EFFECTS OF SINGLE DOSE LOW-LEVEL LASER THERAPY, ON PAIN AND HEALING OF EXTRACTION SOCKET AFTER EXTRACTION OF PREMOLARS IN ORTHODONTIC PATIENTS

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by

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Alhamdulillah, praise is only to Allah for his endless mercy and blessings that we can still breathe the fresh air and survive in this world for gratis. Allah says in Holy Quran: "Your Lord is best aware of you. If He will, He will have mercy on you, or if He will, He will punish you" (Holy Quran 17:54).

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LIST OF ABBREVIATIONS

ALP	Alkaline phosphatase
ATP	Adenosine Tri-Phosphate
CO_2	Carbon Dioxide
COX	Cytochrome c oxidase
Er,Cr:YSGG	Erbium, Chromium: Yttrium Selenium- Gallium Garnet
Er:YAG	Erbium: Yttrium Aluminum- Garnet
GaAlAs	Aluminium Gallium Arsenide
GaAS	Gallium Arsenide
HeNe	Helium–Neon
Ho:YAG	Holmium: Yttrium Aluminum-Garnet
IEC	International Electrotechnical Commission
IL-1 β	Interleukin-1β
IL-6	Interleukin-6
IL-8	Interleukin-8
InGaAlP	Aluminium Gallium Indium Phosphide
IPS	Institut Pengajian Siswazah
J	Joule
LLLT	Low-Level Laser Therapy
m	Meter
MBT	McLaughlin Bennett Trevisi
Nd: YAG	Neodymium: Yttrium Aluminum-Garnet
NiTi	Nickel-Titanium
NO	Nitrous oxide
NSAIDs	Non-Steroidal Anti-inflamatory Drugs
OPG	Osteoprotegerin
RANK	Receptor activator of nuclear factor-kappa B
RANKL	Receptor activator of nuclear factor-kappa B ligand

KESAN TERAPI LASER PERINGKAT RENDAH DOS TUNGGAL TERHADAP SAKIT DAN PENYEMBUHAN SOKET EKSTRAKSI SELEPAS PENGEKSTRAKAN PRAMOLAR PADA PESAKIT ORTODONTIK

ABSTRAK

Pencabutan gigi untuk tujuan ortodontik adalah satu prosedur biasa. Pengekstrakan, sama seperti pembedahan lain membawa beberapa komplikasi dan faktor risiko seperti sakit, bengkak, jangkitan, soket kering, dan lain-lain. Ubat anti-radang bukan steroid (NSAIDs) adalah ubat pilihan untuk mengurangkan kesakitan selepas pengekstrakan, tetapi ia mungkin mempunyai kesan sistemik. Terapi laser tahap rendah (LLLT) didapati berkesan dalam mengurangkan kesakitan selepas pengekstrakan dan mempercepatkan penyembuhan luka pengekstrakan molar ketiga. Daya ortodontik memulakan aktiviti osteoklastik dalam tulang alveolar yang membawa kepada pergerakan gigi yang mana juga memudahkan prosedur pengekstrakan dan juga mengurangkan kesakitan selepas pengekstrakan, tetapi ia masih belum dikaji. Tujuan penyelidikan ini adalah untuk menilai kesan dos tunggal LLLT terhadap kesakitan dan penyembuhan soket pengekstrakan pre-molar sebelum peringkat penjajaran dan perataan, selepas permulaan rawatan ortodontik. Empat puluh empat pesakit Pakistan yang memerlukan pengekstrakan pra-molar bagi rawatan ortodontik, lingkungan umur antara 12 hingga 18 tahun, telah dipilih. Pesakit dibahagikan kepada 2 kumpulan, Kumpulan A dan Kumpulan B. Kedua-dua kumpulan mempunyai bahagian eksperimen (Ae, Be) dan bahagian plasebo (Ap, Bp) yang dipilih secara rawak untuk reka bentuk mulut terbelah. Kumpulan A menjalani pengekstrakan dulu diikuti dengan permulaan rawatan ortodontik manakala Kumpulan B menjalani pengekstrakan selepas penjajaran dan pemerataan gigi. Laser diod Gallium-Aluminium-Arsenik (mod berterusan dengan panjang gelombang 940nm) telah digunakan pada 6 titik bukal dan palatal, 1 cm apikal kepada soket pengekstrakan, serta-merta selepas pengekstrakan. Penyinaran digunakan selama 30 saat pada setiap titik, memancarkan tenaga sebanyak 3 joule. Kesakitan telah direkodkan selama satu minggu dengan skala penilaian nombor dan penyembuhan luka dinilai secara klinikal pada peringkat awal, hari ke-2, ke-7 dan ke-15. SPSS versi 22.0 digunakan untuk menganalisa data. Sisi eksperimen dan plasebo dibandingkan untuk memerhatikan kesan LLLT atas kesakitan dan penyembuhan luka, manakala perbandingan antara kumpulan dibuat untuk melihat kesan penglibatan gigi sebelum ini dengan wayar gerbang, atas kesakitan dan penyembuhan luka. Ujian Mann Whitney U bukan parametrik dan Krusker Wallis digunakan untuk membandingkan keputusan antara semua kumpulan. Kumpulan A mengalami kesakitan yang lebih ketara apabila dibandingkan dengan kumpulan B ($p<0.05$). Perbezaan kesakitan antara sisi eksperimen dan plasebo dalam kedua-dua kumpulan adalah tidak ketara. Tiada perbezaan yang ketara diperhatikan mengenai penyembuhan luka di kalangan semua kumpulan. Kesimpulannya, aplikasi lawatan tunggal LLLT tidak mengurangkan kesakitan selepas pengekstrakan dan tidak menjejaskan penyembuhan luka pengekstrakan pada pesakit ortodontik. Penglibatan pra-molar sebelum ini mengurangkan kesakitan pengekstrakan namun penyembuhan luka tetap tidak terjejas.

EFFECTS OF SINGLE DOSE LOW-LEVEL LASER THERAPY, ON PAIN AND HEALING OF EXTRACTION SOCKET AFTER EXTRACTION OF PREMOLARS IN ORTHODONTIC PATIENTS

ABSTRACT

Tooth extraction for orthodontic purposes is a common procedure. However, extractions, just like other surgeries carry some complications and risk like pain, swelling, infections, and dry socket. Non-steroidal anti-inflammatory drugs (NSAIDs) are preferred medicines to reduce post-extraction pain, but it may have systemic effects. Low-level laser therapy (LLLT) has been found effective in decreasing the post-extraction pain and acceleration of wound healing after third-molar extraction. Orthodontic forces initiate osteoclastic activities in the alveolar bone which causes mobility of the tooth which also eases the extraction and reduces post-extraction pain, but it has not been investigated. The purpose of this research was to evaluate the effect of single dose of LLLT on the pain and healing of extraction socket before alignment and levelling stage, after initiation of orthodontic treatment. Forty-four Pakistani patients, age between 12 to 18 years who needed pre-molar extraction for orthodontic treatment were selected, which were then divided into 2 groups (A and B). Both groups had experimental (Ae, Be) and placebo sides (Ap, Bp), allocated randomly for split mouth design. Group A underwent extractions first, then orthodontic treatment. However, group B underwent extractions after alignment and leveling of teeth. Gallium-Aluminum-Arsenic diode laser (continuous-mode, wavelength 940nm) was applied on 6points buccally and palatally, 1cm apical to the extraction socket, immediately after extraction. Irradiation was applied for 30seconds at each point, emitting energy of 3joules.

After extraction, pain was recorded for a week with numeric rating scale and wound healing was assessed clinically at baseline, 2^{nd}, 7^{th} and 15^{th} day. SPSS version 22.0 was used to analyze the data. Experimental and placebo sides were compared to observe the effect of LLLT on pain and wound healing, while inter group comparison was made to see the effects of prior engagement of tooth with the arch-wire, on pain and wound healing. Non-parametric Mann Whitney U test and Krusker Wallis test were applied to compare the results amongst all the groups. Group A experienced significantly more pain when compared with group B ($p<0.05$). The difference in pain between experimental and placebo sides in both the groups was insignificant. No significant difference was observed regarding wound healing among groups. In conclusion, single visit application of LLLT did not reduce the post-extraction pain and did not affect the extraction wound healing in orthodontic patients. Prior engagement of pre-molars reduced the extraction pain, however wound healing remained unaffected.

CHAPTER 1

INTRODUCTION

1.1 Background

Extracting teeth in orthodontics has always been controversial in order to gain spaces for alignment of teeth. On one hand, dental extractions can cause damage to dentofacial aesthetics and perfect occlusion while on other hand it can bring significant improvement in aesthetics in patients with bimaxillary protrusions.(Angle, 1907). For that reason, since many years the decision of dental extractions in orthodontics has been debatable.

In the past, the father of American orthodontics "Edward Hartley Angle" and his supporters were against the idea to extract teeth for the purpose of treatment of malalignment. However; during early 19th century, Calvin Case (another prominent American orthodontist) claims that extractions required to treat dental malocclusions were only 6 to 7 % (Case, 1913). Dental extractions for orthodontic treatment were reintroduced in 1940s and during 1960s almost fifty percent of the orthodontic cases involved dental extractions in the United States of America (USA) (Salzmann, 1965)**.** In most orthodontic cases in order to treat malocclusion, first pre-molars are preferred for extractions(Moreira and Mucha, 1997); (Paschoal and Santos-Pinto, 2012).

Teeth extraction is possibly a distressful event. Therefore, the conclusion for dental extraction need to made vigilantly because there is a risk of psychological involvement due to daunting thoughts of the procedure (Travess et al., 2004). Teeth extraction is potentially frightful event and it involves emotional effect in the life of patient, as a result, patients who needs dental extraction are mostly scared by its name (Travess et al., 2004).

1.1.1 Risks and complications associated with extractions

Dental extractions are considered as minor oral surgical procedure, which leads to the formation of wounds. Patients may suffer from symptoms like pain and swelling and may have disturbed life for some time right after tooth removal (Paschoal and Santos-Pinto, 2012).

1.1.2 Risk of pain

This minor surgical procedure may lead to complication such as inflammation (Raiesian et al., 2017). Initially, symptoms after tooth removal include pain, bleeding from the socket and swelling (Gupta et al., 2019). The Institute of Medicine Committee (IOM) specified that the symptom of pain is not just an ordinary sensory stimulation in fact pain perception is difficult to understand and it involves central nervous system (CNS) and also modifies the cognitive process and psychological state (Al-Khateeb and Alnahar, 2008). Therefore, for patients undergoing pain, it is important to control the perception of pain (Chang, 2002).

1.1.3 Choice of drugs to control pain

Local and systemic steroids and non-steroidal anti-inflammatory drugs (NSAIDs) are the drugs of choice, to reduce the inflammation and the pain after removal of teeth; nonetheless, these drugs have few adverse reactions like allergic reaction, systemic bleeding, and gastro intestinal issues (Raiesian et al., 2017). With extended use of corticosteroids patient proneness towards infections increases and the process of wound healing delays (Vegas-Bustamante et al., 2008). It seems justifiable to consider a different approach to relieve pain after extraction in order to avoid the side effects of those medications that control pain (Marković and Todorović, 2006).

1.1.4 Risk of dry/alveolar osteitis

One more condition that may be associated with extractions is alveolar osteitis (AO) also known as dry socket. It is seen commonly as a complication following extraction of a permanent tooth. This condition usually happens within 2-4 days following extraction, caused by clot degradation resulting in delay in wound healing along with radiating pain in and around an extraction socket. Halitosis can also be one of the symptom of alveolar osteitis (Kaya et al., 2011).

1.2 Low-Level Laser Therapy

Numerous researches have shown that laser therapy can fasten cell and tissue repair and helps in relieving post-surgical pain (Raiesian et al., 2017). Low-level laser therapy (LLLT) has made its way in dentistry and is being used for the prevention of inflammatory signs and symptoms post-surgically. Nowadays, low-level laser therapy (LLLT) have become popular for its ability to heal wounds faster (Samaneh et al., 2015). Low power laser therapy or low-level laser therapy (LLLT) is amongst innovative method that demonstrates many benefits such as reducing post-surgical pain and swelling, accelerates wound healing, and helps in bone regeneration (Samaneh et al., 2015). It is postulated that low energy laser light has positive effects on reducing pain, and also enhances wound healing (Paschoal and Santos-Pinto, 2012).

1.3 Early Engagement of Tooth to Be Extracted

Early engagement of the tooth by placing brackets and aligning them with the orthodontic wires may lessen the discomfort of tooth extraction. Rai, reported in one of his studies that aligning the tooth to be extracted before extraction resulted in reduction of post-operative pain and enhanced wound healing (Rai, 2016). The reason for decrease in pain after extraction of tooth that was engaged with arch-wire is that orthodontic forces can cause localized inflammation in periodontal ligament (PDL). Simultaneously orthodontic force induces micro trauma to the surrounding tissues and bone softness increases because of reduction in trabecular bone density which results in increment in tooth mobility and less resistance in tooth extraction (Rai, 2016).

1.4 Statement Of Problem

Since the uncomplicated extractions are also associated with the moderate type of pain, there is a need to look for modalities which can reduce the pain and other complications without any side effects (Al-Khateeb and Alnahar, 2008). Low-level laser therapy (LLLT) has not only been found effective in reducing pain related to the extraction of third molars, but it also accelerated the healing of extraction socket (He et al., 2015). However, in most studies, patients were recalled frequently to apply laser, which can be difficult in this demanding world. Single session of LLLT has been found effective in reducing pain related to tooth movement and separator placement (Qamruddin et al., 2016, Qamruddin et al., 2017).

This raises a question whether single session of LLLT would also be effective in reducing the post extraction pain and accelerate the healing of wound after the extraction of pre-molars in orthodontic patients?

Another question would be the bonding and engaging the tooth to be extracted, in the arch-wire before referring for extractions. Would this approach also be effective in reducing the pain related to extraction?

These questions have not been answered in literature.

1.5 Justification of the study

Fear or phobia for dental treatment is a major difficulty in its acceptance (Gazal et al., 2015). Amongst all other dental treatment, tooth extractions has been associated with increased level of dental anxiety (Maulina et al., 2017). Therefore, it is crucial to check a variety of modalities to get a better outcome to reduce these problems to favor humanity. Low-level laser therapy is non-invasive and also have beneficial results. (Qamruddin et al., 2015). LLLT is also being applied on humans for variety of purposes and no adverse effects have been reported (Qamruddin et al., 2018). According to International Electrotechnical Commission (IEC), type 4 lasers are the choice of lasers for being used in medicine and dentistry. Type 4 lasers can produce hazardous affect especially to skin and eyes. Therefore, it is mandatory to use protective equipment like glasses (Alam, 2019)

The benefits of adopting LLLT for post operative pain reduction after extractions, especially in children may reduce the chances of pain and enhance healing of the wound, this warrants further investigation.

The benefits of engaging the tooth to be extracted in the arch-wire has not been much investigated for pain scores. Early engagement of the tooth may ease the extraction and LLLT of that extraction socket may further comfort the patient after extraction. These potential benefits should be investigated to improve the wellbeing of patients.

1.6 Novelty of the research

The results of this research will introduce novel non-invasive techniques to reduce the pain and make the treatment acceptable and comfortable. The consequences of the research will give extra awareness to the healthcare provider about the effects of single dose of LLLT on the pain and healing of the extraction socket after the extraction of pre-molars whether they are extracted before the fixation of brackets or after engaging them in the arch-wire for few months. Subsequently dentists will be able to offer their patients new techniques with evidence.

1.7 Objectives

1.7.1 General Objectives

The main objective of this clinical study was to find out the effect of single dose of low-level laser on pain level and wound healing of extraction socket after removal of the pre-molars in adolescent orthodontic patients.

1.7.2 Specific Objectives

1. To determine and compare the effect of single dose LLLT with non-experimental side in split mouth study design on pain of pre-molar extraction in patients undergoing orthodontic treatment.

2. To determine and compare the effect of single dose LLLT with non-experimental side in split mouth study design on wound healing of extraction socket after pre-molar extraction in patients undergoing orthodontic treatment.
3. To determine and compare the effect of single dose of LLLT with non-experimental side in split mouth study design on pain of pre-molar extraction which was bonded and engaged in arch-wires for alignment and levelling before surgery, in patients undergoing orthodontic treatment.
4. To determine and compare the effect of single dose of LLLT with non-experimental side in split mouth study design on wound-healing of extraction socket of pre-molar which was bonded and engaged in arch-wires for alignment and levelling before surgery, in patients undergoing orthodontic treatment.

1.8 Alternative hypotheses

1. There would be significant reduction in post-operative pain associated with extraction of pre-molar when single session of LLLT was applied.
2. There would be significant improvement in the wound healing of extraction socket associated with extraction of pre-molar when single session of LLLT was applied.
3. Post-operative pain would be significantly lesser in the group where pre-molars were engaged in the arch-wire for alignment and levelling before extraction.
4. Wound healing would be significantly faster in the group where pre-molars were engaged in the arch-wire for alignment and levelling before extraction.

1.9 Research questions

1 Would there be significant reduction in post-operative pain associated with extraction of pre-molar when single session of LLLT was applied?

2. Would there be significant improvement in the wound healing of extraction socket associated with extraction of pre-molar when single session of LLLT was applied?
3. Would there be significantly lesser post-operative pain in the group where pre-molars were engaged in the arch-wire for alignment and levelling before extraction?
4. Would there be significantly faster wound healing in the group where pre-molars were engaged in the arch-wire for alignment and levelling before extraction?

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The choice whether to extract teeth or not for the purpose of alignment is arguable for almost a century (Dardengo et al., 2016). This is the most critical verdict taken by orthodontists (Ribarevski et al., 1996). Decision whether to extract or avoid does not totally depends on the dental crowding, but attention needs to be placed on facial soft tissues as well (Saelens and De Smit, 1998a).

According to Edward Hartley Angle and his followers, extractions can disturb ideal occlusion and ideal facial aesthetics and preferred treating malocclusions without extracting teeth. After the death of Angle, one of his follower Charles Tweed re-evaluated Angles cases and observed that with Angle's non-extraction philosophy, 80% of his cases did not accomplish the objectives. For this reason Charles Tweed favored dental extractions for achieving facial and occlusal harmony (Dardengo et al., 2016). In the 1940's, dental extractions were introduced again in orthodontics. In USA, during 1950s and 1960s, almost half of the orthodontic cases involved dental extractions (Salzmann, 1965).

To treat malocclusions, the decision whether to extract teeth or not varies case to case. Various elements are involved in the decision for dental extraction for orthodontic treatment. These factors includes morphological and etiological characteristic of malocclusion, particular objective to achieve desired outcome and also technique selection for orthodontic treatment (Weintraub et al., 1989). First, orthodontist evaluates each case critically and consider all the factors and takes decision whether the candidates needs tooth extraction for the succession of their orthodontic treatment (Peck and Peck, 1979b)

Many studies are done, and majority reported that among all the teeth pre-molars are preferred for extraction for orthodontic purpose (Ong and Woods, 2001). Many orthodontists picked first pre-molar as their first choice for tooth extraction for orthodontic purpose (Moreira and Mucha, 1997). Pre-molars extractions for orthodontic treatment is also known as therapeutic extraction (Mahtani and Jain, 2020). For relieving severe crowding pre-molars extractions are done (Saelens and De Smit, 1998b, Paschoal and Santos-Pinto, 2012).

Dental treatment procedures increases patient's anxiety and tooth extraction provokes the most stress (Astramskaitė and Juodžbalys, 2017). The judgment to extract a tooth should be made with the knowledge of the consequences of treatment, which includes the psychological impact of the procedure (Travess et al., 2004). Tooth extraction is terrifying and provokes anxiety to the patients (Astramskaitė et al., 2016). Tooth extractions has psychological impact on patient's life (de Jongh et al., 2008)

Tooth extraction is a minor oral surgical procedure (Paschoal and Santos-Pinto, 2012). Pain after tooth extraction is most common complain after this surgery followed by dry socket (Rakhshan, 2015). Dry sockets are also known as alveolar osteitis. It is associated with extreme pain and pain reaches its peak at 12-48 hour post-surgery (Kamal et al., 2021). It is caused by blood clot degradation. When the extraction socket is devoid of blood clot, it delays wound healing with scorching pain around extraction socket. Bad breath or halitosis can also be associated with dry socket (Kaya et al., 2011).

Many individual reported that the pain after tooth extraction as the most intense pain that they have experienced during their life (Rao and Kumar, 2018). Non-steroidal anti-inflammatory drugs and local and systemic steroids are suggested to be prescribed to minimize pain and inflammation after extractions of teeth but they possess some side effects that include systemic bleeding, allergic reactions as well as gastro intestinal issues (Raiesian et al., 2017). Patient susceptibility to infection increases and delay healing with prolonged use of corticosteroids (Vegas-Bustamante et al., 2008). With these observations, it has been justified to look for a method to relieve postoperative pain without usage of these drugs in order to avoid their adverse effects (Marković and Todorović, 2006).

It is reported that low energy laser light has positive effects on reducing pain and enhances wound healing (Paschoal and Santos-Pinto, 2012).

2.2 Low-level laser therapy (LLLT)

Low-level laser therapy or LLLT involves the use of low-levels of red light or near infrared wavelengths to diminish and treat variety of ailments (Castano et al., 2007). This light is absorbed vastly and produces unique beneficial effects in the living tissues. Low-level lasers are different from high power lasers in a way that low-level laser does not have enough energy to destroy or damage any tissue but sufficient power to stimulate or initiate tissue healing (Hawkins et al., 2005). Photobiomodulation is another name for low-level laser therapy (Chung et al., 2012). It is postulated that chromopheres that are present in the cell for example cytochrome c oxidase which is located in mitochondria absorbs low-level of red lights or infrared wavelengths which alters the function of cytochrome c oxidase and that results in manufacturing more adenosine tri phosphates (ATP) , which is a chief source of cellular power which may result in pain relief and healing (Castano et al., 2007). Laser therapy in dentistry is being used for following purpose

- Photobiomodulation
- Curing of filling materials such as composite resin
- Laser scanning (restorative dentistry, orthodontics)
- Detection of dental cavities
- Photo-activated disinfection (PAD) (Parker, 2007)

2.3 Lasers

Now a days the use of lasers in dental practices is common (Doshi-Mehta and Bhad-Patil, 2012). The abbreviation of term laser is 'Light Amplification by the Stimulated Emission of Radiation' (Verma et al., 2012). There are two main properties of laser beams to understand its physiological capability, one of them in monochromaticity which is really high and second one is that the light is extremely collimated (Solon et al., 1961). Cold laser or low-level laser therapy is referred to as low-level because it uses energy concentration from light at lower level to produce bio-stimulatory effects when compared with the different types of laser which are used for thermal coagulation or cutting or ablation of the tissue (Chung et al., 2012). When laser is applied on tissue at lower doses for e.g. 2 J/cm2, proliferation is stimulated and at higher doses for e.g. 16 J/cm2 suppression is been observed (Verma et al., 2012). Low-level laser therapy uses energy at low-level and does not raise the temperature of the treated tissue above normal body temperature i.e. 36.5 degrees centigrade (Doshi-Mehta and Bhad-Patil, 2012).

2.4 History

Soon after the creation of ruby laser in 1960, low-level laser therapy which is also referred to as photobiomodulation came into existence in its contemporary form followed by invention of helium neon laser (HeNe) in 1961 (Chung et al., 2012). Since 1960 after the development of therapeutic laser system for the use for medical and cosmetics purposes, positive effects of low powered irradiation were seen on wound healing and pain relief (Chung et al., 2004).

After the invention of first working laser in 1967 in Semmelweis University, Budapest, Hungary, Dr. Endre Mester wanted to check the effects of low-level laser therapy on cancerous cells in mice. He divided the mice into 2 groups and shaved their backs. One group was exposed to ruby laser with the power of 694-nm. Surprisingly, group that was exposed to laser did not get cancer cell destruction but the hair on their back started to grew faster when compared with untreated group (Hamblin, 2009). This experiment showed the first biostimulatory effect of laser therapy (Hamblin, 2009). As soon as Mester found this effect of LLLT he tried these discoveries on patients with non-healing skin ulcer and found very positive results (Mester et al., 1985); (Chung et al., 2012).

2.5 Properties of laser

There are three main properties of lasers, which are being used by the medical community (Sliney and Trokel, 2012).

2.5.1 Monochromatic emission

Lasers can be differentiated by their colours. Blue or blue-green colour represents the argon laser whereas red represents He-Ne laser (Sliney and Trokel, 2012).

2.5.2 Collimation

This is the 2nd important property that can differentiate lasers from conventional light. This property acts like a magnifying glass that is used as point to focus sun's rays to lit the fire (Sliney and Trokel, 2012). This suggests that there is likelihood of attaining huge power concentrations into narrow stream of light (Solon et al., 1961).

2.5.3 Coherence

High spatial coherence is one of the important properties of conventional lasers (Redding et al., 2012). This property of lasers focuses on a spot of very low divergence to achieve the output of their energies at great distances. This phenomenon often known as ''pencil beam''(Chung et al., 2012).

2.6 Interaction of lasers with tissues:

One of the four interactions can happen once the low-level laser energy reaches the target tissue (Verma et al., 2012)

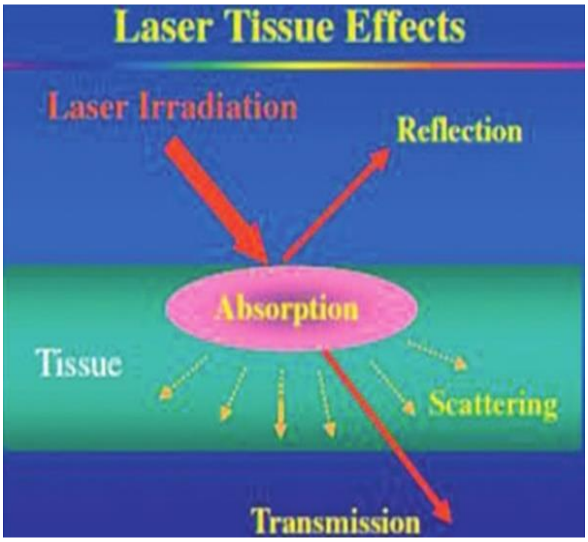

Figure 2.1: Interaction of laser beams on tissue. (Verma, Maheshwari et al. 2012)

a. **Absorption**
b. **Scattering**
c. **Transmission**
d. **Reflection**

2.6.1 Absorption

Rays are absorbed by a specific molecule which is known as chromophore. The energy of this light is altered in different types of energy to achieve work. Chromophores present in intraoral soft tissue include haemoglobin, melanin and water whereas dental hard tissues include hydroxyapatite and water (Verma et al., 2012)

2.6.2 Scattering

The tissue has many inhomogeneous structures that cause scattering of the light. The light scatters because of difference in the index of refraction and also by the variations in optical properties of the cells present in the tissue (Svaasand et al., 1985).

2.6.3 Reflection

Reflection occurs when the laser rays that recoil back from the external layer of the tissues, just like mirror (Trelles and Calderhead, 2005). These reflections of laser beams can be hazardous (Sliney, 1995).

2.6.4 Transmission

Transmission can be explained by considering the example of clear window glass. Light rays that enters the tissue passes through the tissue in unused form and emerges at the opposite of the targeted tissue (Trelles and Calderhead, 2005).

2.7 Mode of action of low-level laser therapy

The exact mechanism that produces therapeutic action of low-level laser therapy is still not very well-known but it seems that low-level laser therapy effects are of the broader range at the cellular, molecular and tissue level (Chung et al., 2012). Chromophores (haemoglobin and melanin) absorb the photons from laser radiation in the cell and electron in the chromophore is stimulated. Electron moves towards high energy orbit from low energy orbit (Sutherland, 2002). There are strong indications that demonstrate that monochromatic light of laser targets the chromophere within the mitochondria initially. The absorbed radiation will result in more mitochondrial products for example ATP, protein, NADH, RNA, and also increases the oxygen utilization, and according to numerous in vitro trials, the rate of cellular respiration is increased when HeNe laser or any other forms of radiation are exposed to mitochondria (Chung et al., 2012). Low-level laser therapy enhances osteoblastic and osteoclastic activities by raising the levels of ALP (alkaline phosphatase) / RANK (Receptor activator of nuclear factor-kappa B)/ RANKL (Receptor activator of nuclear factor-kappa B ligand)/ OPG (Osteoprotegerin). RANK and RANKL are associated with osteoclastic activity and OPG are involved in bone forming activities (Alazzawi, 2018). Biostimulatory effects on bone of low-level laser therapy is directly related to quantity of laser energy applied (Jawad, 2013).

2.8 Use of laser for therapeutic purposes

According to some previous studies, LASER has both excitatory and inhibitory effect on the process of wound healing (Kipshidze et al., 2001). However, it is believed that laser radiation on wounds has beneficial effect, because of the shortage of information regarding best healing parameters, mode of action and dosimetry, it is difficult to explain the exact mechanism (Coombe et al., 2001). According to Medrado et al, (2003) from his experiment it was found that laser irradiation increases collagen deposition and reduces inflammatory reaction and enhances proliferation of myofibroblasts on cutaneous wounds. It's also been suggested that the dose of laser at 8J/cm2 is more efficient than applying laser at 4J/cm (Medrado et al., 2003). Some investigators also noted that detrimental effect of laser at molecular level when HeNe laser was applied at the dose of 10J/cm2 but the application of same laser at the dose of 5J/cm2 increased the speed of wound healing by demonstrating the action of mitochondria which resulted in stimulation of the cell proliferation and migration of fibroblasts at the site of wound (Hawkins and Abrahamse, 2005); (Hawkins and Abrahamse, 2006)**.** GaAs laser produced stimulatory effect on proliferation of fibroblasts at 3J/cm2 (Pereira et al., 2002). From another report it was being suggested that Er:YAG laser produced best effects at dose of 3.37J/cm2 (Pourzarandian et al., 2005). In previous studies, it is found that entrance of light to a depth 0.5-50mm occurs at the wavelength of 630-780 nm and demonstrated excellent possibilities for wound healing (Moore et al., 2005). While, Karu et al (1993) stated that wavelength of light at 660nm being most effective (Karu et al., 1993). Numerous studies published that application of light at wavelength of 660nm improves healing of wound superficially, acne and skin conditions (Kipshidze et al., 2001).

The range of wavelength of light that is used for low-level laser therapy is 600-1070 nm. The range of wavelengths between 600-700 nm are used to treat superficial tissues, whereas longer wavelengths in the range between 780-950 nm affects deeper layers of tissues (Chung et al., 2012). The depth of penetration depends on wavelength of light (Bertoloni et al., 1993). When single dose 940nm wavelength of low-level laser (diode laser) was applied after third molar impaction surgery, it was observed that low-level laser therapy had positive impact on wound healing and also had anti-inflammatory effects (Eroglu and Keskin Tunc, 2016).

2.9 Doses for Low-Level Laser Therapy

The amount and wavelengths of low-level laser light application are important for its beneficial effects. Arndt Schultz law states that "little dosages stimulate the living system, moderate dosages delay and higher dosages destroy" (Ohshiro, 1988). Some researches reported the cellular effects of LLLT after its application, which are as follows (Al-Watban, 2000).

- Bio-activation occurring at $<.06$ J/cm^2 – zero
- Bio-stimulation occurring at 0.12-0.24 J/cm^2
- Bio-activation occurring at 0.24-0.30 J/cm^2 – zero

- Bio-inhibition (release of cellular singlet oxygen) at 0.30-0.60 J/cm^2

The size of laser light delivered to an aimed tissue is described as energy density, which is calculated in J/cm^2. To induce bio-stimulatory effect of laser therapy the energy concentration required in the range of 2-10 J/cm^2, depends on state of aimed tissue (Bjordal, 2008).

Following are the factors:

- Oral mucosa and tissues of oral cavity _2-3 J/cm^2
- Irradiation through the bone (focus – peri apical area) _ 2-4 J/cm^2
- Muscles outside oral cavity and around Temporomandibular joint _ 6-10 J/cm^2 (Bjordal, 2008)

2.10 Analgesic effect of low-level laser therapy

Often patient avoids or delay their dental treatment because of a past experience related to pain that was poorly managed during dental treatment (Mehlisch, 2002). According to previous researches, low-level laser therapy (LLLT) may have analgesic effect when applied clinically (Honmura et al., 1993). The process through which pain is relieved by laser therapy is still poorly understood. Some scholars believes that the dropping of pain may be caused by anti-inflammatory and neuronal effect of low-level laser therapy (Turhani et al., 2006), which includes nerve cell stimulation and lymphocytic respiration and stabilization of transmembrane voltage and discharge of chemical messengers, neurotransmitters in inflammatory tissue (Fork, 1971; Ponnudurai et al., 1987). At first, inhibition of the release of arachidonic acid which results in reduced quantities of PGE2 which is a powerful inflammatory facilitator (Mizutani et al., 2004; Angelieri et al., 2011; Bicakci et al., 2012). Low-level laser therapy has gained popularity for its analgesic effect as well but the exact mechanism through which pain is relieved is unknown. The following are some suggestions, which elaborate how laser irradiation reduces pain.

Laser energy when exposed to target tissue, may cause the discharge of beta-endorphin, which is opioid chemical messenger that is present within the body which has powerful analgesic effect (Arias and Marquez-Orozco, 2006). One more effect of low-level laser therapy is that it stabilizes transmembrane voltages and impedes the stimulation and transmission of pain messages to the brain (Sonesson et al., 2016). Hamba et al. reported that low-level laser thearapy demonstrated the inhibition of the discharges from the C-fibers of the connective tissue of tooth in reaction to stimulus of pain (Wakabayashi et al., 1993). A research on excised rat's sciatic nerve has concluded that even 4 to 8 joules per point can reduce the activity of sodium potassium ATPase and lessens the generation of action potential in small diameter unmyelinated C fibers (Kudoh et al., 1988).

In areas of muscle spasm and chronic inflammation, few enzymes are inactivated by hypoxia and acidosis. Laser energy can reactivate these enzymes for e.g. activation of super oxidase dismutase that can reduce the free radicals which is a source of pain (Bolognani and Volpi, 1992).

Lack of energy (ATP) also contributes to pain. Since low-level laser therapy increases the production of ATPs therefore it can contribute in the analgesic effect of laser through this mechanism (Reza et al., 2011).

There is strong evidence to support the successful use of low-level laser therapy in alleviating pain in various conditions like chronic joint disorders. Patients reported improvement in the pain related to osteoarthritis involving knee joints (Hegedűs et al., 2009). A systematic review of 16 randomized clinical trials on the effect of LLLT on cervical pain has shown positive results in both acute and chronic neck pain. Chronic cervical pain was significantly alleviated immediately after application of LLLT (Chow et al., 2009). Marked improvement in pain related to oral mucositis and cervical dentinal hypersensitivity has also been reported (Sandford and Walsh, 1994; Cauwels and Martens, 2011). A study was done to evaluate the photobiostimulatory effects of single dose of low-level laser therapy on orthodontic tooth movement and pain. It was found that single application of low-level laser therapy can reduce pain and accelerates tooth movement (Qamruddin et al., 2021).

2.11 Low-level laser therapy and wound healing

Low-level diode lasers are biostimulators, have anti-inflammatory, analgesic effects on acute and chronic pain and can stimulate wound healing (Batinjan, 2013). The mechanism of acceleration in wound healing by LLLT has been explained by its action on cells with low redox state. LLLT can change the pH of those cells from acidic to alkaline (Yamamoto, 1996). Nitric oxide (NO) which is produced in mitochondria, binds with Cytochrome c oxidase (COX) resulting in inhibition of cellular respiration. These injured cells are hypoxic, acidic and with low redox state (Brown, 1995) LLLT can photo-dissociate NO from COX and reverse the inhibition of cellular respiration resulting in change in pH, accelerating wound healing (Lane, 2006).

LLLT also enhances the cell proliferation of endothelial cells, fibroblasts, keratinocytes, and lymphocytes. Again, the mode of action is mitochondria dependent (Stadler, 2000). Photo-stimulation of the mitochondria causes upregulation of transcription factors giving rise to increase in growth factors, protein synthesis in nucleus and activation of enzymes (Saygun, 2012; Esmaeelinejad, 2013) regulated by increase in hydroperoxide anion and H2O2 free radicals (Pal, 2007; Sommer, 2015; Eslami, 2017).

2.12 Association of pain and wound healing

Wound healing following tooth extraction is a complex process that involves a highly coordinated interaction of cellular, molecular, biochemical, and physiological mechanisms. Wound healing occurs in four stages. At first, hemostatic phase occurs in which bleeding is stopped followed by inflammatory phase, proliferative phase and lastly remodeling phase (Politis, 2016). Several biologically active compounds such as cytokines, proteases and growth factors are produced during wound healing (Cooper, 1999; George, 2006). During the process of wound healing cytokines such as tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 (IL-1) can cause pain by stimulating nerve ending (Barrientos, 2008). Cytokines are the ligands or signaling molecules that have a vital role in the process of wound healing (Barrientos, 2008). Certain inflammatory cytokines such as IL-10 and IL-4 have been associated with pain behaviors and with the development of chronic pain (Zhang, 2007). Recent clinical studies suggest that increased levels of wound cytokines are related to increased pain during wound healing procedure (Gardner, 2017). Cytokines communication pathway includes numerous monitoring checkpoints, which frequently involve feedback inhibition, because of this process tissue tries comes back to its dormant non-inflammatory state (Hanada, 2002). Possible feedback inhibition mechanism involves negative regulation and dephosphorylation. Negative regulation occurs when proteins such as SOCS3 (suppressor of cytokine signaling 3), CIS (cytokine-inducible SH2-containing protein), SOCS1 (suppressor of cytokine signaling 1) sends negative signals in cytokine mediated signaling pathways. Dephosphorylation occurs by protein phosphatases which weakens the signaling mechanism and controls cytokine responses (Yoshimura, 2018).

Low level laser therapy also helps in regulating cytokines feedback mechanism by reducing pro-inflammatory cytokines. For instance, TNF-α and IL-1β. Excess of TNF-α and IL-1β can lead to extreme inflammation (Dos Anjos, 2019). A clinical trial was done on rats with the injury in spinal cord, LLLLT was applied and results showed that LLLT reduced inflammation and encouraged their recovery (Song, 2017). LLLT has positive effects on wound healing and cytokine regulation. Wound healing occurs in four stages. At first, cooagulation and hemostasis followed by inflammation then proliferation and then remodeling. LLLT acts on inflammatory phase by reducing the levels of pro-inflammatory cytokines which results in reduction in pain intensity during wound healing (Gardner, 2017). Overall, LLLT promotes wound healing at molecular level and reduce pain (Hussein, 2011).

2.13 Low-level laser therapy and post extraction pain and healing

The analgesic effect of LLLT on post extraction pain is controversial. Shenawy compared the effects of LLLT with the NSAIDs and recommended low power laser as the best modality to reduce post-operative pain after non-surgical extraction of lower third molars (El-Shenawy et al., 2010). More recently Hamid also used LLLT (GaAlAS) 810nm with 9J energy to reduce post lower third molar extraction pain and found significant results (Hamid, 2017). In contrast, other research showed no favorable effects in reducing pain when LLLT was applied after the extraction of impacted mandibular third molars. In a split mouth study, Mozatti applied superpulsed laser on the wound after extraction of molars and found significant acceleration in healing (Mozzati, 2011).

Extractions of pre-molars bilaterally is a common practice in orthodontics and the patients are mostly children who fear post-operative pain after removal of teeth. Prescription of analgesics is the common practice to make them comfortable after the extractions. Since today, only two studies have been conducted to evaluate the effect of LLLT on the pain and healing of the extraction wound of pre-molars in orthodontic patient, which reported no significant difference in pain and healing among lased and experimental sides supposedly due to insufficient sample size (Paschoal and Santos-Pinto, 2012). However, the other research found significant reduction in pain and faster wound healing in lased group when compared with cryotherapy and controls (John, 2020). Therefore, the purpose of this study is to evaluate the effects LLLT on pain and wound healing after pre-molar extraction in orthodontic patients.

2.14 Pre-molar extraction in orthodontics

Before doing orthodontic treatment, sound diagnosis and planning necessitate numerous complicated decisions. Initially, the demand for treatment needs to be significantly assessed. If orthodontic treatment is considered essential, the orthodontist then ought to decide, amongst other issues, if successful treatment will need tooth removal (Peck and Peck, 1979a). Extraction of tooth is of utmost importance in orthodontics to gain space in jaw to correct dental crowding and malocclusion (Jago, 1974). Nearly all extractions in orthodontics involved some combination of pre-molars (Keim et al., 2002). Pre-molar extractions are preferred because of its suitable position that is neither