

Clinical Efficacy of a Novel Lipid-based Thymoquinone Gel: A Two-arm, Single-blinded, and Randomized Study

Saima Y. Khan¹, Imran Ahmad²

¹Department of Pediatric and Preventive Dentistry, Dr. Z.A. Dental College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India, ²Jina Pharmaceuticals Inc, Libertyville, IL, USA

Received : 11-02-21
 Revised : 28-05-21
 Accepted : 13-06-21
 Published : 28-06-21

ABSTRACT

Objective: To evaluate the clinical efficacy of topical 0.1% lipid-based Thymoquinone (TQ) gel, as an antiplaque and antigingivitis agent. **Materials and Methods:** A randomized clinical trial was carried out in the Outpatient Department of Pediatric and Preventive Dentistry, Dr. Z.A. Dental College, India with a purposive sample of 60 healthy children of 12–18 years of age, having a gingival and plaque index ≥ 1 ; the children were divided into two groups by simple randomization. Group A (30 subjects) was advised to apply peanut amount of Chlorhexidine (CHX) gel, twice a day for 2 min. Group B (30 subjects) was advised the same but with a peanut amount of 0.1% lipid-based TQ gel. The clinical parameters were the recording of plaque and gingival indices, prior to the treatment (baseline) and at the 14th and 28th day, respectively (post-treatment). The total duration of the study was 28 days with follow-up. **Results:** Data analysis was done by employing IBM Statistical Package for Social Sciences Windows software, version 20 (IBM Corp, Armonk, NY, USA). The 0.1% lipid-based TQ gel had a statistically significant reduction ($P < 0.001$) in plaque index as compared with CHX gel during the 0–14th day and 0–28th day, respectively. Similarly, 0.1% lipid-based TQ gel showed a statistically significant reduction in gingival index ($P = 0.005$) during the 0–14th day and a statistically significant reduction during the 0–28th day ($P < 0.001$), as compared with CHX gel. **Conclusions:** Statistically significant reduction in plaque ($P < 0.001$) and gingival index ($P < 0.001$) with the usage of 0.1% lipid-based TQ gel.

KEYWORDS: Chlorhexidine, gingival index, plaque index, Thymoquinone

INTRODUCTION

A common complaint of parents coming to the Pediatric Dentistry clinic is that their children hardly devote time to brushing. Consequently, gingival inflammation sets in due to insufficient use of oral hygiene measures.^[1,2] To maintain oral hygiene, different mechanical and chemical plaque control aids have been advocated. The use of mechanical plaque aid depends on the skill, dexterity, performance, and motivation of the operator. This is difficult for the pediatric patients to solely depend on.^[3] The chemical plaque aids are generally considered as an adjunct to the mechanical aids, of which Chlorhexidine (CHX) is the most frequently

used, despite its inherent drawbacks such as staining of teeth on continuous usage, alteration of taste and microflora, and formation of painful desquamative lesions on the mucosa.^[4] The World Health Organization works with a mission to save lives and preserve health, especially in developing and underdeveloped countries, on the affordability and accessibility of commercially available drugs such as antibiotics, antimicrobials, and antivirals and it recommends the possibility of using

Address for correspondence: Dr. Saima Yunus Khan, Department of Pediatric and Preventive Dentistry, Dr. Z.A. Dental College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India.
 E-mail: dr.saima.y.khan@gmail.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Khan S, Ahmad I. Clinical efficacy of a novel lipid-based Thymoquinone gel: A two-arm, single-blinded, and randomized study. J Int Soc Prevent Communit Dent 2021;11:376-81.

Access this article online	
<p>Quick Response Code:</p> 	<p>Website: www.jispcd.org</p>
	<p>DOI: 10.4103/jispcd.JISPCD_49_21</p>

plant/herb extracts as an alternative.^[5-7] One of the most widely used herbal medicines, tagged as the “Miracle herb of the century,” is *Nigella sativa*.^[7,8] *N. sativa* is also called the Black Seed. Several compounds have been isolated from *N. sativa*, but the most important reported active constituent is Thymoquinone (TQ). TQ has many favorable inherent pharmacological properties, such as anticancer, anti-inflammatory, antioxidant, antibacterial, and antiviral properties to name a few.^[7,9,10] The results have revealed that TQ has a therapeutic effect on oral and dental diseases. However, the poor solubility and high lipophilicity of TQ restricts its maximal utilization in the oral environment. Hence, topical lipid-based TQ gel will be of immense benefit as it provides sustained release of TQ to the target site with no toxicity to the normal tissues.

Considering the favorable pharmacological property of TQ as an anti-inflammatory and antibacterial agent, a comparative study—between the gold standard CHX gel and 0.1% lipid-based TQ gel—was planned. To the best of our knowledge, no clinical study has been performed to evaluate the clinical efficacy of topical lipid-based TQ gel. The null hypothesis is that there would be no significant difference in plaque and gingival indices recording from baseline to the end of the study between 0.1% lipid-based TQ and CHX gel.

MATERIALS AND METHODS

The study was conducted in the Outpatient Department of Pediatric and Preventive Dentistry, Dr. Z.A. Dental College, India. The ethical clearance from the Institutional Committee (D. No. 266/FM/IEC/13.2.2021) was obtained before the start of the study. The study was performed following the principles stated in the Declaration of Helsinki and Good Clinical Practice guidelines, Indian Council of Medical Research guidelines; all parents of the patients provided written and oral informed consent. The trial was registered with the Clinical Trials Registry, India (CTRI/2021/04/033045).

A prospective, single-blinded, and simple randomized study was planned; it included a purposive sample of 60 participants who were equally divided into two groups. The total duration of the study was 28 days with follow-up. A double-blind study could not be planned, as it was mandatory to inform the parents and their children about the drug and its constituents. The subjects were bound by the following inclusion and exclusion criteria:

INCLUSION CRITERIA

- 1) Subjects of both the genders who were 12–18 years of age
- 2) Subjects with permanent teeth in occlusion
- 3) Subjects in good general health other than gingivitis

- 4) Subjects willing to participate and who had given written and oral informed consent
- 5) Subjects diagnosed with mild to moderate type of gingivitis on score (≥ 1 for both gingival and plaque index)

EXCLUSION CRITERIA

- 1) Subjects with known hypersensitivity to any of the study drugs
- 2) Subjects should not have received any periodontal therapy within the preceding six months.
- 3) Subjects with any underlying systemic disease
- 4) Subjects currently on orthodontic treatment—fixed or removable
- 5) Subjects not willing to comply with the protocol-specified therapy and recall visits
- 6) Subjects with history of smoking
- 7) Subjects on any regular medication that could decrease the salivary flow

PREPARATION OF THE TQ GEL

Lipid-based 0.1% TQ gel was supplied by Intas Pharmaceuticals Ltd., Ahmedabad, India. In brief, the lipid soy phosphatidylcholine and alpha tocopherol were dissolved in ethanol. TQ and propylene glycol were added to the lipid mixture and stirred for 15 min under light protection. Carbopol Ultrez 10 was also added with the sprinkling technique, and the whole bulk was hydrated until it was free from lumps. The final volume was made up with propylene glycol and filled in Lami tubes.^[11,12]

STUDY DESIGN

Informed consent (written and oral) was obtained from all the participants. All the patients were subjected to oral prophylaxis (ultrasonic scaling) and then they were randomly distributed to either of the study groups: group A or B.

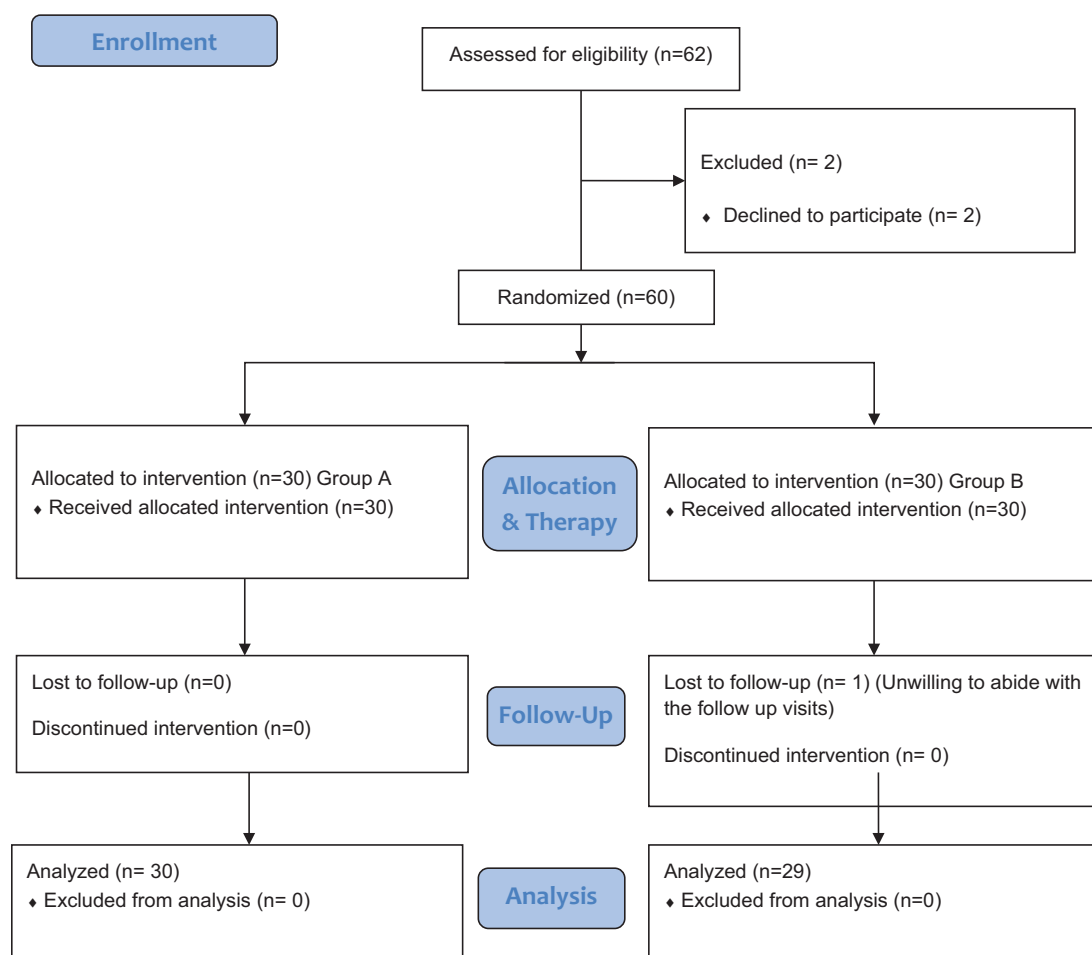
Group A: participants were advised to apply locally only on gums, twice daily for 2 min, peanut amount of CHX gluconate gel (Hexigel, CHX gluconate gel, 1% w/w, ICPA Health Products Ltd.), 30 min after morning and night brushing.

Group B: participants were instructed to massage/apply locally for 2 min peanut amount of 0.1% lipid-based TQ gel (Intas Pharmaceuticals Ltd., Ahmedabad), twice a day, 30 min after morning and night brushing, only on the gums. Both the groups were instructed not to eat and drink for 30 min after the use of the gel.

Index score recording: the patients of both the groups, prior to ultrasonic scaling, were assessed for plaque index^[13] and gingival Index.^[13] This formed the baseline score (pre-treatment). To remove bias, the recording of

the indices on all visits (pre- and post-treatment) was carried out by a single independent investigator, who did not know about the study and the group distribution. The standardization and validity of this independent investigator was done before the start of the study, on two visits: one week apart. The mean Kappa value was found to be 0.84. The oral prophylaxis (ultrasonic) and the random distribution of the patients into either of the groups, that is, group A or B, was done by the main investigator. After ultrasonic scaling, all the enrolled patients were subjected to common oral hygiene instructions. The enrolled patients were recalled back again on the 14th day and 28th day (post-treatment), respectively, for the reassessment of plaque and gingival index. The patients were instructed to bring back the chemical plaque aid assigned to them, on both the recall visits.

CONSORT FLOW DIAGRAM



STATISTICAL ANALYSIS

The mean plaque and gingival index was analyzed by using Student's *t* test for normally distributed data and Wilcoxon W and Mann–Whitney U test for skewed data. A *P* value of less than 0.05 was considered as significant. Data analysis was done by employing IBM Statistical Package for Social Sciences, Windows software, version 20 (IBM Corp, Armonk, NY, USA).

RESULTS

The data were found to be normally distributed by Kolmogrov–Smirnov and Shapiro–Wilk test. There was a dropout of one case in group B at the 14th day, as the patient expressed unwillingness to abide by the follow-ups, whereas group A continued without any dropout. Figure 1 shows the mean \pm standard error of mean of percentage reduction of plaque index of group A and B at different time intervals: 0–14th day,

14th–28th day, and 0–28th day, respectively. At 0–14th day, the mean with standard error of mean percentage reduction of plaque index for group A was 9.1 ± 1.1 ; however, for group B, it was 26.2 ± 2.1 . At 0–28th day, the plaque index percentage reduction for group A was 16.8 ± 0.96 and for group B it was 51.8 ± 3.2 , respectively. Figure 2, similarly, shows the mean \pm standard error of mean of percentage reduction of gingival index of group A and group B at different time intervals: 0–14th day, 14th–28th day, and 0–28th day, respectively. At 0–14th day, the mean with standard error of mean of percentage reduction for gingival index for group A was 16.1 ± 2.3 and for group B it was 28.5 ± 3.5 . At 0–28th day, group A showed a reduction of 27.1 ± 2.8 and group B showed a reduction of 64.2 ± 3.3 , respectively. Table 1 illustrates the intergroup comparison by *t* test for equality of means at 0–14th day, 14th–28th day, and 0–28th day, respectively. Group B had a highly statistically significant reduction ($P < 0.001$) in plaque index, as compared with group A during 0–14th day with a *df* = 57, confidence interval

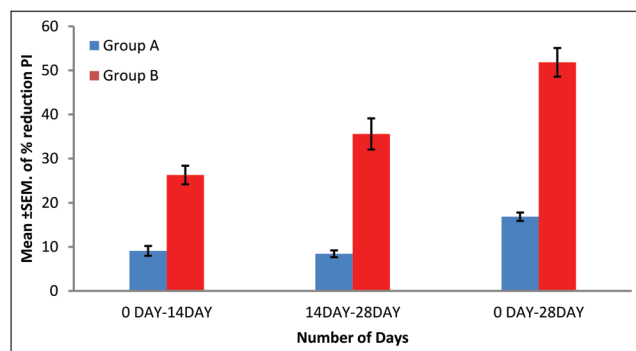


Figure 1: Distribution of mean \pm standard error of mean percentage reduction of PI of group A and group B at the 0–14th day, 14th–28th day, and 0–28th day, respectively. Group A represents chlorhexidine gluconate, and group B represents Thymoquinone gel. PI = plaque index

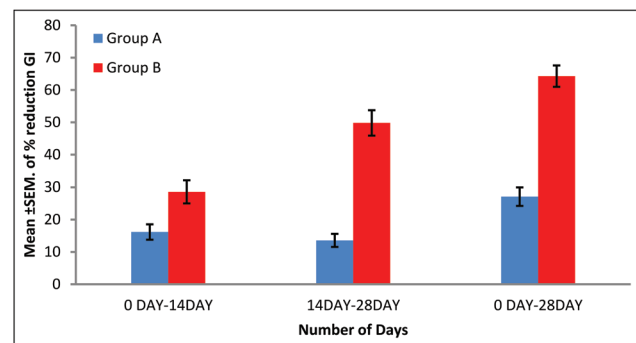


Figure 2: Distribution of mean \pm standard error of mean percentage reduction of GI of group A and group B at the 0–14th day, 14th–28th day, and 0–28th day, respectively. Group A represents chlorhexidine gluconate, and group B represents Thymoquinone gel. GI = gingival index

(CI; -27.8 to 12.4) and 0–28th day, respectively, with a *df* = 57, CI (-41.6 to 28.2). Similarly, group B showed a statistically significant reduction in gingival index ($P = 0.005$) at *df* = 57, CI (-20.8 to 3.9) during 0–14th day and a highly statistically significant reduction during 0–28th day ($P < 0.001$) at *df* = 57, CI (-45.9 to 28.4) as compared with group A.

DISCUSSION

The commercially available antibiotics/antimicrobials have an adverse effect. The World Health Organization recommends the possibility of using plant/herb extracts as an alternative. *N. sativa* is frequently recommended and tagged as the “Miracle herb of the century.”^[7,8] The active constituent of *N. sativa* is TQ. The results have revealed that TQ has a therapeutic effect on oral and dental diseases.

Based on the results of the present study, the formulated null hypothesis is rejected as there was a statistically significant reduction in plaque ($P < 0.001$) and gingival index ($P < 0.001$), with the usage of 0.1% lipid-based TQ gel.

The role of TQ in different concentrations has been used by researchers in their studies of chronic periodontitis. A study was conducted with a TQ-impregnated periodontal chip^[14] incorporated in chitosan and used in chronic periodontitis. Researchers concluded that clinical benefits were achieved when TQ chips were used as an adjunct with scaling and root planing in the treatment of chronic periodontitis and for their maintenance visits too.

Kapil, *et al.*^[15] evaluated the efficacy of 0.2% TQ gel in the treatment of chronic periodontitis, when placed subgingivally. Plaque index, gingival index, probing pocket depth, relative attachment level, and alkaline phosphatase were used as clinical and biochemical parameters. A statistically significant reduction was observed in plaque and gingival indices from baseline in the group in which TQ was used as an adjunct to scaling and root planing, hence supporting our study.

In another clinical research report,^[16] the periodontal pockets were filled with 0.1% w/w TQ gel. They also advocated the adjunctive use of TQ gel with scaling and root planing in chronic periodontitis. They also ascertained that TQ, after a few weeks, failed in the treatment due to instability in the oral environment. This is the point that the present conducted research tried to overcome with topical lipid-based formulation. In recent years, topical lipid-based formulations have gained attention as a drug delivery system because they are stable, nontoxic, and biodegradable, and they

Table 1: Intergroup comparison by *t* test for equality of means at the 0–14th day, 14th–28th day, and 0–28th day, respectively

	Levene's test for equality of variances		<i>t</i> test for equality of means						
	F	Sig.	<i>t</i>	df	<i>P</i> value	Mean difference	Standard error difference	95% confidence interval of the difference	
								Lower	Upper
Percentage reduction of plaque index 0–14th day	8.2	0.006	-7.2	57	<0.001	-17.1	2.35	-21.8	-12.4
Percentage reduction of plaque index 14th–28th day	45.0	0.000	-7.6	57	<0.001	-27.1	3.5	-34.2	-20.0
Percentage reduction of plaque index 0–28th day	38.8	0.000	-10.4	57	<0.001	-34.9	3.3	-41.6	-28.2
Percentage reduction of gingival index 0–14th day	6.3	0.015	-2.9	57	<0.005	-12.3	4.2	-20.8	-3.9
Percentage reduction of gingival index 14th–28th day	7.5	0.008	-8.3	57	<0.001	-36.2	4.3	-44.9	-27.5
Percentage reduction of gingival index 0–28th day	1.9	0.165	-8.5	57	<0.001	-37.1	4.3	-45.9	-28.4

Significant $P < 0.05$; highly significant $P < 0.01$

cause sustained release of the drug to the target site and improve the therapeutic efficacy of the drug^[15,16] supporting the present research.

The strength of the present study was the use of a novel lipid-based herbal product, which emerged as a promising alternative to CHX gel. As a limitation, a few pediatric patients did complain of taste, which can be taken care of in the future formulations. Apart from taste, no other complaint was reported. Overall, 0.1% lipid-based TQ gel can be recommended as a primary preventive measure and should be used as an adjunct to the mechanical aids for plaque.

CONCLUSION

The overall observations of the present conducted research are promising, with a statistically significant reduction in plaque ($P < 0.001$) and gingival index ($P < 0.001$) with the usage of 0.1% lipid-based TQ gel. Long-term studies with a larger sample size are warranted to authenticate the effect of 0.1% lipid-based TQ gel, as an antiplaque and antigingivitis gel. Further research can be planned with secondary aims, such as use in other oral conditions.

IMPORTANCE TO PEDIATRIC DENTISTS

A promising alternative to CHX gel.

ACKNOWLEDGEMENT

The authors wish to thank all the patients, their family members, and the staff who participated in this study.

FINANCIAL SUPPORT AND SPONSORSHIP

Nil.

CONFLICTS OF INTEREST

The first author Dr. Saima Yunus Khan has no conflicts of interest. Coauthor Dr. Imran Ahmad is serving on the Board of Directors in several Pharma companies: 1. Jina Pharmaceuticals Inc., USA; 2. Novum Pharmaceutical Research Services, USA; 3. Accord Healthcare, USA; 4. Lambda Therapeutic Research Ltd., India; and 5. Head of Scientific Advisory Board at Intas Pharmaceuticals Ltd., India. The product that was tested in the current research belongs to Intas Pharmaceuticals Ltd., India, where there can be possible but not essential conflicts of interest. The reader should remain aware about the same.

AUTHORS' CONTRIBUTIONS

Dr. Saima Yunus Khan was involved in the idea conception, study design, acquisition, interpretation, and article writing and editing. Dr. Imran Ahmad was involved in the analysis, feasibility of drugs, and article writing and editing.

ETHICAL POLICY AND INSTITUTIONAL REVIEW BOARD STATEMENT

All procedures have been performed as per the Declaration of Helsinki (revised in 2008). The ethical clearance from the Institutional Committee (D. No. 266/FM/IEC/13.2.2021) was obtained before the start of the study. The trial was registered with the Clinical Trials Registry, India (CTRI/2021/04/033045).

PATIENT DECLARATION OF CONSENT

Informed consent (written and oral) was obtained from all the parents and participants.

DATA AVAILABILITY STATEMENT

Not available.

REFERENCES

1. Kala BS, Gunjan C, Disha N, Shobha P. Treatment of periodontal disease – A herbal approach. *International Journal of Pharmaceutical Science Review and Research* 2015;33:126-36.
2. Aspalli S, Shetty VS, Devarathamma MV, Nagappa G, Archana D, Parab P. Evaluation of antiplaque and antigingivitis effect of herbal mouthwash in treatment of plaque induced gingivitis: A randomized, clinical trial. *J Indian Soc Periodontol* 2014;18:48-52.
3. Rao NJ, Subhas KR, Kumar KS. Role of phytotherapy in gingivitis; a review. *Journal of Pharmacology and Pharmacotherapeutics* 2012;8:1-5.
4. Peter S. *Plaque Control. Essentials of Preventive and Community Dentistry*. 1st ed. 4th reprint. Darya Ganj, New Delhi: Arya (Medi) Publishing House; 2002. p. 368.
5. Al-Attass SA, Zahran FM, Turkistany SA. Nigella sativa and its active constituent Thymoquinone in oral health. *Saudi Med J* 2016;37:235-44.
6. World Health Organization. *Traditional medicine strategy 2002–2005*. Geneva (CH): WHO Publications; 2006. p. 1-6.
7. Ahmad I, Tripathi J, Sharma M, Karchulli MS, Umer L. Nigella sativa - a medicinal herb with immense therapeutic potential (a systematic review). *International Journal of Biological & Pharmaceutical Research* 2014;5:755-62.
8. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, *et al*. A review on therapeutic potential of Nigella sativa: A miracle herb. *Asian Pac J Trop Biomed* 2013;3:337-52.
9. Yimer ME, Turn KB, Karim A, Rehman NU, Anwar F. Nigella sativa (Black cumin); a promising natural remedy for wide range of illness. *Evidence based complementary and alternative Medicine* 2019;1:1-16.
10. Khan AS, Khan AM, Kamal MA, Damanhour GA, Mirza Z. Panaces seed “Nigella”: A review focusing on regenerative effects for gastric ailments. *Saud Journal of Biological Sciences* 2016;23:542-53.
11. Faustino C, Pinheiro L. Lipid systems for the delivery of amphotericin B in antifungal therapy. *Pharmaceutics* 2020;12:29.
12. Sheikh S, Ahmad A, Ali S, Paithankar M, Raval RC, Shah K, *et al*. Topical delivery of lipid based amphotericin B gel in the treatment of fungal infection: A clinical efficacy, safety and tolerability study in patients. *Journal of Clinical and Experimental Dermatology Research* 2014;5:1-5.
13. Løe H. The gingival index, the plaque index and the retention index systems. *J Periodontol* 1967;38(Suppl):610-6.
14. Al-Bayat F, Kamruddin A, Ismail M, Abdulla M. Formulation and evaluation of a new biodegradable periodontal chip containing Thymoquinone in a chitosan base for the management of chronic periodontitis. *Journal of Nanomaterials* 2013;2013, article ID:397308.
15. Kapil H, Suresh DK, Bathla SC, Arora KS. Assessment of clinical efficacy of locally delivered 0.2% Thymoquinone gel in the treatment of periodontitis. *Saudi Dent J* 2018;30:348-54.
16. Khalil AA, Alaaeldin E. Clinical and biochemical evaluation of Thymoquinone gel in the treatment of chronic periodontitis. *International Journal of Advanced Research* 2019;7:83-93.