supplemented with sheep blood (5%), bacitracin (1%), colistin (1.537%), nalidixic acid (1.5%), hemin (0.5% of 0.1% solution) and vitamin K₁ (0.1% of 0.1% solution) [18] and kept in an anaerobic condition of 10% hydrogen, 10% carbon dioxide and 80% nitrogen at 37 °C for 48hrs [18]. After 48 h the colony of the both pathogens was formed on the sheep blood agar plates and showed haemolysis effect on sheep blood showed in fig. (fig. 3). To determine the antimicrobial activity and the dose with a comparison to standard 1% metronidazole, the brain heart infusion medium was prepared by dissolving the same in distilled water. Then sample from columbia agar media was taken in brain-heart infusion broth (Oxoid) supplemented with 5 mg/l haemin (Sigma, Poole, UK), and incubated for 24 h. Ten percent inoculum was transferred to 9 ml of the same medium and incubated for 3 d. All cultures were allowed to grow at 37 °C in the above mentioned anaerobic condition.

Then 1 ml from the inoculums was added to 8 ml of the same concentration of brain-heart infusion broth (Oxoid) in six separate test tubes and numbering was done like PF₂, PF₃, PF₄, PF₅, PF₆, PF₇. And in one test tube, 1 ml of inoculum in 9 ml medium was taken and called as a positive control (PF₁). In PF₂ 1 ml of 1% w/v concentration of standard metronidazole is added and taken as standard. In other five test tubes 1 ml of aqueous solution of extract in concentration of 0.010 gm/ml, 0.015 gm/ml, 0.020 gm/ml, 0.025 gm/ml, and 0.030 gm/ml was added accordingly and again kept overnight at 37°C in incubator maintaining anaerobic condition in the similar manner described above. Then optical density was measured. Results were tabulated in fig. (fig. 4) showing the minimum concentration of extract that shows the satisfactory antimicrobial activity.

To establish the bio-active compounds with a comparison to standard quercetin (PF₈) of the same concentration as that of the optimized concentration of extract was tested against *P. gingivalis* by following the above-mentioned method (fig. 4).

Formulation of various EETP loaded dental gel formulations

Different concentration of carbopol-934P and HPMC K₄M containing gel was formulated by modified cold mechanical process [19, 20]. Finally, 0.2%w/w ethanol extract of *T. purpurea* Linn. was added with continuous stirring.

Chitosan gel was prepared by following dispersion technique; glycerol (5%w/w) and glacial acetic acid (3%w/w) was added with continuous mixing using a mortar and pestle followed by addition of the 0.2%w/w ethanol extract of *T. purpurea* Linn.

All the formulations were stored in a screw capped wide mouthed beaker covered with aluminium foil in a cool and dark place [22, 23].

Characterizations of various EETP loaded dental gel formulations

pH determination

Digital pH meter was used to determine the pH of the formulations by dipping the electrode in 25 ml of distilled and deionized water containing 2g of formulated EETP loaded dental gel. The pH measurements were performed three times, and the average was noted as the result depicted for each formulation [24, 25].

Viscosity determination

Viscosity determination was carried out using Brooke-field viscometer (TV-10) connected with a thermally-controlled water bath to maintain the temperature at 37 °C. Prior to each experiment, the viscometer was equilibrated with the sample for 10 min and at a shear rate of 20rpm. The viscosity measurements obtained was the average of three readings for each formulation (n = 3).

Gelation temperature measurement

Gelation temperature was determined by following tube tilting method [26]. In this method 2g of aliquots of formulated gel was transferred to a test tube covered with aluminium foil and immersed in a temperature controlled water bath at 4°C. The samples were heated by increasing the temperature of water bath by 1°C till there was stable meniscus on 90°tilting of the test tubes after equilibration for 5 min at every 10°C temperature increased. The data depicted was the average of three readings for each formulation (n = 3).

Swelling index studies

Swelling index studies were performed at 37°C using a thermo jacketed cell containing phosphate buffer saline pH 6.8 as swelling medium. About 2g of EETP loaded dental gel was taken and the study was carried out for 6 h. After 6 h, the gels were scrapped from aluminium foil and the weight was determined by removing the adhered water from the surface by blotting immediately. The swelling Index of the dental gel forming polymer was determined using the relationship [27];

$$\%St = \frac{(W_t - W_o) \times 100}{W_o}$$

Where 'St' represents the swelling of the dental gel at time 't'.

W₀ and W_t represent the initial and final weight of gelling solution and gel respectively.

All measurements were performed in triplicate (n=3) and the data noted is the average of the three readings.

Determination of spreadability

Spreadability study was performed by determining the "Slip" and "Drag" characteristics of EETP loaded dental gel [28] using a wooden block consisting of a pulley at one end. The formulations were kept on one slide and sandwiched by another slide across a length of 6 cm among the two slides. A uniform thin layer was formed by placing a 100 gm weight upon the upper slide. Then the weight was removed, and the adhered excess formulation to the slide was scraped off and a 20 gm loaded pulley was used to slip the upper slide to 6 cm distance and to separate away to the direction of applied force from the slide fixed on the board of the apparatus at the bottom. The time

taken for complete separation of the two slides was recorded. The experiment was repeated for three times and the average was noted for each formulation. A shorter interval indicates better spreadability [29].

$$\text{Spreadability} = m \times l/t$$

Where, 'm' represents the weight tied to the upper slide (20 gm)

'l' represents the length of glass slide (6 cm)

't' represents the time taken to separate the upper slide from the lower slide

Mucoadhesive strength determination

Mucoadhesive strength determination was done to study the mucoadhesive characteristics of prepared EETP loaded dental gel by following the method developed by Choi *et al.* and Yong *et al.* [30, 31]. The collected sheep cheek mucosal tissue was cleaned with distilled water followed by phosphate buffer saline pH 6.8 which is isotonic to the tissues and allowed to stand for sometimes at the same pH to remove all soluble components. After confirmation of integrity of the treated sheep cheek mucosal tissues by microscopic investigation [32], 2g EETP loaded dental gel was made sandwiched in between the two slides using cyanoacrylate adhesive and the mucoadhesive force of the formulated EETP loaded dental gel was determined in terms of the detaching force in the weight of water required to separate the glass plates.

In vitro release study

In vitro release studies of EETP loaded dental gels were carried out by using sigma dialysis membrane (MWCO-3500) bag. In this study, the membrane to be used was soaked overnight in phosphate buffer saline solution of pH 6.8 to allow the pores to open. Then the soaked membrane was filled with 2 gms of the EETP loaded dental gels and kept submerged in a beaker containing 60 ml of phosphate buffer saline pH 6.8 at 37±1°C temperature on a magnetic stirrer with continuous stirring at 100rpm to maintain the sink condition of the receptor medium. The samples were withdrawn at specific time intervals from the aliquot reservoir and the same volume was replaced with similar pre-warmed aliquot. The samples were assayed spectrophotometrically to quantify the amount of flavonoid at 421 nm released through the membrane.

Drug release kinetic study

The release kinetics of the flavonoid as active constituents from EETP loaded dental gel was resolved on the graphical plotting of *in vitro* release data in different models of drug release kinetics; Zero order kinetic model (Equation 1) as percentage of drug (cumulative amount) released in time, First order kinetic model (Equation 2) as log cumulative percentage of drug released in time and Higuchi kinetic model (cumulative percentage of drug released in square root of time) (Equation 3).

$$C = k_0 t \text{-----} \text{(equation 1)}$$

Where k_0 represented zero order rate constant in a unit of concentration/time,

t represented as time in hrs.

Zero order graph yield a straight line with a slope of k_0 and intercept the origin of the axis [33].

$$\log C = \log C_0 - kt/2.303 \text{----} \text{(equation 2)}$$

Where, C_0 represents the initial concentration of drug,

k represented first order rate constant and t represents time in hrs [34].

$$Q = kt^{1/2} \text{-----} \text{(equation 3)}$$

Where K represents the constant for the design variables of the system,

t represents time in hrs.

Hence the rate of drug release was found to be proportional to the reciprocal of the square root of time [35].

Hixon-Crowell cube root law (equation 4) data plot used to describe the drug released with surface area changed in terms of the diameter of the particle and plotted as the cube root of the percentage of drug remaining in the matrix in time [36].

$${}^3\sqrt{Q_0 - Q_t} = K_{HC} t \text{-----} \text{(equation 4)}$$

Where, Q_0 represented as the initial amount of the drug in formulation,

Q_t represented as the amount of drug released in time t in hr,

K_{HC} represented as Hixon-Crowell rate constant [36].

Exponential drug release to the elapsed time was simply explained by Korsmeyer *et al.* [37] to describe the drug release by plotting as the log cumulative percentage drug release in log time (equation 5) and the exponent 'n' was calculated from the slope of the linear graph [37].

$$Q/Q_0 = Kt^n \text{-----} \text{(equation 5)}$$

Where, Q/Q_0 represents the fraction of drug released at time t,

K represents the constant comprising the geometrical structural characteristics of drug/polymer system n represents the exponent of the drug released [37].

The diffusion exponent describe the diffusion model in a semi-empirical way as $n \leq 0.5$, the Fickian diffusion model describe the release mechanism and the non-Fickian model of diffusion described the drug release mechanism as the release of the drug follow both the diffusion and the polymer dissolution mechanism where release rate is inversely proportional to polymer concentration when $0.5 < n < 1.0$. The decrease in the release constant allows long time hydration resulting stretching of the polymer matrix which causes a decrease in rate of drug release. If n was found to be 1, the release kinetic would have been following the zero-order models and if n was found to be greater than 1, then the release mechanism would have been super case-II transport, the model that is considered when unknown release mechanism or different mechanisms control the drug release [37].

The different kinetic model describes drug release from a controlled release preparation by using different mathematical equations like; zero order diffusion model (describe the concentration independent drug release) [33] and first order diffusion model (describe the concentration dependent drug release) [34].

In accordance with Higuchi model of drug release from the polymer bed is directly proportional to the square root of time and hinge on Fickian type of diffusion [35]. But according to Hixon-Crowell cube root law, the diameter of the particle changes with time which dissolve or erode over time [36]. The values of the release exponent (n), and correlation coefficient (R^2) can be calculated from all kinetic models.

In vitro permeation study

Preparation of mucosal tissue

Sheep cheek mucosa was collected from a nearby local slaughter house. A section of mucosal tissue was cut from the sheep cheek mucous membrane and was thoroughly washed with distilled water followed by isotonic phosphate buffer pH 6.8 then soaked in phosphate buffer saline pH 6.8 to remove all the soluble components [32].

Measurement of thickness of sheep cheek mucosal membrane

The sheep cheek mucus membrane was stained with haematoxylin-eosin and thickness measurement was done microscopically. Initially, the paraffin was melted at 65-70 °C to fill into the steel mould. Then at the bottom of the steel mould, the sheep cheek mucosa was placed to cut the section to a thickness of 5-7µm by placing the wax block in the microtome holder after detaching the steel mould by gentle heating followed by cooling. Then the cut

section was transferred to a glass slide and stained with haematoxylin-eosin for 30 min followed by washing with isopropyl alcohol and finally with running water. Then air dried stained mucosa was placed under a microscope to measure the average thickness of the sheep cheek mucosa which was found to be 1.4×10^6 cm.

In vitro permeation determination

In vitro permeation of active constituent of EETP loaded dental gel was determined by Keshary-Chien (KC) diffusion cell containing 25 ml of pH 6.8 phosphate buffer [38, 39] using 2.0 ml of EETP loaded dental gel sheep cheek mucosal membrane. The samples were assayed spectrophotometrically to quantify the amount of flavonoid at 421 nm released through the membrane. The released drug percentage (cumulative) at every interval of time was calculated from the average of three determinations [40, 41] and plotted as cumulative amount drug release (Q) in time (t).

Data analysis of permeation studies of EETP loaded dental gel

The permeation flux at steady state was made out from the linear portion of the curve of the cumulative amount active medicament permeating from unit skin surface area (Q) in time (t) and the curve was extrapolated to abscissa to determine the lag time (L_t). The partition coefficient of flavonoid content in the formulation contained extract of *T. purpurea* Linn. was calculated as described by following equation [42].

$$\text{Partition coefficient} = \frac{C_s - C_{eg}}{C_{eg}} \times \frac{1000}{W_e}$$

Where, C_s signified as the initial flavonoid concentration in aliquot, C_{eg} as equilibrium flavonoid concentration and W_e represented as the mucous membrane weight taken for study.

Reckoning of Permeability coefficient (P) was assessed from derived relation from the first law of diffusion by Fick and expressed by the following equation [43].

$$P = \frac{\text{steady state flux (J)} \times \text{thickness of skin (h)}}{\text{concentration of drug (C)}}$$

Reckoning of Diffusion Coefficient (D) was assessed from derived relation from Fick's second law using equation [44]:

$$D = h^2/4t$$

Where, h and L_t signified as the thickness of skin and lag time respectively.

Extrudability study

Extrudability study of the formulated EETP loaded dental gels were performed basing upon the weight required to extrude minimum of 0.5 cm ribbon of gel in 10 sec in the percentage of weight from the lacquered aluminum collapsible tube. More the quantities of the weight of gel extrude, the better will be the extrudability. The extrudability of each formulation was measured in triplicate and average values were calculated from the following formula; [45]:

$$\text{Extrudability} = \frac{\text{weight applied to extrude gel (gm)}}{\text{area of applied force (cm}^2\text{)}}$$

Syringeability

Syringeability test was tested with the help of no-22 by inserting one gram of formulation in the syringe and it was pressed to check whether it is passing through it or not.

In vitro antimicrobial activity

In vitro antimicrobial study of the EETP loaded dental gel on clinically isolated *Porphyromonas gingivalis* and comparison of antimicrobial efficacy with marketed 1% metronidazole gel was carried out. Ten percent pathogenic inoculum was transferred to 9 ml of the same medium and incubated for 3 d. All cultures were grown at 37 °C in above mentioned anaerobic condition. Then 1 ml inoculum from that liquid media was added to 8 ml of the same concentration of Brain-Heart infusion broth (Oxoid) in seven

separate test tubes for each pathogen and numbered as PGF₁X₁, PGF₁X₂, PGF₂X₁, PGF₂X₂, PGF₃X₁, PGF₃X₂ and PGM as a negative control. The test tube containing 1 ml inoculum in 9 ml medium for each pathogen is called as a positive control and named as PGB. In PGB 1g of marketed metronidazole, gel solution was added and taken as standard. On the other hand 1g of 2.5%w/v solution of formulated EETP loaded dental gels i.e. DF₁X₁, DF₁X₂, DF₂X₁, DF₂X₂, DF₃X₁, DF₃X₂ were added respectively to the test tubes namely PGDF₁X₁, PGDF₁X₂, PGDF₂X₁, PGDF₂X₂, PGDF₃X₁, PGDF₃X₂ and again kept overnight in 37°C in incubator maintaining anaerobic condition in an above-mentioned manner. Then optical density was measured and noted (fig. 10).

Stability study

All the formulated EETP loaded dental gels were tested for stability by storing at 40°C/75% RH for one month and observed for any change in pH, viscosity, swelling index, mucoadhesive strength, spreadability, drug content and drug release after one month. During storage, the proper storage condition was maintained by storing in clean, dry, airtight containers and keeping away from light [46].

RESULTS AND DISCUSSION

Quantification of flavonoid

From scanning graph, the flavonoid absorbance was found to be 0.182 at 421 nm (fig. 1) and a standard curve was shown in fig. 2. The total quantification count was calculated as 51.17 mg QE/gm.

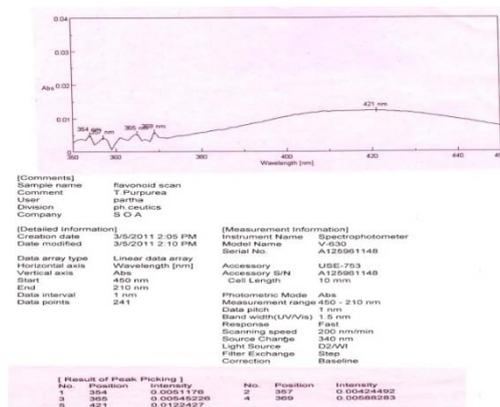


Fig. 1: UV Scanning of flavonoids

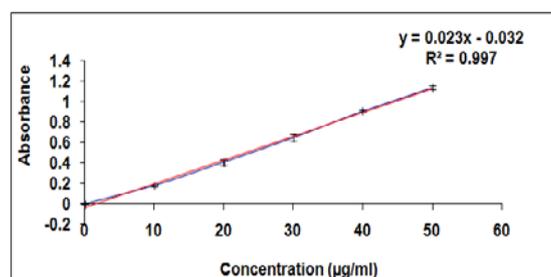


Fig. 2: Standard curve of quercetin

Anti-gingival activity study and dose determination of EETP

From the fig. (fig. 3) the presence of black colour colony and haemolysis on sheep blood agar medium confirms the presence of *P. gingivalis* [47]. From the optical density study, it is found that +ve control (PF₁) showed the optical density of 0.416 ± 0.02 , standard (PF₂) showed OD of 0.095 ± 0.04 and the 0.020 gm/ml i.e. PF₅ shows the activity nearer to the standard and OD was found 0.091 ± 0.02 . So this concentration (0.020 gm/ml) was optimized to be used as the

concentration of extract to be used in all the EETP dental gel formulations (fig. 4.).



Fig. 3: Black colony and haemolysis effect of *P. gingivalis*

Formulation of various EETP loaded dental gel formulations

Basing on the quantification data, 0.2%w/v ethanol extract of *Tephrosia purpurea* Linn. was used for the development of dental gel formulations using varying concentrations of carbopol 934P [0.35% (DF₁X₁) and 0.40% (DF₁X₂)], HPMC-K₄M [3.5% (DF₂X₁) and 4.0% (DF₂X₂)] and Chitosan [4.0% (DF₃X₁) and 4.5% (DF₃X₂)](table 1).

Characterizations of various EETP loaded dental gel formulations

DF₁X₁, DF₁X₂, DF₂X₁, DF₂X₂, DF₃X₁ and DF₃X₂ all the formulations showed there was no alteration in pH i.e. pH 6.8.

The viscosity of various placebo dental gel formulations is directly proportional to the concentration of polymer but inversely proportional with the concentration of propylene glycol. Gelation temperature decreased with the increasing concentration of polymer but slightly increased with increased propylene glycol concentration.

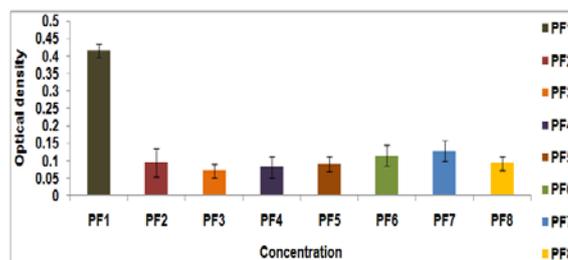


Fig. 4: Antibacterial activity of different plant extracts on *P. gingivalis*

Values are mean±SEM of 3 replications, PF₁-positive control, PF₂-standard control, PF₃-0.010 gm/ml of extract, PF₄-0.015 gm/ml of extract, PF₅-0.020 gm/ml of extract, PF₆-0.025 gm/ml of extract, and PF₇-0.030 gm/ml of extract

For DF₁X₁, DF₁X₂, DF₂X₁, DF₂X₂, DF₃X₁ and DF₃X₂ viscosity was found 3718±2.09mpa s, 4242±2.33mpa s, 3641±1.99mpa s, 4024±2.05mpa s, 3425±2.31mpa s, and 3991±2.09mpa s (fig. 5). This may be due to the change of mechanical property of polymer [48].

Table 1: Composition of various optimized EETP loaded dental gel formulations

S. No.	Composition (% W/W)	DF ₁ X ₁	DF ₁ X ₂	DF ₂ X ₁	DF ₂ X ₂	DF ₃ X ₁	DF ₃ X ₂
1	Extract	0.2	0.2	0.2	0.2	0.2	0.2
2	Carbopol-934P	0.35	0.40	--	--	--	--
3	HPMC-K4M	--	--	3.5	4.0	--	--
4	Chitosan	--	--	--	--	4.0	4.5
5	Acetic acid	--	--	--	--	4.5	4.5

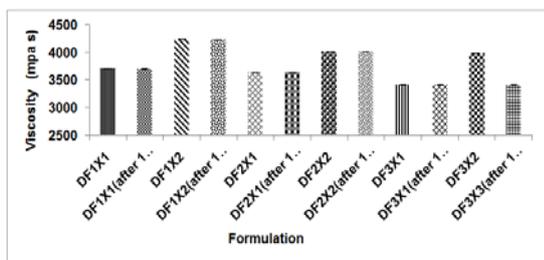


Fig. 5: Viscosity of various EETP loaded dental gel formulations

Values are mean±SEM of 3 replications, DF₁X₁ and DF₁X₂- formulation contain Carbopol-934P 0.35% and 0.40%, DF₂X₁ and DF₂X₂-formulation contain HPMC-K₄M 3.5% and 4.0%, DF₃X₁ and DF₃X₂-formulation contain Chitosan 4.0% and 4.5%

At higher gelling temperature the dental gel formulations may be in sol form within the dental cavity and at lower gelling temperature the dental gel formulations will be in gel form. The dental gel formulations in sol form possess the property of flowing out or wash out easily. So when the dental gel formulations are in sol form the residence time within the dental cavity is less and release will be less, so bioavailability is less as at 37 °C, the behaviour of the formulations changed, depending on the polymer concentration. The mono-molecular micelle forms at a lower concentration of polymer and poly-molecular micelle forms at high polymer concentration and micelle come closer to minimize their interaction with water cause the mechanical and molecular arrangement change [47]. For DF₁X₁, DF₁X₂, DF₂X₁, DF₂X₂, DF₃X₁ and DF₃X₂ gelling temperature was found to be 37.04±0.51°C, 36.55±0.51°C, 37.03±0.34 °C, 36.46±0.76 °C, 37.05±0.42 °C, 36.61±0.61°C (fig. 6).

Swelling index of DF₁X₂ i.e. 45.06±0.41% was found more than the swelling index of DF₁X₁ i.e. 42.42±0.65%. Swelling index of DF₂X₂ i.e. 41.58±0.58% was found more than swelling index of DF₂X₁ i.e. 40.65±0.14%. Swelling index of D₃X₂ i.e. 43.32±0.54% was found more than swelling index of DF₃X₁ i.e. 39.86±0.54 % (fig. 7).

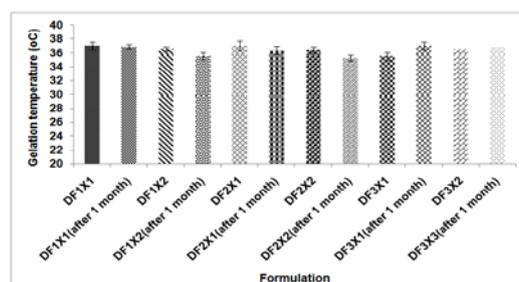


Fig. 6: Gelation temperature of various EETP loaded dental gel formulations

Values are mean±SEM of 3 replications, DF₁X₁ and DF₁X₂- formulation contain Carbopol-934P 0.35% and 0.40%, DF₂X₁ and DF₂X₂-formulation contain HPMC-K₄M 3.5% and 4.0%, DF₃X₁ and DF₃X₂-formulation contain Chitosan 4.0% and 4.5%

Spreadability of DF₁X₂ i.e. 25.21±0.58 gm. cm/sec, was found more than the spreadability of DF₁X₁ i.e. 23.92±0.18 gm. cm/sec. spreadability of DF₂X₂ i.e. 26.12±0.67 gm. cm/sec was found more than spreadability of DF₂X₁ i.e. 24.47±0.95 gm. cm/sec. Spreadability of DF₃X₂ i.e. 24.85±0.35 gm. cm/sec, was found more than spreadability of F₃X₁ i.e. 24.05±0.92 gm. cm/sec. (fig. 8).

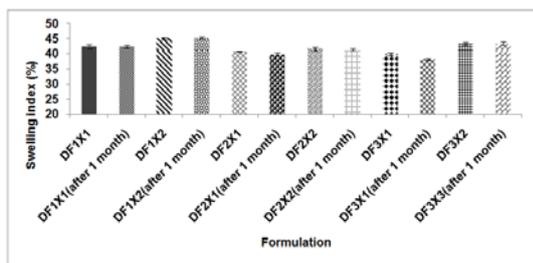


Fig. 7: Swelling index of various EETP loaded dental gel formulations

Values are mean±SEM of 3 replications, DF₁X₁ and DF₁X₂-formulation contain Carbopol-934P 0.35% and 0.40%, DF₂X₁ and DF₂X₂-formulation contain HPMC-K₄M 3.5% and 4.0%, DF₃X₁ and DF₃X₂-formulation contain Chitosan 4.0% and 4.5%

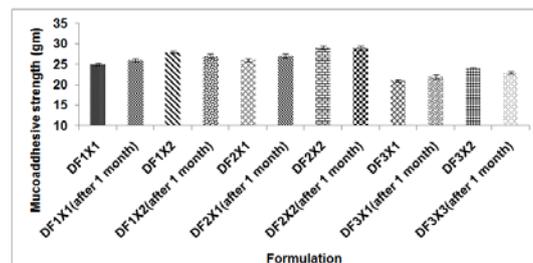


Fig. 9: Mucoadhesive strength of various EETP loaded dental gel formulations

Values are mean±SEM of 3 replications, DF₁X₁ and DF₁X₂-formulation contain Carbopol-934P 0.35% and 0.40%, DF₂X₁ and DF₂X₂-formulation contain HPMC-K₄M 3.5% and 4.0%, DF₃X₁ and DF₃X₂-formulation contain Chitosan 4.0% and 4.5%

The mucoadhesive strength of DF₁X₂ i.e. 28±0.32 gm was found more than mucoadhesive strength of DF₁X₁ i.e. 25±0.21 gm. Mucoadhesive strength of DF₂X₂ i.e. 29±0.43 gm was found more than that of DF₂X₁ i.e. 26±0.33 gm. Mucoadhesive strength of DF₃X₂ i.e. 24±0.21 gm was found more than F₃X₁ i.e. 21±0.24 gm (fig. 9). This can lead to a strong adhesion which may lead to the prolong drug release as more the mucoadhesive strength less would be the washout effect of the formulation, hence of active pharmaceutical ingredient.

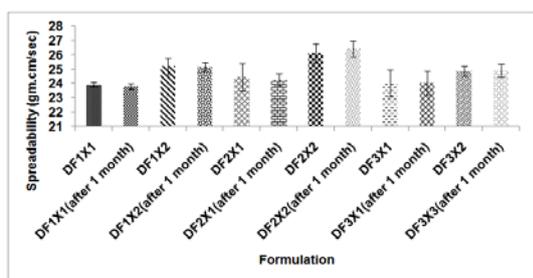


Fig. 8: Spreadability of various EETP loaded dental gel formulations

Values are mean±SEM of 3 replications, DF₁X₁ and DF₁X₂-formulation contain Carbopol-934P 0.35% and 0.40%, DF₂X₁ and DF₂X₂-formulation contain HPMC-K₄M 3.5% and 4.0%, DF₃X₁ and DF₃X₂-formulation contain Chitosan 4.0% and 4.5%

In vitro release study of various EETP loaded dental gel formulations

The cumulative percentage drug release in the formulation DF₁X₁ and DF₁X₂ was found to be 94.043±0.379 and, 94.514±0.211 respectively after 8 hr. The drug release from gel has sustained the drug more than 7 hr. Formulation DF₁X₁ liberated 63.559±0.369% of its drug content within 2.0 hr. formulation DF₁X₂ liberated 63.090±0.131 of its drug content in 2.0 hr. [fig. 10 (a)]

The cumulative percentage drug release in the formulation DF₂X₁ and DF₂X₂ was found to be 97.276±0.326 and, 97.435±0.078 respectively after 8hr. The drug release from gel has sustained the drug more than 7 hr. Formulation DF₂X₁ liberated 65.886±0.662% of its drug content within 2.00 hr; formulation DF₂X₂ liberated 62.595±0.317 of its drug content in 2.0hr. [fig. 10 (a)]

The cumulative percentage drug release in the formulation DF₃X₁ and DF₃X₂ was found to be 93.697±0.090 and 93.719±0.130 respectively after 8hr. The drug release from gel was sustained the drug more than 7hr. Formulation DF₃X₁ liberated 63.051±0.111% of its drug content within 2.0 hr; formulation DF₃X₂ liberated 63.666±0.185% of its drug content within 2.0hr. [fig. 10 (a)]

From *in vitro* drug release study it was obtained that the drug release of DF₁X₁, DF₁X₂, DF₂X₁, DF₂X₂, DF₃X₁ and DF₃X₂ dental gel were found to be the best which can be explained by First order release kinetic as the plot produced the highest linearity (R²= 0.9811, 0.9832, 0.9762, 0.9749, 0.9749 and 0.9784 respectively) [Table 2, fig. 10 (b)].

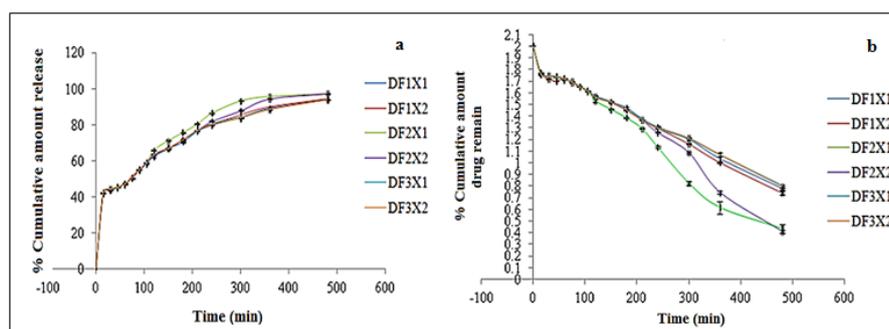


Fig. 10 (a): Zero order plot of various EETP loaded dental gel formulations, (b): First order plot of various EETP loaded dental gel formulations

Values are mean±SEM of 3 replications, DF₁X₁ and DF₁X₂-formulation contain Carbopol-934P 0.35% and 0.40%, DF₂X₁ and DF₂X₂-formulation contain HPMC-K₄M 3.5% and 4.0%, DF₃X₁ and DF₃X₂-formulation contain Chitosan 4.0% and 4.5%

The result evidenced that the rate of drug release was concentration dependent. However, the drug release kinetic was found well matched with Higuchi Model release kinetic from the curve; indicating the release of the drug increased with the square root of time [35,36]. The log cumulative percentage drug released in log time plotted for Korsmeyer-Peppas equation also demonstrate a good linearity with anomalous kinetic (R²= 0.9436, 0.9490, 0.8348, 0.8343, 0.8280 and 0.8252 respectively). The 'n' value was found

greater than 0.5 confirmed the release of flavonoid from all EETP loaded dental gels followed non-Fickian Diffusion Kinetic [table 2, fig. 10 (e)]. Hence, the process of drug release includes two steps, first, step supposed to be the swelling of gel and second step may include the overall diffusion of the active component [48, 49]. However, the data obtained from drug diffusion study confirmed that DF₂X₁ and DF₂X₂ dental gel formulations are the best amongst the rest.

Table 2: Drug release kinetics of various EETP loaded dental gel formulations

Formulation	Kinetic model					Hixson-Crowell	
	Zero order R ²	First order R ²	Higuchi R ²	Korsmeyer-Peppas R ² n		R ²	n
DF ₁ X ₁	0.7593	0.9811	0.9314	0.8288	0.6455	0.9436	0.0053
DF ₁ X ₂	0.7612	0.9832	0.9355	0.8408	0.6588	0.949	0.0065
DF ₂ X ₁	0.7712	0.9762	0.9388	0.8348	0.6506	0.9651	0.0061
DF ₂ X ₂	0.7886	0.9749	0.9456	0.8343	0.6506	0.9651	0.0061
DF ₃ X ₁	0.7886	0.9749	0.9314	0.8280	0.6442	0.9402	0.0051
DF ₃ X ₂	0.7511	0.9784	0.9294	0.8252	0.6430	0.9338	0.0050

Values are mean±SD of 3 replications

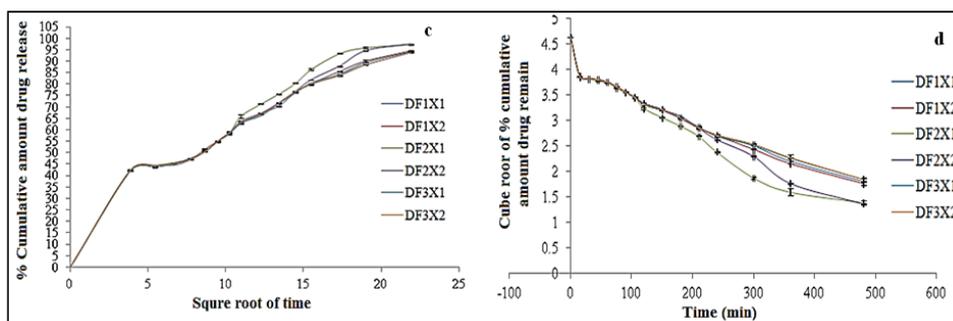


Fig. 10 (c): Higuchi plot of various EETP loaded dental gel formulations, (d): Hixson-Crowell cube root plot of various EETP loaded dental gel formulations

Values are mean±SEM of 3 replications, DF₁X₁ and DF₁X₂-formulation contain Carbopol-934P 0.35% and 0.40%, DF₂X₁ and DF₂X₂-formulation contain HPMC-K₄M 3.5% and 4.0%, DF₃X₁ and DF₃X₂-formulation contain Chitosan 4.0% and 4.5%

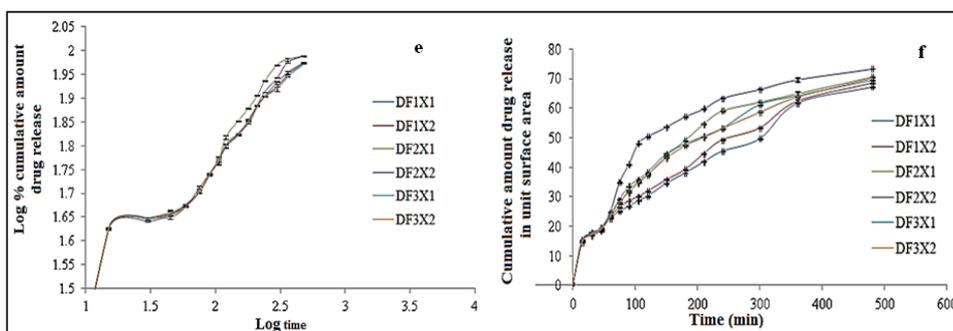


Fig. 10 (e): Korsmeyer-Peppas plot of EETP loaded dental gel formulations, (f): Cumulative amount drug release in unit surface area of EETP loaded dental gel formulations

Values are mean±SEM of 3 replications, DF₁X₁ and DF₁X₂-formulation contain Carbopol-934P 0.35% and 0.40%, DF₂X₁ and DF₂X₂-formulation contain HPMC-K₄M 3.5% and 4.0%, DF₃X₁ and DF₃X₂-formulation contain Chitosan 4.0% and 4.5%

In vitro permeation study of various EETP loaded dental gel formulations

However, from the permeation study, it was seen that a descending order of cumulative amount permeation in the unit surface area for all the dental gel formulations was found. (DF₂X₂, DF₂X₁, DF₃X₂, DF₃X₁, DF₁X₂, DF₁X₁) [Table 3, fig. 10(f)]. Permeation flux is also high for DF₂X₂ and DF₂X₁ i.e. $0.1426 \pm 0.0031 \mu\text{g} \cdot \text{cm}^{-2} \cdot \text{min}^{-1}$ and $0.1426 \pm 0.0028 \mu\text{g} \cdot \text{cm}^{-2} \cdot \text{min}^{-1}$ and lowest flux was found in DF₁X₁ dental gel ($0.1263 \pm 0.0034 \mu\text{g} \cdot \text{cm}^{-2} \cdot \text{min}^{-1}$).

Extrudability study of various EETP loaded dental gel formulations

Extrudability of all the EETP loaded dental gels decreased. However, the rate of decrease in extrudability is inversely proportional to the concentration of propylene glycol. Moreover, the increase in the concentration of propylene glycol increases the force required for extruding the dental gel from the collapsible tubes. Hence, this was confirmed that all the dental gel formulations extrude satisfactorily [table 4].

Table 3: In vitro permeation parameters of various EETP loaded dental gel formulations

S. No.	Formulation	Permeation Flux(J) ($\mu\text{g} \cdot \text{cm}^{-2} \cdot \text{min}^{-1}$)	Lag time (t_L MIN)
1	DF ₁ X ₁	0.1263 ± 0.0034	15 ± 0.5
2	DF ₁ X ₂	0.1309 ± 0.0037	15 ± 0.5
3	DF ₂ X ₁	0.1426 ± 0.0028	15 ± 0.5
4	DF ₂ X ₂	0.1426 ± 0.0031	15 ± 0.5
5	DF ₃ X ₁	0.1357 ± 0.0027	15 ± 0.5
6	DF ₃ X ₂	0.1372 ± 0.0033	15 ± 0.5

Values are mean±SD of 3 replications.

Table 4: Extrudability of various EETP loaded dental gel formulations

S. No.	Formulation	Extrudability(gm/cm ²)
1	DF ₁ X ₁	16.05±0.032
2	DF ₁ X ₂	15.65±0.030
3	DF ₂ X ₁	16.67±0.010
4	DF ₂ X ₂	14.12±0.010
5	DF ₃ X ₁	15.85±0.050
6	DF ₃ X ₂	14.67±0.020

Values are mean±SD of 3 replications

Syringeability study of various EETP loaded dental gel formulations

All the formulation easily and uniformly passed through needle no-22.

In vitro antimicrobial activity of various EETP loaded dental gel formulations

The antimicrobial study of all the EETP loaded dental gels resulted in high level of antimicrobial property than that of the standard marketed metronidazole (1% w/v) gel. DF₂X₂ was found to have the highest antimicrobial property [fig. 11].

Stability study

All the EETP loaded dental gel formulations were subjected for stability study for one month. After one month, all the evaluation parameters of dental gel formulation was found same or no change (fig. 5-9) as compared with that of the previous data taken at the time of preparation of the formulation thereby confirming a stable product after one month.

The *in vitro* drug release study data after one month is given in fig. (fig. 12).

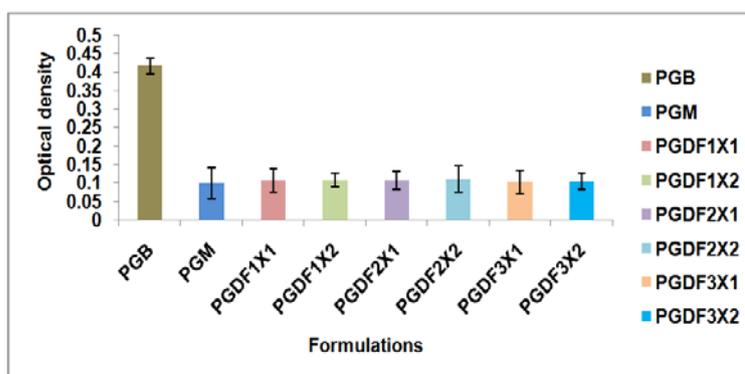


Fig. 11: Optical density of different formulations on P. gingivalis culture

Values are mean±SD of 3 replications, PGB-positive control, PGM-negative control, PGF₁X₁-DF₁X₁ solution, PGF₁X₂-DF₁X₂ solution, PGF₂X₁-DF₂X₁ solution, PGF₂X₂-DF₂X₂ solution, PGF₃X₁-DF₃X₁ solution, PGF₃X₂-DF₃X₂ solution

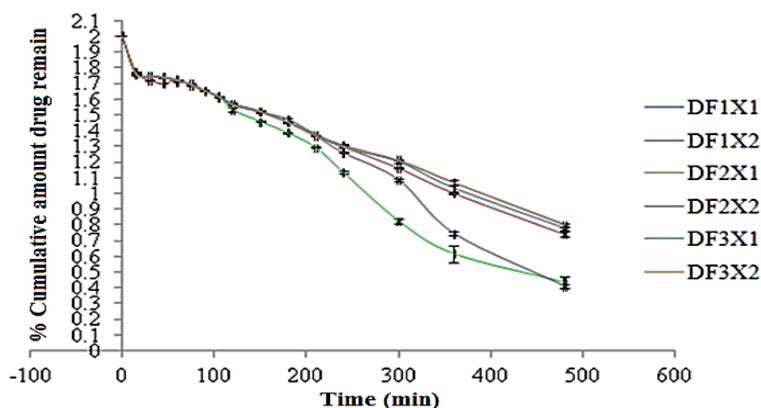


Fig. 12: In vitro release study to determine Stability study of various EETP loaded dental gel formulations

Values are mean±SEM of 3 replications, DF₁X₁ and DF₁X₂-formulation contain Carbopol-934P 0.35% and 0.40%, DF₂X₁ and DF₂X₂-formulation contain HPMC-K₄M 3.5% and 4.0%, DF₃X₁ and DF₃X₂-formulation contain chitosan 4.0% and 4.5%

CONCLUSION

Among all the dental gel formulations containing ethanol extract of *Tephrosia purpurea* Linn., DF₂X₂ (HPMC-K₄M-3.5%w/w, PG-20%w/w, NaOH-1.0%w/w, glycerol-1.0%w/w) showed the highest drug release i.e.97.435±0.078% in 8hr. resulting in first order release kinetics (R²-0.9749).

All the EETP loaded dental gels were found to follow non-Fickian drug diffusion model which was further confirmed by permeation study; the maximum drug permeation was found to be 91.220±0.065% in 8hr.

The antimicrobial study revealed that DF₂X₂ was having the highest antimicrobial activity as compared to the marketed formulation. The

extrudability and syringe ability data showed the satisfactory result for DF₂X₂.

The stability study after one month showed a little or no change of characters as that of the initial study, hence, stable. However, the reported data reveals the absence of detailed structured study of EETP contained in dental gel development in literature, and this assay seemed EETP as a component and safe dental gel that would be more acceptable and beneficial to assess its usefulness accurately.

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CONFLICT OF INTERESTS

Declared none

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