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High Performance Liquid Chromatographic Method for Determination of Ketoprofen Concentration in Patient Extracted Impacted Third Molar

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ABSTRACT

Aims of the study: Evaluation of ketoprofen concentration by using (HPLC) and compare the efficacy of pre and postoperative effect of ketoprofen and clinical outcome on patients undergoing surgery of impacted third molar. ketoprofen was tablet 100 mg.

Materials and methods: The study was carried out on 39 patient aged (23.94±5.06) years and different sex (23 females and 16 males) . These subjects were divided into three groups as the following:

Group 1: The study group consists of (13) patients with impacted third molar operation received ketoprofen tablet (100mg), 30 minute preoperative,

Group 2: (13) patients with impacted third molar operation received ketoprofen tablet 100 mg 30 minute postoperative.
Group 3: (13) patients The placebo group with impacted third molar operation received glucose 30 minute postoperative as a control group. The blood plasma taken from patient after 1.5 hour of taken drug and undergoing chemical preparation to be measure the ketoprofen concentration by using (HPLC).And the clinical outcome (facial swelling, pain and trismus(mouth opening)) will be measured after 24 and 48 hour after operation. All groups will take paracetamol tablet post-operatively in the first 24 hour after surgery ,and we calculate the number of paracetamol tablet needed by each group. Data were analyzed using paired t-test, ANOVA, and Duncan multiple analysis range test.

Result: the result showed that the plasma concentration of ketoprofen by (HPLC) device when given post operatively the concentration of drug was (2.5±1.09 ppm) is much higher than the plasma concentration when the ketoprofen (0.25±0.236 ppm) is given pre operatively. The clinical outcome (pain ,swelling and trismus, patient sleep)show improvement when given the ketoprofen post operatively than pre operatively and control group. With lower number of analgesia (paracetamol) used when ketoprofen taken post-operatively than in patient take ketoprofen pre-operatively and in control group.

Conclusion: ketoprofen when given to patient with impacted third molar surgery as postoperative show a good plasma concentration and improvement in patient clinical outcome.

Key words: Ketoprofen, NSAIDs ,HPLC, Impacted third molar.

INTRODUCTION

A surgical trauma in the oral cavity always causes tissue injury characterized by hyperemia, vasodilatation, increased capillary permeability with liquid accumulation in the interstitial space and granulocyte and monocyte migration (1,2) due to the increased osmotic pressure in capillaries (Starling law). Edema is the expression of exudates or transudation, and in surgery, probably both the events occur.(3)Transudation in fact is secondary to blood flow slowing (i.e. hyperemia, vasodilatation, stenosis, etc.), while a superimposed infection is responsible for exudates(4). Extension of the incision as well as tissue manipulation and length of surgery could affect the entity of swelling. According to previous published data, postoperative swelling and pain are significantly lower following a smaller incision(5,6)When impacted third molars are removed, post-surgery is characterized by limitation in the mouth opening, pain, reduced

masticatory capability and swelling of variable degree(7). The latter represents a serious issue as it affects the ability of the patient to interrelate and to return to the routine working life, especially during the first 3 days following oral surgery(8,9)The introduction of Non-steroidal anti-inflammatory drugs (NSAIDs) e.g. ketoprofen has significantly altered the management of postoperative pain in dentistry(10). Ketoprofen is The NSAIDs are a group of chemically dissimilar agents that differ in their antipyretic, analgesic, and anti-inflammatory activities. They act primarily by inhibiting the cyclooxygenase (COX) enzymes that catalyze the first step in prostanoid biosynthesis. This leads to decreased prostaglandin synthesis with both beneficial and unwanted effects. All of the NSAIDs act by inhibiting the synthesis of prostaglandins (11,12)

MATERIALS AND METHODS

The study was carried out on 39 patient undergoing impacted third molar surgery, aged (23.94±5.06) years either sex(23 females,16 males) patients. All patients were healthy and they had no history of any systemic diseases. the patients attended to Oral and Maxillofacial Department/ Periodontal unit in College of Dentistry at University of Mosul, the period was from 29/9/2013 to 1/5/2014 diagnoses were established according to the clinical findings, and diagnosed with impacted third molar.

Patients were divided into 3 groups:

1. Group 1 consisted of thirteen patients with impacted third molar surgery received Ketoprofen tablet (100mg) 0.5 h pre operatively and then after 1.5 hour we take a blood sample.

2. Group 2 consisted of thirteen patients with impacted third molar surgery, received Ketoprofen tablet (100mg) 0.5 h post operatively and then after 1.5 hour we take a blood sample.

3. Group 3 consisted of 13 patients with impacted third molar surgery, received (100mg) glucose 0.5 h post operatively. after 1.5 hour we take a blood sample

We given analgesia (paracetamol) to all groups post operatively and then calculate the number of paracetamol needed in the first 24 hour to measure the intensity of pain for each group.

Facial swelling measurement:

Facial swelling was analyzed by comparing the two sides of the patient's face. Four facial measurements using plastic-measuring tape to approximate millimeters were assessed to be a baseline data for recording of facial swelling (objective). These facial measurements included the following; Tragus-pogonion (Figure 1), Tragus-commisure (Figure 2), Gonion-Pogonion (Figure 3), and Gonion-lateral canthus (Figure 4).

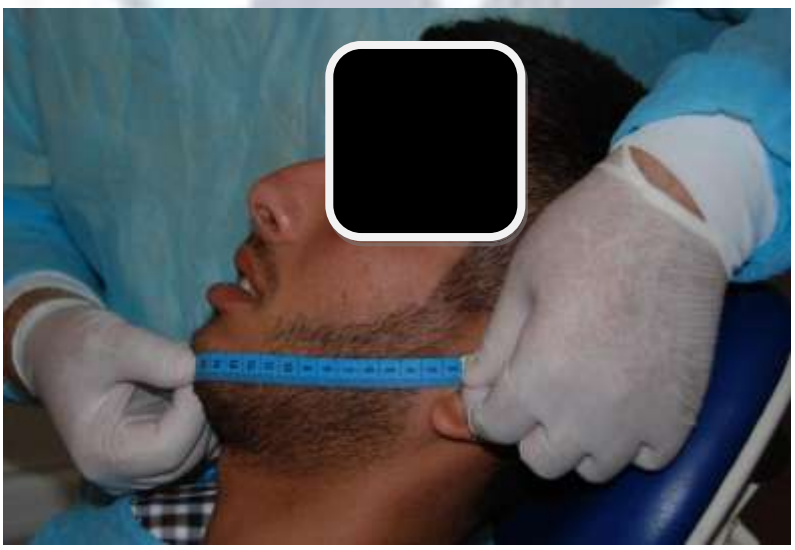

Figure (1): Tragus-pogonion measurement.

Figure (2): Tragus-commissure measurement.

Figure (3): Gonion-pogonion measurement.

Figure (4): Gonion-lateral canthus measurement.

Trismus was determined by checking the extent to which the patient could open his/her mouth. Mouth opening (Trismus) was assessed by recording the pre- and postoperative maximum mouth opening, measured as the distance from the incisal edge of upper central incisors to lower corresponding ones. Trismus assessment was done by electronic digital vernier (Figure 5).

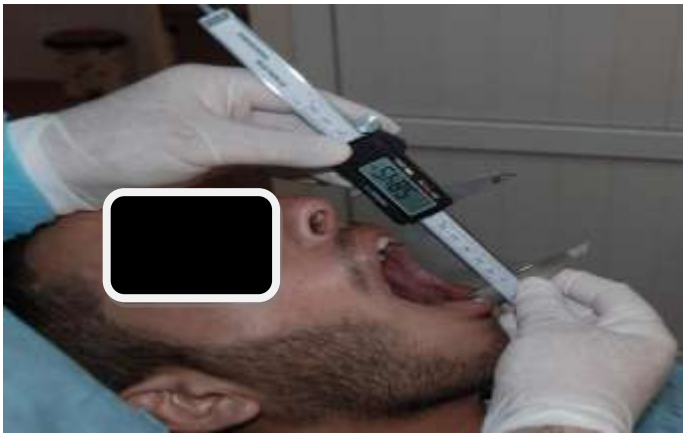

Figure (5): Maximum mouth opening measurement.

Chemical procedure for blood sample preparation for HPLC:

Five ml of blood sample was collected for two group before (pre-operative) and after (postoperative) surgical operation. A rubber tourniquet was placed around the upper arm, scrubbed site of injection several times with alcohol-saturated cotton, and allows drying. A venopuncture occurred with sterile disposable syringe via sterile needle through the skin to the vein and allowed blood to draw inside syringe. The blood sample was transferred to a sterilized plane heparinized tube and allowed for 30 minutes for clotting then centrifuged at 3000rpm for 10 minutes in centrifuge, to isolate the plasma from whole blood. Protein was separated using plasma and acetonitrile in ratio of 1:1;this mixture was then vortex for 10 minutes ,centrifuged at 3000 rpm for 10 minutes. Supernatant was then filtered by 0.45 micrometer membrane filter. The obtained plasma was then used to produce the desired concentrations. The samples was transferred by micropipette to Eppendorf tube and stored in deep freeze at -20°C to be thawed for analysis by HPLC.(High Performance Liquid Chromatographic system (LC 20A, Shimadzu Corp, Japan).

All procedures were carried out under local anesthesia. The patients were given appropriate postoperative instructions. The post-operative complications were recorded based on history and clinical examination. Radiographs if necessary were taken. **Visual analog scale** was used for pain, Vertigo and effect of surgery on **patient sleep** according to scale which was divided into three grades, mild (1-3), moderate (4-7) and severe(8-10). **Facial swelling and Trismus.**

Statistical analysis

The data were expressed as mean ± SD , difference between three experimental groups were statistically analyzed by using paired t-test , one way analysis of variance (ANOVA) followed by Duncan multiple analysis range test. The level of significance was at $p < 0.05$.

RESULTS

The results showed that the of plasma concentration of ketoprofen (**Standard** was ± ppm) and the of area under the curve was (±) at ($p \leq 0.05$) While the concentration and area under the curve of c**ontrol group** was (0.00±037p.p.m) and (0.00±0.31) respectively. The plasma concentration of ketoprofen (**Preoperative** was 0.25±0.23 ppm) and the mean of area under the curve (2.83±0.931) that significantly difference from the plasma concentration of ketoprofen (**Postoperative** 2.5±1.09 p.p.m and from the area under the curve of postoperative ketoprofen (8.45±0.224)) respectively. . As in Figure(1,2,3,4) and Table(1)

The mean and SD of intensity of pain by **Visual analog scale** registered by the patient feeling of pain after 24and 48h from surgery were lower in patient take ketoprofen (2.9±1.75 and 4.7±1.36 respectively) as **postoperative** than the mean of patients take ketoprofen as **preoperative** 24 and 48h (5±3.21 and 5.9±1.49 respectively) and with **control group** 24 and 48h (6.5±1.53 and 7.9±2.54) respectively at ($p \leq 0.05$).while no found any significant difference between all group after 72h. **Table (2)**.

The number of analgesic tablet (paracetamol) taken by the patient is significantly lower in patients take ketoprofen **postoperative** (2.12±0.64) tablet and in **preoperative** ketoprofen patients group was (2.93±1.45) than in **control group** was (3.76±1.02) at ($p \leq 0.05$) **Table(3)**

The mean and SD of **trismus** (Mouth opening) in millimeter in the first 24 and 48hour after surgery which showed the lower level and significant difference with patients take ketoprofen as postoperative than those patients take ketoprofen

as preoperative and with patients in control group while no significantly difference between preoperative and control group. ($p \leq 0.02$). **Table (4)**

The mean and SD of measurement in first 24 and 48hour after surgery , swelling parameters in postoperative ketoprofen taken (**Tragus-Pogonion) were** (11.53±0.90 and 11.34±0.94) centimeters respectively and for preoperative was (12.56±0.95 and 11.96 ±0.98) centimeters respectively and for control group were (13.73±1.07, 12.96±1.05) centimeters respectively.

Tragus-Commissure for postoperative 24h and 48 h in postoperative ketoprofen taken were (15.38±1.06 and 14.79±1.16) centimeters respectively and for preoperative 24h and 48 h were (16.00±1.19 and 15.57±1.036) centimeters and for control group 24 and 48 h were (16.96±1.128 and 16.03±1.025) centimeters respectively.

Gonion- lateral for postoperative ketoprofen group after 24hand 48 h were (11.34±1.42 and 10.76±1.34) centimeters respectively and for preoperative 24 and48h were (12.19±0.77 and 11.61±.78) centimeters respectively and for control group 24h and 48h (12.53±1.02 and 1.95±1.109) centimeters respectively.

And Gonion- pogonion for post-operative ketoprofen group 24h and 48h were (14.30±0.96 and 13.73±1.012) centimeters respectively and for preoperative 24 and 48h were (14.86 ±1.265 and 13.90±1.24) centimeters respectively and for control group 24 and 48h were (15.69 ±0.95 and 14.88±1.01) centimeters respectively.

From the above swelling parameters, we showed that the lower level and significant difference with patients take ketoprofen as postoperative as the patients take ketoprofen as preoperative and with patients in control group. **Table (5,6).** The mean and SD of effect of surgery on patient sleep is lower in patients take ketoprofen as a **postoperative** than in **preoperative** ketoprofen patients group and with patients in **control group** at $p \leq 0.05$. **Table (7)**

DISCCUSION

Ketoprofen is a non-steroidal anti-inflammatory drug (NSAIDs) and these group of drugs are structurally diverse group of agents with analgesic, antipyretic and anti-inflammatory properties. The primary mechanism of action of these drugs is believed to be the inhibition of enzymes of the cyclo-oxygenase (COX) family, which are involved in the biosynthesis of prostaglandins.(13,14) Ketoprofen is a member of the aryl propionate group of NSAIDs, and has well established analgesic and anti-inflammatory effects.(15)

In this study the plasma concentration of ketoprofen high significant when given post operatively to patient have impacted third molar surgery than when given ketoprofen as preoperative drug, this study is in agreement with a previous studies which suggest that the impacted third molar surgery correlated with inflammation result in rapid elevation of pro inflammatory mediator like prostaglandin, leukotrines chemokines and cytokines like IL and TNF(16).

The biosynthesis of Prostanoids as a pro inflammatory mediater will highly elevate as a result of infection, truma and inflammation which is a direct result of impacted third molar surgery.(17) Prostanoids (i.e., prostaglandin [PG]E_2, PGD_2, $PGF_{2\alpha}$, thromboxane A2 [TXA_2] and prostacyclin [PGI_2]) are special second messengers owing to their ability to cross the cell membrane, diffuse through the extracellular space, and interact with high-affinity G-protein-coupled receptors located on the same cell or in neighboring cells.(18) Prostanoids play important roles in many cellular responses and pathophysiologic processes, such as modulation of the inflammatory reaction and its resolution, erosion of cartilage and juxta-articular bone, GI cytoprotection and ulceration, angiogenesis and cancer, hemostasis and thrombosis, renal hemodynamic and progression of kidney disease, as well as other protection and progression of atherosclerosis.(19).

By this study, the plasma concentration of ketoprofen is inversely correlated with pain and inflammatory response and other patient clinical outcome after the impacted third molar surgery so: By this study the higher plasma concentration of ketoprofen when given as postoperative drug improve patient clinical condition like: The patient suffer a less pain, swelling, .trims, vertigo, less feeling sleep disturbance than the patient take ketoprofen pre operatively and then the control group. us is, due to the role of ketoprofen in improvement of patient clinical outcome after the impacted third molar surgery, this may be return to increase the concentration of ketoprofen when given postoperative of the surgery.

CONCLUSION

By HPLC method We found that the blood plasma concentration of Ketoprofen when giving postoperatively is more than blood plasma concentration of Ketoprofen when giving as preoperatively and this result is supported by measuring the clinical outcome (swelling, pain, and trismus, patient sleep and vertigo) and inflammation associated with impacted third molar surgery .

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FIGURES USED:

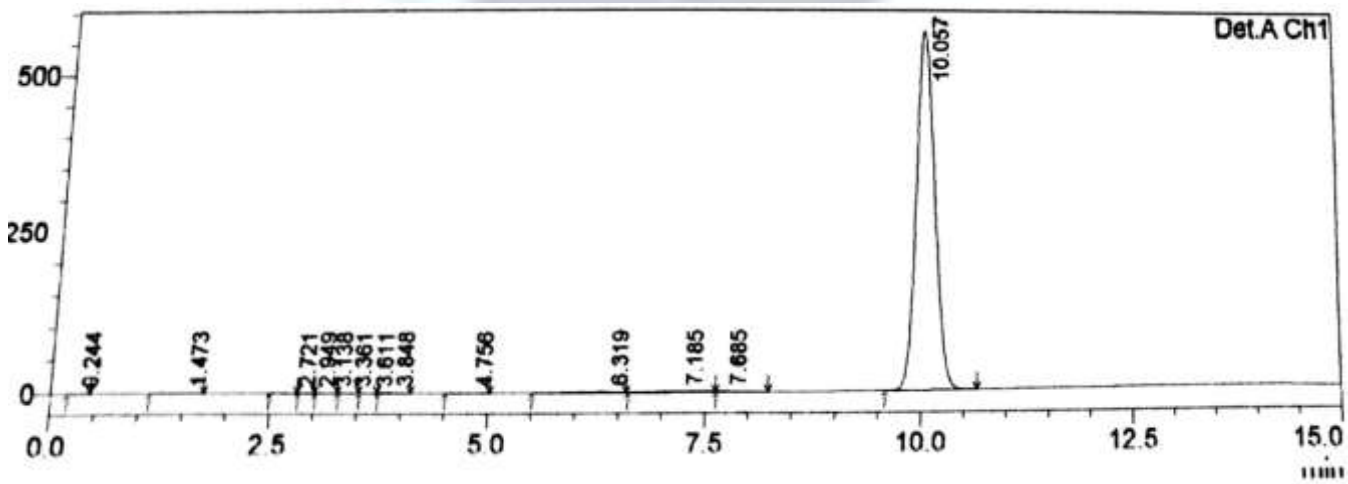

Figure 1: (standard ketoprofen measurement by HPLC)

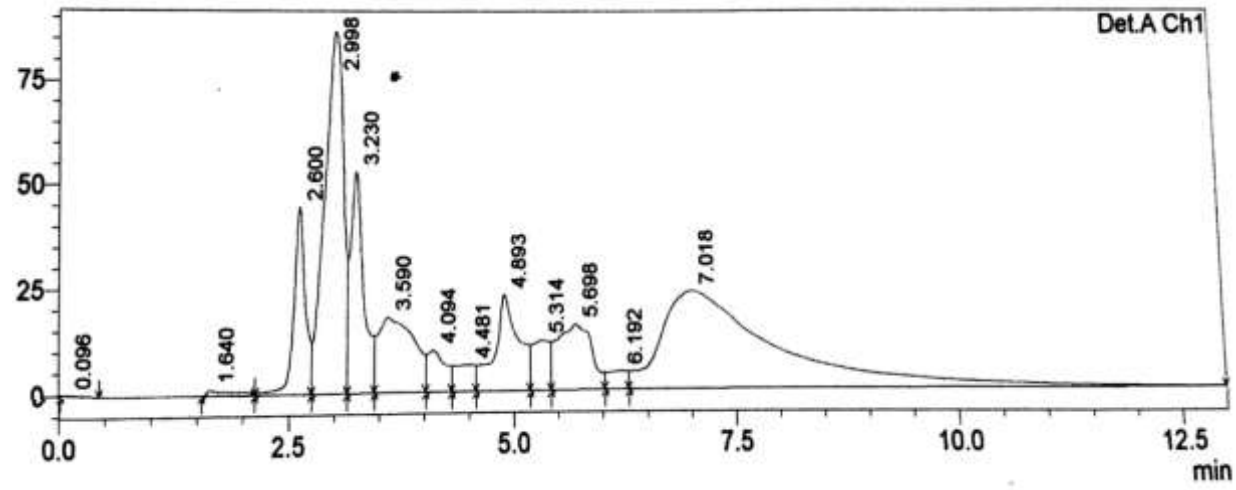

Figure 2: (control measurement obtained by HPLC)

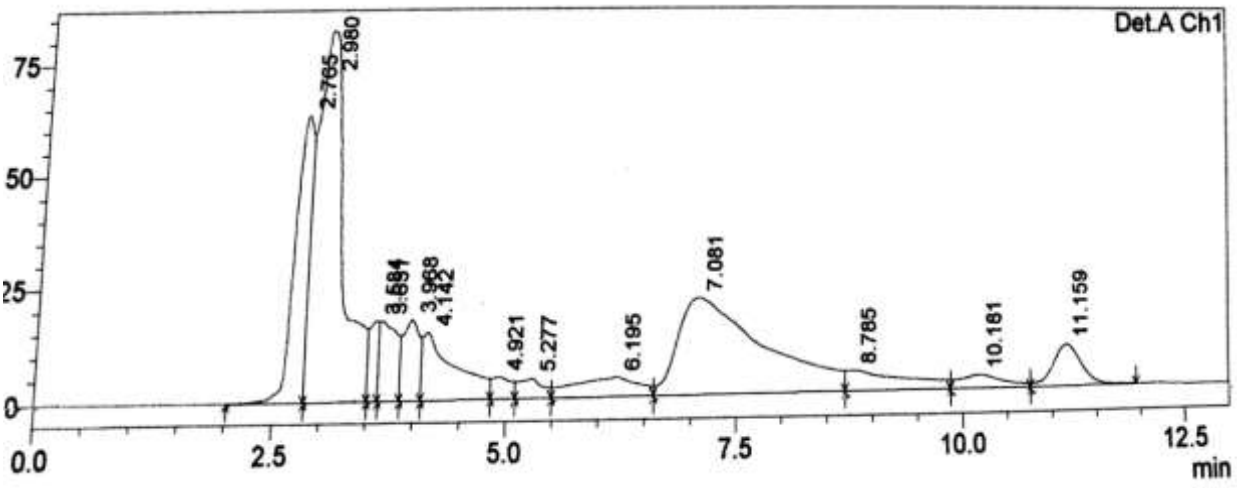

Table 3: (pre operative measurement of ketoprofen obtained by HPLC)

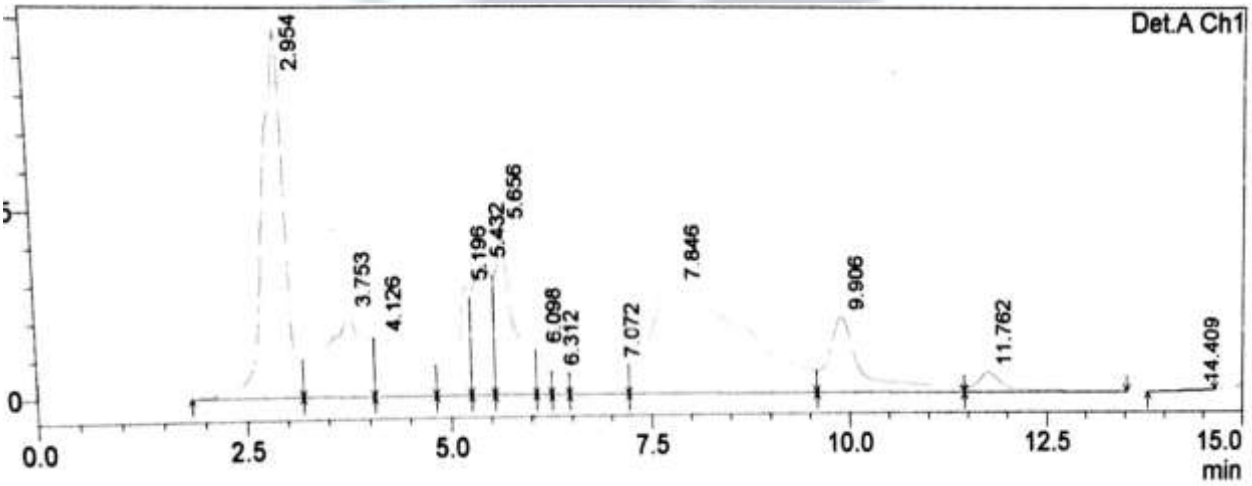

Table 4 : (post operative measurement of ketoprofen obtained by HPLC)

Table(1): Comparison between Concentration (p.p.m) and area under the curve (AUC) of ketoprofen in pre and postoperative and control groups.

Groups	concentration(p.p.m)	Area under the curve(AUC)
control	0.00±0.37	0.00±0.31
pre operative	0.25±0.23*****	2.83±0.93
post operative	2.58±1.09***a**	8.45±0.22

Value are Mean ±SD for three groups.
***** significant with control at p≤0.05.
a: significant with postoperative group at p≤0.05.
ppm: part per million

Table (2): Pain intensity score (Visual analog scale)

Groups	Pain score after 24hr	Pain score after 48hr
Control	6.53±1.53	7.92±2.54
Pre operative	5.00±3.21	5.92±1.49
Post operative	2.92±1.75***a**	4.76±1.36*****

Value are Mean ±SD for three groups.
***** significant with control at p≤0.05.
a: significant with postoperative group at p≤0.05.

Table (3): Number of analgesia tablet (paracetamol)

Groups	number tablet (paracetamol)
Control	3.76±1.02
Pre operative	2.93±1.45
Post operative	2.12±0.64*a

Value are Mean ±SD for three groups.
***** significant with control at p≤0.05.
a: significant with postoperative group at p≤0.05.

Table(4): (Trismus) : Measurement of Mouth opening in Millimeter (mm) after 24 and 48 of surgery

Groups	mouth opening **in Millimeter (mm) after** 24 hour	mouth opening **in Millimeter (mm) after** 48 hour
Control	22.04±1.03	30.22±1.07
Pre operative	23.03±1.06	31.57±1.0167
Post operative	25.33±0.96*	33.93±0.62*

Value are Mean ±SD for three groups.
***** significant with post operative at p≤0.05.

Table (5): (SWELLING) Measurement of swelling (Tragus-Pogonion, Tragus-Commissure) In (centimeters **CM)**

Groups	Tragus-pogonion Measurement in 24Hour C.M	Tragus-pogonion Measurement in 48Hour C.M	Tragus commissure measurement24Hour in C.M	Tragus commissure measurement48 Hour in C.M
control	13.73±1.07	12.96±1.05	16.96±1.12	16.03±1.25
Ketoprofen pre-operative	12.56±0.95	11. 96 ±0.98	16.00±1.19	15.57±1.33
Ketoprofen post-operative	11.53±0.90	11.34±0.98	15.38±1.06	14.79±1.16

* means significant difference at ($p \leq 0.05$).

Table (6): (SWELLING) Measurement of swelling (Gonion latera, Gonion pogonion) In (centimeter CM)

Groups	Gonion lateral Measurement in 24Hour C.M	Gonion lateral Measurement in 48Hour C.M	Gonion pogonion measurement 24Hour in C.M	Gonion pogonion measurement48 Hour in C.M
control	12.53±1.02	11.95±1.10	15.69 ±0.95	14.88±1.01
Ketoprofen pre-operative	12.19±0.77	11.61±0.78	14.86 ±1.26	13.90±1.240
Ketoprofen post-operative	11.34±1.42***a**	10.76±1.342***a**	14.30±0.96	13.73±1.012

Value are Mean ±SD for three groups.
***** significant with control at p≤0.05.
a: significant with postoperative group at p≤0.05.

Table (7): Effect of surgery on patient sleep

Groups	Effect on patient sleep	Frequency	Percent %	Mean & SD Of effect on sleep
control	**Mild**	1	7.7%	2.00±0.70
	Moderate	4	30.7%	
	Sever	8	61.6%	
	Total	13	100%	
Ketoprofen pre-operative	**Mild**	2	15.4%	1.84±0.53
	Moderate	7	53.9%	
	Sever	4	30.7%	
	Total	13	100%	
Ketoprofen post-operative	**Mild**	6	46.3%	1.2308±0.4385*a
	Moderate	5	38.3%	
	Sever	2	15.4%	
	Total	13	100%	

Value are Mean ±SD for three groups.
***** significant with control at p≤0.05.
a: significant with postoperative group at p≤0.05.