Ozone is found in nature consisting of three oxygen atoms (O₃), a higher energetic form than normal atmospheric oxygen (O₂), discovered in 1840 [1,2]. Ozone is created when an oxygen molecule receives an electric discharge breaking it into two oxygen atoms. The individual atoms combine with another oxygen molecule to form an O₃ molecule. It is occurred in the environment either in gaseous form or as ozonated water [3]. Ozonated water is known as an antiseptic, powerful oxidant and strong antimicrobial agent towards bacteria, fungi, viruses and protozoa [4]. Ozone in the aqueous phase has advantages that are its potency, lack of mutagenicity, rapid microbicidal effects, ease of handling. Ozone has been advocated for treatment of gum infection (Sandhaus, 1969) [5] and root caries (Bayson et al, 2000).

Ozone has limitation as it is irritating to the respiratory system [6]. Very low concentrations may cause headache, irritation or dryness of the nose, throat and eyes [7]. Higher concentration may cause lung congestions, oedema, haemorrhage, changes to the blood and loss of vital lung capacity. It is irritating to the eyes and can cause redness, pain and blurred vision [8].

Effect of Ozone as Root Canal Irrigant
Ozone works best when there is less organic debris remaining. Therefore, the recommendation is to use either ozonated water or ozone gas at the end of the cleaning and shaping process. It is advised to use any conventional irrigants during the earlier phase and finally irrigate with ozonated water using ultrasonics and also ozonated oil as a medicament.

Mechanism of action of Ozone against microorganism
Ozone is a powerful oxidizing agent, far stronger than O₂. It is also unstable at high concentrations, decaying to ordinary diatomic oxygen. It has a varying half-life length, depending upon atmospheric conditions (temperature, humidity, and air movement).

\[ 2 \text{O}_3 \rightarrow 3 \text{O}_2 \]

This reaction proceeds more rapidly with increasing temperature and increased pressure. Stratospheric ozone is created and destroyed primarily by ultraviolet (UV) radiation. The air in the stratosphere is bombarded continuously with UV radiation from the Sun. When high energy UV rays strike molecules of ordinary oxygen (O₂), they split the molecule into two single oxygen atoms. The free oxygen atoms can then combine with oxygen molecules (O₂) to form ozone (O₃) molecules.

\[ \text{O}_2 + \text{UV light} \rightarrow 2 \text{O} \]
\[ \text{O} + \text{O}_2 + \text{M} \rightarrow \text{O}_3 + \text{M} \ (\text{where M indicates conservation of energy and momentum}) \]

The same characteristic of ozone that makes it so valuable – its ability to absorb a range of UV radiation – also causes its destruction. When an ozone molecule is exposed to UV energy it may revert back into O₂ and O. During dissociation, the atomic and molecular oxygens gain kinetic energy, which produces heat and causes an increase in atmospheric temperature. Ozone production is driven by UV radiation of wavelengths less than 240 nm. Ozone dissociation typically produces atomic oxygen (O) that is stable when exposed to longer UV wavelengths, up to 320 nm, and visible light wavelengths of 400-700 nm. Longer wavelength photons can penetrate deeper into the atmosphere, creating regions of ozone production and destruction. When an ozone molecule absorbs even low energy UV, it splits into an ordinary oxygen molecule and a free oxygen atom.

\[ \text{O}_3 + \text{UV, visible light} \rightarrow \text{O} + \text{O}_2 \]

The free oxygen atom may then combine with an oxygen molecule, creating another ozone molecule, or it may take an oxygen atom from an existing ozone molecule to create two ordinary oxygen molecules.

\[ \text{O} + \text{O}_2 \rightarrow \text{O}_3 \] or \[ \text{O}_3 + \text{O} \rightarrow \text{O}_2 + \text{O}_2 \]

Processes of ozone production and destruction, initiated by ultraviolet radiation, are often referred to as Chapman Reactions. Most O₃ destruction takes place through catalytic processes rather than Chapman Reactions. Ozone is a highly unstable molecule that readily donates its extra oxygen molecule to free radical species such as nitrogen, hydrogen, bromine, and chlorine. These compounds naturally occur in the stratosphere, released from sources such as soil, water vapor, and the oceans.

\[ \text{O}_3 + \text{X} \rightarrow \text{XO} + \text{O}_2 \ (\text{where X may be O, NO, OH, Br or Cl}) \]

Ozone is produced naturally by the following natural methods.
1. The first is from electrical discharges. Ozone is created when an oxygen molecule receives an electrical discharge breaking it into two oxygen atoms. The individual atoms combine with another oxygen molecule to form an O₃ molecule [9].

2. The second from ultraviolet rays emitted from the sun which plays the role of electrical discharge over oxygen present in stratosphere, thus creating the ozone layer which absorbs most of the ultraviolet radiation emitted by the sun [10].

Ozonisation: It is important to avoid the formation of chlorates. The mechanism of chlorate formation is:

\[
\text{O}_3 (\text{ozone}) + OCl \rightarrow 2\text{O}_2 + \text{Cl}^- \\
\text{O}_3 + OCl \rightarrow \text{O}_2 + \text{ClO}_2^- \text{ ionic products of ozonization of NaOCl} \\
\text{O}_3 (\text{ozone}) + \text{OCl}_2 \rightarrow \text{O}_2 + \text{ClO}_3^- 
\]

When NaOCl at pH 10–12 interacts with organic tissue it produces three reactions:

- Saponification, resulting in the creation of lipids
- Amino acid neutralization reaction
- Chloramination reaction

These three reactions are important in the disinfection of the root canal system. Studies have shown that the saponification reaction occurs only if we have enough free radicals. In our normal bleach solution, these free radicals are not found in high amounts, so the solution is called “lazy” because of its low saponification rate.

**Ozone as an Antimicrobial Agent**

Ozone is one of the most powerful antimicrobial agents available for use in medicine or dentistry [11]. As failure of root canal therapy is mainly caused by microorganisms, it is not surprising that there are enormous advantages to killing these pathogens. Numerous research have proved the antimicrobial effectiveness of ozone as a gas and as ozonated water [12,13]. Ozone has shown antimicrobial efficacy against resistant pathogens by neutralising them or preventing their growth. Hems examined the antibacterial effect of gaseous and aqueous ozone against E. faecalis in root canals. A significant reduction of the remaining bacteria was observed following the application of aqueous ozone. Nagayoshi et al advocated that ozonated water had almost the same antibacterial action as 2.5% NaOCl in endodontic therapy, particularly when used with the ultrasonics [4]. Huth et al also informed the possible advantages of employing ozone in root canal management in high concentrations [3]. Another study evaluated the capability of ozone to eradicate E. faecalis, observed that its antimicrobial effectiveness was not equivalent to that of NaOCl. Estrela et al described, ozone have no antimicrobial action against E. faecalis [14].

**Aqueous ozone Vs Gaseous ozone**

In dentistry, ozone has been used in either gaseous or aqueous form to eliminate microorganisms in root canals. Cardoso et al investigated the effectiveness of aqueous ozone to eradicate E. faecalis and Candida albicans from root canals. They demonstrated that aqueous ozone can eliminate bacteria.

One study, evaluated the efficacy of aqueous ozone against E. faecalis in bovines. The root canals were irrigated with 4 mg/L aqueous ozone for 10min. The root canal irrigation with aqueous ozone caused a considerable decrease in the amount of remaining bacteria.

In a study conducted by Ihsan Hubbezoglu, in which he used higher concentration of aqueous ozone with manual irrigation technique. As a result, a significant reduction of E. faecalis was detected in root canals. Nagayoshi examined the effect of aqueous ozone against E. faecalis and Streptococcus mutans in bovines. After aqueous ozone irrigation with an ultrasound technique for 10min, the
viability of E. faecalis and S. mutans invading dentinal tubules significantly reduced. Huth et al conducted a study to evaluate the effect of aqueous and gaseous ozone on the specific endodontic pathogens [19]. Aqueous ozone completely eliminated E. faecalis and C. albicans when used in concentrations down to 5 microgram mL⁻¹, whereas lower concentration reduced substantially but did not eliminate them totally. I need case of P. micros, aqueous ozone down to 2.5 microgram mL⁻¹ led to complete eradication. Ozone gas in concentrations down to the tested minimum of 1 gm-3 for 1 min almost completely eliminated the microorganism with a mean reduction of more than 99%[3].

In another part of the study, he tested the antimicrobial action of ozone against E. faecalis, C. albicans and P. aeruginosa mono species bio films. Application of aqueous ozone for 1 min was dose dependently effective against the microorganisms. When gaseous ozone was used, it's dose dependent effectiveness against different species were revealed. E. faecalis and C. albicans was almost eliminated by the highest gas concentration. Against P. aeruginosa, gaseous ozone 4 gm-3 was significantly less effective than NaOCl. Gaseous and aqueous ozone completely eliminated the tested pathogens.

Antimicrobial Efficacy of Ozone Vs Sodium hypochlorite
Ozone has a very good antimicrobial efficiency. A study have concluded that ozonated water had almost the equal antimicrobial effectiveness as 2.5% NaOCl for endodontic irrigation. They also showed low grade of toxicity against bacterial cells. Among the irrigating solutions, ozone has some interesting features; debriefing action, bactericidal effect, angiogenesis stimulation capability and high oxidising power.

Study by Hems et al found that NaOCl was superior to ozonated water in killing E. faecalis in both culture and biofilm [16]. Ibrahim and Abdullah studied that 1.31% NaOCl might allow passage of oxidation of ozonated water, thus increasing their antibacterial effect compared to 1.31% NaOCl or ozonated water alone [17].

Oxygen has a dramatically toxic effect to microaerophilic and anaerobic bacteria. Virtej and colleagues [19] compared the antimicrobial performance of four systems used as root canal irrigants. At the end of the study, the Endox device showed the least antibacterial effect with significant difference to MTAD and HealOzone. The authors concluded that ozone has great potential endodontic antimicrobial use and that MTAD and HealOzone seem to be effective as 3% NaOCl in reducing mixed bacterial infection in the root canal system [18].

Nagayoshi and colleagues [19] found nearly the same antimicrobial activity and a lower level of cytotoxicity of ozonated water as compared with 2.5% NaOCl. In the study, they examined the effect of ozonated water against E. faecalis and S. mutans infections in vitro in bovine dentin. When the specimen was irrigated with sonication, ozonated water had nearly the same antimicrobial activity as 2.5% sodium hypochlorite (NaOCl) [20].

Muller and colleague found 5% NaOCl superior to gaseous ozone in eliminating microorganism organised in a cariogenic biofilm. This study reported less than one log reduction of bacteria after using ozone gas above biofilms in culture media, which was only a similar reduction to that achieved by using 0.2% chlorhexidine or photo activated disinfection [21]. In vitro root canal contents and caries, unlike artificial biofilms, contain many molecules such as iron, which can increase the antimicrobial effectiveness of ozone in teeth and can help produce the powerful hydroxyl radicals in vivo to further increase the antimicrobial effectiveness of ozone.

Biocompatibility of Ozone Vs other irrigants
A high level of biocompatibility if aqueous Ozone on human oral epithelial (BHY) cells, gingival fibroblast (HGF-1) cells and periodontal cells have been published [22]. Huth and colleagues [23-26] investigated whether gaseous ozone and aqueous ozone exerted any cytotoxic effect on BHY cells and HGF-1 cells. Ozone was found to have toxic effect on both cell types. Essentially no cytotoxic signs were observed for aqueous ozone. NaOCl and H2O2 resulted in markedly reduced cell viability. As a result, aqueous ozone had the highest level of biocompatibility of the tested antiseptics. In addition, ozone gas applied into the moist root canal, as currently performed with Healozone device, dissolves in canal fluids, thereby resulting in aqueous ozone, which then enters into contact with tissues. Irrigation of the root surface of avulsed teeth did not reveal any negative effect on periodontal ligament cell proliferation [24]. As a result, the healing accelerating effect of ozonated water did not result in any detrimental effects on cells.

CONCLUSION
Many research are being carried out for proving the use of ozone as a root canal irrigant. Ozone is effective, cheap, easy and fast treatmet to help disinfect root canals. Ozone is much stronger than chlorine. Ozone is the most powerful antimicrobial and oxidant that can be used in endodontics, and as aqueous ozone revealed the highest level of biocompatibility compared with commonly used antiseptics. Ozone has a place in the 21st century oral health care and we should use its proven powerful antimicrobial efficacy and potent oxidant ability to reduce microorganisms during root canal therapy.

REFERENCES