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Comparison of The Efficiency of Pulsed Electromagnetic Field and Transcutaneous Electric Nerve Stimulation in Reducing Pain During Initial Orthodontic Teeth Alignment: A Single-Blind, Randomized Control Trial

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ABSTRACT

OBJECTIVES: To collate the efficiency of Transcutaneous Electric Nerve Stimulation (TENS) and Pulsed Electromagnetic Field Therapy (PEMF) during the initial phase of the orthodontic treatment in alleviating the pain. **MATERIALS AND METHODS:** The split mouth randomized, prospective, clinical trial was performed in forty-eight patients, at the start of the orthodontic treatment. Randomization was done twice, one for allotment of the type of intervention and second, for selection of the experimental and control side. After placement of initial 0.014 Niti arch wire, Transcutaneous electric nerve stimulation was done by the clinician on the dental chair for patients in TENS group and two nights of pulsed electromagnetic field therapy were prescribed for those in PEMF group. Pain scores were assessed with the Numerical rating scale at 0, 2, 6, 24 and 48 hours. **RESULTS:** Maximum pain was discovered at 6 hours in PEMF experimental group, whereas the TENS group had most pain at 2 hours. The pain scores in TENS and PEMF when compared, were found to be statistically identical at every time interval. **CONCLUSION:** Both TENS and PEMF have been shown to be viable methods for managing orthodontic pain that could be effectively used by the patients with very minimal side effects

KEYWORDS: Orthodontic Pain, Transcutaneous Electric Nerve stimulation, TENS, Pulsed Electromagnetic field, PEMF, Initial alligment.

INTRODUCTION

Pain is a highly unpleasant feeling that occurs as a result of noxious stimuli. Fear of discomfort remains the most prevalent reason for not undergoing orthodontic treatment. Various studies have documented that physical discomfort during orthodontic treatment had a detrimental impact on patient's compliance during the treatment and their quality of life. Despite its significant clinical significance, Krukemeyer et al reported that orthodontic pain is largely disregarded and under appreciated along with the number of patients who used analgesics between the appointments.

Various orthodontic procedures starting from the separator placement, placement of initial alignment and levelling wire, headgear or facemask application have all been found to be associated with some

amount of pain. Stress on teeth causes an inflammatory response with pain and bone resorption, which is necessary for tooth movement.1-3 First week immediately after treatment with initial arch wires, the patients are uncomfortable and experience pain due to the force application and it gradually reduces to normal levels in 7 days. So, intervention during this stage prevents treatment discontinuation and enhances patient co-operation.

Different methods have been proposed to relieve orthodontic pain ranging from oral analgesics,4 plastic wafers,5 anaesthetic gels, xylitol chewing gums,6 vibratory stimulation of the periodontal ligament,7 transcutaneous electric nerve stimulation, Low-level laser therapy7 and Pulsed electromagnetic field. Although the pharmacological approach is found to be the most effective, Nonsteroidal anti-inflammatory medicines (NSAIDs) disrupt the osteoclastic mechanisms responsible for tooth movement and reduce the efficacy of orthodontic treatment. So, the non-pharmacological methods of pain reduction play an important role in enhancing the treatment.

Among the non-pharmacological methods available, Pulsed electromagnetic field (PEMF) and Transcutaneous Electric Nerve Stimulation (TENS) devices are gaining more importance as they are user-friendly and have minimal side effects. TENS generates an electrical stimulation that is quicker than a pain impulse which reaches the substantia gelatinosa in the dorsal horn, closing the pain gate and reducing pain intensity. Initially, Roth and Thrash devised a nonpharmacological, non-invasive TENS technique that used large external sponge-pad electrodes or internal probe electrodes. Although this device was effective in reducing periodontal pain after separator placement, they were relatively large and expensive.8 Since then, portable and less priced TENS devices have been developed to effectively manage orthodontic pain.

PEMF is a non-invasive treatment that lowers pain and swelling by generating 'short bursts of current' without affecting the body's main physiological functions. PEMF is widely used in the fields of orthopedics and plastic surgery to treat pain, inflammation, and bone repair following surgery.9-14 PEMF devices have been effectively utilized in dentistry to control pain caused by TMJ dysfunction,15 as well as post-operative pain management and soft tissue healing following third molar extractions.16 Only one Randomized clinical trial in the orthodontic literature looked at the efficiency of PEMF after the initial 0.014 NiTi arch wire was placed, and it found PEMF to be useful in lowering discomfort following arch wire placement.

However, no literature is available to compare the efficacy of these two important modalities in reducing orthodontic pain. So this clinical trial aimed to compare the effectiveness of these two non-pharmacological methods in reducing the pain during the initial arch wire placement.

MATERIALS AND METHODS

The study was approved by the Ethical committee of Meenakshi Ammal Dental College (MADC/IRB-XXXI/2019/491). The randomized Prospective Clinical trial was done in a split-mouth design in the Department of Meenakshi Ammal Dental College, Chennai from 2020 to 2022

Patient selection

Patients, ranging in age from 16 to 24, reporting to the department in need of orthodontic treatment were selected. All of the subjects had routine dental exams and had good oral hygiene. Anterior crowding in the lower arch was estimated using vernier calliper (Figure 1) and patients who had moderate to severe (4-9 mm) anterior crowding in the lower arch, according to Little's irregularity index18 were selected

(Table 1). The study protocol was verbally explained and a consent form was obtained from the patients and only those willing were included in the study.

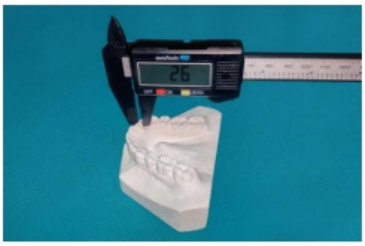


Figure 1: Vernier caliper used to assess crowding in dental cast

Table 1: Little's irregularity index - Scoring

DEGREE OF CROWDING	LITTLE'S INDEX
0 mm	Perfect alignment
1-3 mm	Minimal crowding
4-6 mm	Moderate crowding
7-9 mm	Severe crowding
10mm >	Very severe crowding

The following were the criteria for inclusion:
• Patients in the age group between 16 to 24 years
• Patients receiving fixed orthodontic treatment
• Patients in the initial stage of orthodontic treatment
• Patients who had good periodontal status
• Patients who had moderate to severe crowding

The following were the criteria for exclusion:
• Presence of local infections or any other dental pain
• Patients under pain or anxiety medications
• Patients with cardiac pacemakers and cardiac arrhythmias
• Patients with systemic diseases
• Epileptic patients

SAMPLE SIZE

G power version 3.1.9.2 of the Sampling programme was used to evaluate the sample size. Effect size (Cohen d) was calculated from the mean difference in the test groups for mean values. The sample size was evaluated to be 24 per group with an alpha (type 1 error) of 5% and power of the study (type 2 error) as 95%

RANDOMIZATION

Sealed envelopes were used for randomization. Randomization was done twice, both for the selection of the device and for the selection of the experimental and control sides.

INTERVENTION

The trial was carried out at the start of the orthodontic treatment with initial arch wire – 0.014 Niti (G4TM Niti EuropaTM Form I; G&H Wire Company, Franklin, USA) with 0.022 x 0.028" slot MBT prescription brackets (Mini DiamondTM; ORMCO Corporation, California, USA).

TRANSCUTANEOUS ELECTRIC NERVE STIMULATION

The TENS device used in this study is designed by UltraCare PRO (Zealmax Innovations Pvt. Ltd, Gujarat, India).(Figure 2) It is a dual-channel rechargeable TENS unit made for pain relief and had a compact, portable design made for easy operation with four reusable self-adhesive electrode pads to be used, two on each side. The device produced a rhythmic pulse and a maximum current of 10mA with a net neutral charge at a pulse rate of 90hz and a pulse width of 200uS. Patients were informed that they would be testing a pain-relieving gadget that delivered a modest electric current and were also told that the stimulus might be anything from sub-sensory to a little tingle.

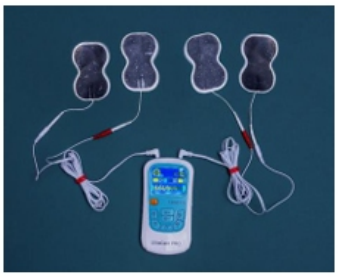


Figure 2: Transcutaneous Electric Nerve Stimulation

Following randomization immediately after the placement of the arch-wire, for those patients who had chosen TENS, two electrodes were placed on the experimental side and two electrodes on the contralateral side.(Figure 3)Electrical impulses were generated only on the experimental side for two intervals of 5 minutes with a 2-minute break in between the two applications.

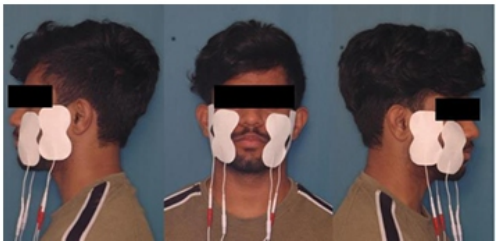


Figure 3: TENS electrodes placed on the cheeks ion device (UltraCarePRO) used in the study

PULSED ELECTRO MAGNETIC FIELD

The PEMF device utilized in the study was ActiPatchTM (Bioelectronics Corporation Ltd, USA). It was a tiny, portable device that weighed about 8 g and had a pulse rate of 1,000 pulses per second with each pulse lasting 100 microseconds. The device (Figure 4) had an antenna that was 12cm in diameter, with a treatment area of 100 sq. cm, a carrier frequency of 27.12 MHz, a 720-hour on/off capacity and a power of 73 microwatts per cm2. The device has an active power unit that generates the electromagnetic waves and a wire loop that transmits these waves over the loop area.

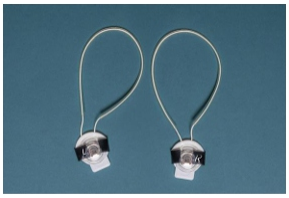


Figure4:Pulsed Electro Magnetic Field device(Actipatch^{TM})used in the study.

The devices were marked as R and L for the right and left sides respectively and after randomization, the control PEMF device was rendered inert by interposing a translucent sheet between the circuit and the battery supply, which served as a placebo. The LED lights on all devices were covered with the same-coloured tape, making it impossible to tell which gadgets were experimental and which were placebo. Patients were requested to wear the device extra orally, bilaterally on the cheeks with bio-adhesive tapes for 8 hours a day for two consecutive days(Figure 5) Patients were shown how to handle and use the gadget and were told to stick to the survey schedules religiously.

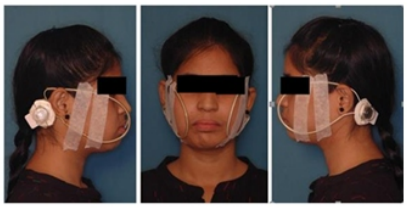
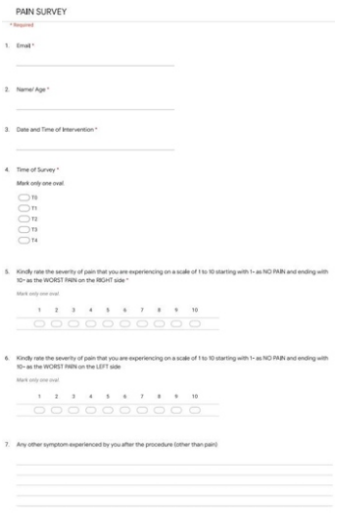


Figure 5: PEMF device placed on the cheeks bilaterally using bio-adhesive tapes

SURVEY

Pain evaluation was done over a period of 48 hours for a total of five times each. The first assessment was made immediately after the arch-wire placement at 0 hours (T0), and then subsequent assessments were made at 2 hours (T1), 6(T2), 24 (T3), and 48 (T4) hours after the first evaluation for both the groups.

Throughout the trial, a Google survey form was established for the goal of collecting data on the patient's pain perception.(Figure 6) To ensure that replies were collected at precise time intervals, the patient was provided a link to the survey form by SMS and Whatsapp. The pain measure utilized was the Numeric Rating Scale, which ranged from 0 to 10, with 0 indicating no pain and 10 indicating the most severe pain possible.


PAIN SURVEY

* Required

1. Email *

2. Name/ Age *

3. Date and Time of Intervention *

4. Time of Survey *

Mark only one oval.

T0
T1
T2
T3
T4

5. Kindly rate the severity of pain that you are experiencing on a scale of 1 to 10 starting with 1- as NO PAIN and ending with 10- as the WORST PAIN on the RIGHT side *

Mark only one oval.

1 2 3 4 5 6 7 8 9 10

6. Kindly rate the severity of pain that you are experiencing on a scale of 1 to 10 starting with 1- as NO PAIN and ending with 10- as the WORST PAIN on the LEFT side

Mark only one oval.

1 2 3 4 5 6 7 8 9 10

7. Any other symptom experienced by you after the procedure (other than pain)

Figure 6: Pain survey form used in the study

RESULTS

STATISTICALANALYSIS

The data were tabulated in Microsoft Excel 2010 and statistical analysis was performed in statistical package version 4.1.1 (10 - 08- 2021 release) from Core Team (2021): A language and environment for statistical computing' Foundation for Statistical Computing, Vienna, Austria. Descriptive statistics were given by Mean, Standard Deviation, Minimum, Maximum and Mode. Control and Test groups were compared using Mann Whitney U test (PEMF vs PEMF Control; TENS vs TENS Control). The pain score over five different time periods – T0, T1, T2, T3,
T4 for every group were analyzed by Friedman's repeated measures ANOVA with post hoc Conover test. PEMF and TENS at every time interval were compared using Mann Whitney U test. P value less than 0.05 was considered significant.

Descriptive Statistics

Table 2. Pain scores in PEMF Experimental Group

	T0	T1	T2	T3	T4
Mean	2.5652	3.3478	3.6087	2.8696	2.4783
SD	1.9265	2.0362	2.0832	1.2175	1.2011
Min	1	1	1	1	1
Max	7	7	7	5	5
Median	2	3	3	3	2
Mode	1	2	2	2	2

Table 2 shows the descriptive statistics of pain scores in the PEMFexperimental group. Maximum pain was found in T2 time interval.

Table 3. Pain Scores in PEMF-Control Group

	T0	T1	T2	T3	T4
Mean	2.5652	3.3478	3.6087	4.0000	4.4348
SD	1.9265	2.0362	2.0832	1.3143	1.5905
Min	1	1	1	2	2
Max	7	7	7	7	7
Median	2	3	3	4	4
Mode	1	2	2	5	4

Table 3 shows the descriptive statistics of pain scores in the PEMFcontrol group. Maximum pain was found in T4 time interval.

Table 4. Pain Scores in TENS Experimental Group

	T0	T1	T2	T3	T4
Mean	2.5652	3.2609	2.9565	2.2609	1.9130
SD	1.9265	1.6016	1.2961	0.8100	0.9002
Min	1	1	1	1	1
Max	7	7	5	4	4
Median	2	3	3	2	2
Mode	1	2	2	2	2

Table 4 shows the descriptive statistics of pain scores in the TENSexperimental group. Maximum pain was found in T1 time interval.

Table 5. Pain Score in TENS-Control Group

	T0	T1	T2	T3	T4
Mean	2.5652	3.6522	4.1739	3.0870	2.3043
SD	1.9265	1.4957	1.2304	0.9960	0.8221
Min	1	2	2	1	1
Max	7	7	6	5	4
Median	2	3	4	3	2
Mode	1	3	3	4	2

Table 5 shows the descriptive statistics of pain scores in the TENS control group. Maximum pain was found in T2 time interval

Table 6. Comparison of Groups at every time interval

	P Values of Mann Whitney U Test				
Group	T0	T1	T2	T3	T4
PEMF vs PEMF Control	1	1	1	0.0057*	0.00009*
TENS vs TENS Control	1	0.2979	0.0043*	0.0056*	0.0801*

The difference between control and PEMF was not significant till T2.However, at times T3 and T4 the groups were significantly different

In the case of TENS vs TENS Control, there was a significantdifference from T2 itself.

Table 7. Statistical Analysis of pain score in every group over thetime intervals – PEMF Experimental

	T 1	T 2	T 3	T 4	T 5
Sample size	23	23	23	23	23
Median	2	3	3	3	2
Sum of ranks	54	78.5	84.5	70	58
Mean of the ranks	2.347826	3.413043	3.673913	3.043478	2.521739
χ^2-score	15.485714				
Degree of Freedom (df)	4				
p-value	0.003792916228277				
The result is very Significant at p < 0.05					

Table 8. Post hoc analysis with pairwise comparisons usingConover's test

	T 1	T 2	T 3	T 4	T 5
T 1	1	3.86E-08	4.81E-11	1.66E-04	3.28E-01
T 2	3.86E-08	1	1.44E-01	3.95E-02	2.46E-06
T 3	4.81E-11	1.44E-01	1	5.90E-04	4.35E-09
T 4	1.66E-04	3.95E-02	5.90E-04	1	4.06E-03
T 5	3.28E-01	2.46E-06	4.35E-09	4.06E-03	1

All pairs are statistically significant

Tables 7 & 8 show the statistical Analysis of pain score in every group over the time intervals – PEMF Experimental Group. The groups significantly differed (P=0.003). Further, the difference in pain score between every time interval was significant.

Table 9. Statistical Analysis of pain score in every group over the time intervals – PEMF-Control

	T 1	T 2	T 3	T 4	T 5
Sample size	23	23	23	23	23
Median	2	3	3	4	4
Sum of ranks	44	64	68.5	81	87.5
Mean of the ranks	1.913043	2.782609	2.978261	3.521739	3.804348
χ^2-score	23.863517				
Degree of Freedom (df)	4				
p-value	8.51E-05				
The result is very Significant at p < 0.05					

Table 10. Post hoc analysis with pairwise comparisons using Conover's test

	T 1	T 2	T 3	T 4	T 5
T 1	1	2.96E-06	2.55E-08	1.31E-14	0
T 2	2.96E-06	1	2.64E-01	5.39E-05	7.59E-08
T 3	2.55E-08	2.64E-01	1	2.43E-03	8.00E-06

T 4	1.31E-14	5.39E-05	2.43E-03	1	1.08E-01
T 5	0	7.59E-08	8.00E-06	1.08E-01	1

All values are statistically significant

Tables 9 & 10 show the statistical Analysis of pain score in every group over the time intervals – PEMF control group. The groups significantly differed (P<0.0001). Further, the difference in pain score between every time interval was significant.

Table 11. Statistical Analysis of pain score in every group overthe time intervals – TENS Experimental

	T 1	**T 2**	**T 3**	**T 4**	**T 5**
Sample size	23	23	23	23	23
Median	2	3	3	2	2
Sum of ranks	63	91	79	62.5	49.5
Mean of the ranks	2.73913	3.956522	3.434783	2.717391	2.152174
χ^2-score	22.912088				
Degree of Freedom (df)	4				
p-value	0.000131851008993				
The result is very Significant at p < 0.05					

Table 12. Post hoc analysis with pairwise comparisons using Conover's test

	T 1	T 2	T 3	T 4	T 5
T 1	1	2.99E-10	1.06E-04	8.99E-01	9.34E-04
T 2	2.99E-10	1	3.07E-03	1.67E-10	0
T 3	1.06E-04	3.07E-03	1	6.69E-05	5.14E-11
T 4	8.99E-01	1.67E-10	6.69E-05	1	1.40E-03
T 5	9.34E-04	0	5.14E-11	1.40E-03	1

All values are statistically significant

Tables 11 & 12 show the statistical Analysis of pain score in every group over the time intervals – TENS experimental Group. The groups significantly differed (P=0.0001). Further, the difference in pain score between every time interval was significant

Table 13. Statistical Analysis of pain score in every group overthe time intervals – TENS-Control

	T 1	T 2	T 3	T 4	T 5
Sample size	23	23	23	23	23
Median	2	3	4	3	2
Sum of ranks	49.5	84.5	96.5	68	46.5
Mean of the ranks	2.152174	3.673913	4.195652	2.956522	2.021739
χ^2-score	37.964736				
Degree of Freedom (df)	4				
p-value	1.14E-07				
The result is very Significant at p < 0.05					

Table 14. Post hoc analysis with pairwise comparisons using Conover's test

	T 1	T 2	T 3	T 4	T 5
T 1	1	2.22E-15	0	2.07E-06	4.12E-01
T 2	2.22E-15	1	1.41E-03	1.82E-05	0
T 3	0	1.41E-03	1	1.03E-11	0
T 4	2.07E-06	1.82E-05	1.03E-11	1	6.44E-08
T 5	4.12E-01	0	0	6.44E-08	1

All Values are statistically significant

Tables 13 & 14 show the statistical Analysis of pain score in every group over the time intervals – TENS –Control Group. The groups significantly differed (P<0.0001). Further, the difference in pain score between every time interval was significant.

Table 15. Statistical Analysis of Comparing PEMF andTENS test groups for every time interval

T0	T1	T2	T3	T4
1	0.839536447	0.400420566	0.108222609	0.088986313

(All values are insignificant)

In table 15, when pain scores in TENS and PEMF were compared, it was observed that pain score at every time interval in TENS and PEMF was statistically similar. It implies that TENS and PEMF are similar in controlling pain.

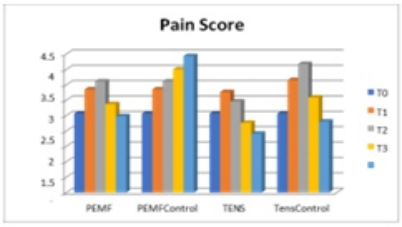


Figure 7: Inferential Statistics

DISCUSSION

Orthodontic pain is a subjective phenomenon and is affected by multitude of factors such as age, gender, pain threshold, and psychological factors. Recent evidence suggests that age and sex have a substantial influence on pain perception from adolescence onwards due to the emergence of differences in pain response especially after puberty. 19 Exclusively female orthodontic patients were studied by Jung et al. and he found that PEMF was effective. As some studies show the influence of age and gender on pain during orthodontic treatment whereas few of them show no gender or age preferences.20 The absence of consensus is due to the heterogeneity of the population and age of the subjects. Thus, in the current study, both males and females were involved and not separate d by gender.

The subjectivity of pain in relation to age has been investigated with mixed results. The effect of aging on pain is not well documented. In orthodontics, Bergius et al found that older patients had higher pain levels, but the relationship was not linear. Hence, the study's age group was limited to 16 to 24 years old to avoid age - related differences.

A split- mouth design was utilized in this study to exclude all components relating to subject differences. During orthodontic treatment, separator placement, arch-wire insertion, activation, elastic wear, application of orthopedic forces, and debonding are some of the procedures that induce pain. Owing to this the patients perceive pain as pressure, tension or tooth discomfort. Thus, pain and discomfort during the initial stages of the treatment can consequentially diminish the patient' s co -operation throughout the treatment and might potentially result in early discontinuation. As a result, the emphasis of this research was on pain perception during early tooth movement caused by 0.14" NiTi wire in the aligning and levelling phase.

Several authors have presented the scope and importance of pharmacological management of pain during orthodontic treatment. Various NSAIDs have been studied regarding their efficacy for the above purpose. In addition, the dosage to be used, duration of action of these drugs have also been reported. Ibuprofen, paracetamol, and acetylsalicylic acid have been commonly used. However, it has been reported that NSAIDs are cleared from blood much before the orthodontic movement starts, as these drugs are administered only initially during the therapy. 21 Oscar et al, 22 in their study demonstrated that nonsteroidal anti -inflammatory analgesics like aspirin and ibuprofen reduce the number of osteoclasts by blocking prostaglandin production, resulting in less orthodontic tooth movement. Pharmacological management with anaesthetic agents like local lidocaine/ prilocaine were also tried. 20,23

To overcome the disadvantages of the drug therapy, various non- pharmacological modalities like, chewing gum, 6 plastic bite wafers, 5 vibratory pressures 7 and laser therapy were utilized for management of orthodontic pain. These methods have been studied extensively in the literature and have reported several disadvantages. Chewing gum, plastic wafers and vibration require high amount of patient cooperation. Low- level laser therapy is associated wit h higher cost factor. 7

PEMF therapy is described as a non-invasive technique that produces short bursts of electrical current in tissues without causing heat or altering key biologic systems, making it beneficial as a supplementary therapy in the treatment of postoperative pain and edema. The common frequency used in clinical practice is 27.12 MHz, and it is described to have no known negative effects. However, in the past, PEMF appliances were not portable and were hence used only in a clinical setup. Recently, portable devices are made available thus, the concept of its home–use has emerged. It is reported to be more cost-effective, smaller, wearable, and even disposable. PEMF therapy can now be used to treat postoperative pain and edema not just in the dental office but also at home, providing dentists with a more adaptable pain management option.

PEMF increases blood and lymph flow by increasing nitric oxide release via nitric oxide synthase, which is generated in response to an increased rate of calcium ion binding to calcium- regulated protein. It also inhibits the generation of growth factors and promotes wound healing and tissue repair by acting on the cGMP second messenger. 24 In the literature, it is reported that there was no discomfort, tingling, or heating reported by the patients. 17 As PEMF is an extraoral device which is not esthetically acceptable by patients, they were prescribed for exclusive night time wear.

Another non-pharmacological pain control modality is the use of TENS. It is worthwhile to note that FDA has approved TENS as early as 1972, for pain control. The principle of TENS is as follows: During TENS therapy, pulsed electrical current would be generated and delivered across the intact skin surface, by using electrodes to stimulate superficial nerves for localized pain relief. Since its advent, TENS is frequently used by health professionals for acute and chronic pain management.

TENS generates an electrical stimulus that is faster than a pain impulse that reaches the substantia gelatinosa located at the dorsal horn, closing the pain gate and reducing pain intensity. TENS also causes opiate- like peptides, like endorphins, to be activated. TENS has been used to manage pain in dentistry in several trials. 8

Thus in this split mouth study the patients were divided into groups and subjected to PEMF and TENS respectively for orthodontic pain management.

In this study, pain scores at five different time intervals were recorded. Among which three of the pain scores – T0, T1 and T2 were taken on the day of the initial strap- up at 0, 2 and 6 hours respectively, after the placement of the arch-wire. T3 was the pain score recorded after 24 hours and T4 was recorded at 48 hours after the first arch-wire placement.

Koritzansky et al 26 in their study on pain and discomfort in orthodontic treatment found that within four hours of initial arch-wire placement, the pain begins, intensifies over a period of 24 hours and subsides in less than seven days which was also evident in the control group of the current study where pain score increased upto T2 and then gradually reduced after 24 hours. Similar findings were reported by Ngan et al and Scheurer et al 27,28: a considerable rise in pain after 24 hours, followed

Because pain is subjective, there is no gold standard for assessing it. The Numerical rating scale (NRS), verbal rating scale and visual analog scale are three pain-rating scales that are routinely used for pain evaluation. For pain studies, Hoggart 29 found that Verbal rating scale or Visual analog scale are not as effective as Numerical rating scale. Since the visual analog scale was not available on google survey the patients were requested to fill in the survey using the Numerical Rating scale (NRS) in google survey forms. This helped in assessing the pain scores and patients were duly reminded before each recording.

In the PEMF group, the findings showed that no significant difference was evident at the time intervals T1 and T2 between the experimental and control group and at 24 hours (T3), and 48 hours (T4) after the initial orthodontic wire was inserted, so PEMF devices demonstrated a considerable potential to reduce orthodontic discomfort after 24 hours, which concurred with the findings of the study by Jung et al in 200717. In PEMF experimental group, maximum reduction was seen in T3 time interval which was also similar to the observation by Niezgoda et al. 13

In the TENS experimental group, at T1 (2 hours) there was no significant reduction in pain, which was similar to the findings by Roth. 8 Desai et al, 30 conducted a study to assess and compare the effects of TENS treatment and piroxicam on the amount of discomfort caused by orthodontic separator installation and have evaluated the pain score at 2 hours, 6 hours, 24 hours, and 48 hours after the separator was installed. The pain reported by patients in the piroxicam group grew steadily from 2 to 48 hours, but the pain in the TENS group was dramatically decreased starting a t 6 hours. Similar to this study, the findings in the present study showed that TENS was effective in reducing the pain from T2 (6

hours) following the initial arch-wire placement.

At the end of T4, (48 hours) TENS device was found consistently effective in comparison to the control group. This finding correlated with the findings of Roth et al, 8 who found that a single application of TENS was proven to be as effective as two or three TENS treatments in decreasing pain for more than 48 hours. Melzack31 has also proved that a single TENS application can provide long- lasting analgesia. Johnson et al 32 suggest patients should take breaks from treatment and alter the positioning of electrode pads over time for effective usage. So, in this clinical trial, TENS device application was done for two 5 - minute periods with a 2 - minute rest between each session, on the same day of initial arch-wire activation.

In PEMF and TENS test groups, maximum pain reduction was seen in the T3 (P=0.005) and T2 (P=0.004) intervals respectively. This was to be expected because the time of intervention with TENS is on the dental chair, immediately following arch-wire placement and was effective with only 10 minutes of active usage, while PEMF application can only be done at night on the day of initial archwire placement. This means that TENS was more effective than PEMF in reducing the severity of pain immediately after the arch-wire placement. Further research is needed to maximize the efficiency of PEMF with only a short effective application time making it viable for use at chairside or to be designed more aesthetically for normal day-to- day usage.

Every group was analyzed for statistical difference in pain scale over the time intervals using Friedman's repeated measures ANOVA, the difference was found to be statistically significant in both PEMF and TENS experimental groups. The shape of the pain change curve showed a gradual decline change between the control and the experimental group as the pain scores showed reduction in the control group only at T3 and T4. Whereas the apex of the curve was advanced in both intervention group.

No previous literature is available that compares the efficiency of these two modalities in reducing orthodontic pain. When PEMF and TENS were compared in this study, they were all statistically similar, implying that both treatments were equally efficient in reducing the pain score caused due to orthodontic therapy.

While NSAIDs and non- pharmacological means of pain control are compared, NSAIDs may still offer better efficacy. However, the adverse effects of NSAIDs along with the safety margin concerns, point the attention to non-pharmacological means. TENS and PEMF treatments have significantly more benefits than drawbacks since they are non-invasive, safe, and effective, which leads to better patient acceptance. So, with respect to the results of the study, we can say that both PEMF and TENS offer similar pain control.

However, the major limitation of this study is that the method and duration of application of both the devices were different. PEMF is designed for use over a longer period to be effective, while TENS is only applied for a short duration of about 5 minutes. Also, since the TENS application is done by the clinician on the chair, there is a lesser chance of incorrect device usage with the TENS, as opposed to PEMF where patient compliance is paramount. Additionally, this clinical trial only took into account the non-extraction cases and further studies could be conducted to include extraction cases and also compare the pain scores between extraction and non - extraction cases. Furthermore, age and gender-specific studies could also be conducted at different stages and phases of orthodontic treatment. In addition, pain is a subjective phenomenon and the threshold to pain drastically varies between different patients. So, further research is needed to better standardize all the possible variables to more reliably and accurately evaluate and compare the efficiency of these two devices in relieving orthodontic pain.

CONCLUSION

Within the limitations of the study following conclusions were made:

1. The pain experienced during the initial 48 hours after placement of conventional 0.016/0.014 Ni-Ti arch-wire during initial alignment and levelling after intervention with Pulsed electromagnetic field using a Numerical rating scale was mildly reduced compared to control.
2. The pain experienced during the initial 48 hours after placement of conventional 0.016/0.014 Ni-Ti arch-wire during initial alignment and levelling after intervention with Transcutaneous electric nerve stimulation using a Numerical rating scale was much reduced compared to the control.

On comparing the efficiency of Pulsed electromagnetic field and Transcutaneous electric nerve stimulation in reducing pain during initial teeth alignment, it was found that both the techniques were equally efficacious and the difference was statistically insignificant.

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Smear Layer Removal with Herbal Preparation and 17% edta as a Root Canal irrigant

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A B S T R A C T

INTRODUCTION :

Root canal treatment aims to eliminate all the microbial loads from the root canal system and prevent the infection

Aim : To compare and evaluate smear layer removal of smear layer removal with herbal preparation and 17% EDTA as a root canal irrigant in human root dentin"

Objectives :

1) To evaluate smear layer removal after irrigation with 17%EDTA,

2) To evaluate smear layer removal after irrigation with herbal prepartation,

3) To compare the smear layer removal after irrigation with 17%EDTA, herbal prepartation, combination of both in extracted single rooted teeth

Conclusion: Irrespective of technique used, EDTA as compared to NEW HERBAL FORMULATION was more efficiently removed in coronal and middle third as compared to apical third of the root canals.

Introduction: For the successful endodontic treatment, it is necessary to obtain a sterile root canal. The vital part of this process is to remove all the contents of the root canal mechanically. Root canal treatment aims to eliminate all the microbial loads from the root canal system and prevent the infection. Although cleaning and shaping have been shown to greatly reduce the number of bacteria in infected canals, complete disinfection of canals is difficult to achieve.

Rationale: The smear layer occludes the orifices of the dentinal tubules and also hinders the penetration of intracanal medications and sealers into the dentinal tubules. So, the removal of the smear layer is important for fluid tight seal and success of the root canal treatment.

Aim : To compare and evaluate smear layer removal of smear layer removal with herbal preparation and 17% EDTA as a root canal irrigant in human root dentin"

Objectives :

Primary objective :

4) To evaluate smear layer removal after irrigation with 17%EDTA,

5) To evaluate smear layer removal after irrigation with herbal prepartation,

Other objective :

1) To compare the smear layer removal after irrigation with 17%EDTA, herbal prepartation, combination of both in extracted single rooted teeth

Primary Research question:

Is herbal preparation with and without 17 % EDTA can remove smear layer from the apical third of human root dentin as a root canal irrigant?

Hypothesis:

The Herbal preparation with and without 17% EDTA will be effective in smear layer removal in apical third of canal in human root dentin as a root canal.

Focused PICO question / PICO format :

P- Human single rooted teeth

I- Herbal preparation for irrigation with and without 17% EDTA

C-17% EDTA

O- smear layer removal in apical third of root canal In the present systematic review, population is Human single rooted teeth; the intervention is Herbal preparation for irrigation with and without 17% EDTA; the comparison is 17% EDTA; and the outcomes is smear layer removal in apical third of root canal.

Method

Inclusion and exclusion

INCLUSION CRITERIA:-

Freshly extracted teeth

Single root teeth

Caries free

Absence of cracks

EXCLUSION CRITERIA:-

Endodontic treated

Grossly carious

Fractured teeth

SEARCH STRATEGY

For identification of studies included or considered for this review, detailed search strategy was developed for the database searched. Search was initiated with the combination of controlled vocabulary-free text terms. The keyword employed in this search was broadly classified into five categories describing population, intervention, comparison, outcome, and the type of study.

The electronic searches have been made into four databases viz., Medline through PubMed, The Cochrane Central Register of Controlled Trials, Google Scholar, and Scihub.

The article published in the period between the dates of inspection of each database was considered, by using various combination of following MeSH terms and keywords. The search terms are AND, OR ((17% EDTA) OR (Smear layer removal) OR Herbs, OR Herbal, ((17% EDTA) AND ((smearlayer removal in apical third of root canal)).

COCHRANE search strategy:

The search terms used are AND, OR ((17% EDTA) OR (Smear layer removal) OR Herbs, OR Herbal, ((17% EDTA) AND ((smearlayer removal in apical third of root canal)).

PUBMED search strategy:

The search terms used are AND, OR ((17% EDTA) OR (Smear layer removal) OR Herbs, OR Herbal, ((17% EDTA) AND ((smearlayer removal in apical third of root canal)).

GOOGLE SCHOLAR search strategy:

The search terms used are AND, OR ((17% EDTA) OR (Smear layer removal) OR Herbs, OR Herbal, ((17% EDTA) AND ((smearlayer removal in apical third of root canal)).

SCI-HUB search strategy:

The search terms used are AND, OR ((17% EDTA) OR (Smear layer removal) OR Herbs, OR Herbal, ((17% EDTA) AND ((smearlayer removal in apical third of root canal)).

STUDY SELECTION

A title identified from the search was screened by one reviewer with a subsequent duplicate independent checking of their abstracts/full-texts retrieved by the electronic search against the eligibility criteria by another reviewer.1

Substantial agreement between reviewers in the study selection process was obtained. After the same reviewers independently reviewed the full-text articles of the previous included studies, and studies which did not present any of the exclusion criteria were selected.2

Additionally, all references of the selected studies were manually screened for potentially relevant additional studies. Any possible discrepancies encountered during this process that is, inclusion or exclusion criteria, were resolved by discussion between the reviewers who selected the included studies. If a disagreement persisted, the judgment of a third reviewer was considered decisive.

DATA EXTRACTION AND DATA ITEMS

Information on authors' names, year of publications, study design, sample, inclusion criteria, groups of intervention, type of treatment, follow-up period, method of dentin hypersensitivity stimulation and method of pain assessment and result was independently extracted by two reviewers.3 Data regarding the included studies was also independently extracted by the reviewers based on a previously defined protocol in a specific form in the Microsoft Office Excel 2007 software (Microsoft Corporation, USA).

RISK OF BIAS IN INDIVIDUAL TRIALS

To evaluate the risk of bias in individual studies, different tools were used for randomized controlled trials (RCTs). The risk of bias of the included trials was assessed using Cochrane's risk of bias tool (33). It was used for RCTs after initial calibration. A main risk of bias assessment was included in the systematic review pertaining to each trial's primary outcome.

Risk of bias within studies:

Risk of bias within the studies was evaluated independently by two review researchers. The Cochrane risk of bias tool was used for randomized controlled trials (RCTs) and the studies were classified as low risk of bias, unclear and high risk bias. The following domains were assessed.

Cochrane Risk of Bias Tool for Randomized Controlled Trials:

1. Reaching risk of bias judgements for bias arising from the randomization process

Low risk of bias	Allocation was adequately concealed. AND There are no baseline imbalances across intervention groups at baseline appear to be compatible with chance. AND An adequate (random or otherwise unpredictable) method was used to generate allocation sequence. OR There is no information about the method used to generate the allocation sequence.
Some concerns	Allocation was adequately concealed. AND There is a problem with the method of sequence generation. OR Baseline imbalances suggest a problem with the randomization process. OR No information is provided about concealment of allocation. AND Baseline imbalances across intervention groups appear to be compatible with chance. OR No information to answer any of the signalling questions.
High risk of bias	Allocation sequence was not concealed. OR No information is provided about concealment of allocation sequence.
	AND Baseline imbalances suggest a problem with the randomization process.

2. Reaching risk of bias judgements for bias due to deviations from intended intervention (effect of assignment to intervention)

Low risk of bias	Participants, carers and personnel were unaware of intervention groups during the trial. OR Participants, carers or personnel were aware of intervention groups during the trial but any deviations from intended intervention reflected usual practice. OR Participants, carers or personnel were aware of intervention groups during the trial but any deviations from intended intervention were unlikely to impact on the outcome. AND No participants were analyzed in the wrong intervention groups (that is, on the basis of intervention actually received rather than of randomized allocation).
Some concerns	Participants, carers or personnel were aware of intervention groups and there is no information on whether there were deviations from usual practice that were likely to impact on the outcome and were imbalanced between intervention groups. OR Some participants were analyzed in the wrong intervention groups (on the basis of intervention actually received rather than of randomized allocation) but there was little potential for a substantial impact on the estimated effect of intervention.
High risk of bias	Participants, carers or personnel were aware of intervention groups and there were deviations from intended interventions that were unbalanced between the intervention groups and likely to have affected the outcome. OR Some participants were analyzed in the wrong intervention groups (on the basis of intervention actually received rather than of randomized allocation), and there was potential for a substantial impact on the estimated effect of intervention.

3. Reaching risk of bias judgements for bias due to missing outcome data

Low risk of bias	No missing data. OR Non-differential missing data (similar proportion of and similar reasons for missing data in compared groups). OR Evidence of robustness of effect estimate to missing data (based on adequate statistical methods for handling missing data and sensitivity analysis).
Some concerns	An unclear degree of missing data or unclear information on proportion and reasons for missing in compared groups.
	AND There is no evidence that the effect estimate is robust to missing data.
High risk of bias	A high degree of missing data. AND Differential missing data (different proportion of or different reasons for missing data in compared groups). AND There is no evidence that the effect estimate is robust to missing data.

4. Reaching risk of bias judgements for bias in measurement of the outcome

Low risk of bias	The outcome assessors were unaware of the intervention received by study participants. OR The outcome assessors were aware of the intervention received by study participants, but the assessment of the outcome was unlikely to be influenced by knowledge of the intervention received.
Some concerns	There is no information available to determine whether the assessment of the outcome is likely to be influenced by knowledge of the intervention received.
High risk of bias	Reported outcome data are likely to have been selected, on the basis of the results, from multiple outcome measurements (e.g. scales, definitions, time points) within the outcome domain, or from multiple analyses of the data (or both).

Thresholds for Converting the Cochrane Risk of Bias Tool to AHRQ (Agency for Healthcare Research and Quality) Standards (Good, Fair, and Poor)

Good quality: All criteria met (i.e. low for each domain). Using the Cochrane Risk of Bias tool, it is possible for a criterion to be met even when the element was technically not part of the method. For instance, a judgment that knowledge of the allocated interventions was adequately prevented can be made even if the study was not blinded, if EPC team members judge that the outcome and the outcome measurement are not likely to be influenced by lack of blinding.

Fair quality:

One criterion not met (i.e. high risk of bias for one domain) or two criteria unclear, and the assessment that this was unlikely to have biased the outcome, and there is no known important limitation that could invalidate the results.

Poor quality:

One criterion not met (i.e. high risk of bias for one domain) or two criteria unclear, and the assessment that this was likely to have biased the outcome, and there are important limitations that could invalidate the results.

Following studies selected for the present systematic review shown low risk of bias in randomization process, missing outcome data, measurement of outcome and reported results. Good and fair quality obtained in quality assessment with unclear intended interventions.4

STUDY FINDING'S

RAMA S KALLURU, N DEEPAK KUMAR, SHAFIE AHMED, EMANUEL SOLOMON SATHISH, THUMU JAYAPRAKASH, ROOPADEVI GARLAPATI, BUTTI SOWMYA, K NARASIMHA REDDY in 20142 evaluated the microhardness of human dentin by using four irrigating solutions. In the present study a total of 40 extracted mandibular premolars were selected and sectioned horizontally in the middle third of the root. Forty specimens of 4 mm thickness were embedded in acrylic resin and polished.5 Four test groups, each group containing ten specimens were immersed in respective irrigating solution and subjected to vicker'smicrohardness test at T0, T2 and T5min.2 The data obtained were analyzed using the one way ANOVA followed by Tukey HSD method with ap=0.05 as the level for statistical significance. There was no statistically significant difference in mean values between four experimental irrigating solutions. And the Authors concluded that mixture of Tetracycline isomer i.e. Doxycycline, Citric acid and a Detergent (Tween 80) MTAD not altered the microhardness of root canal dentin significantly and seems to be an appropriate irrigating solution, because of its harmless effect on the microhardness of the root canal dentin.

HEBATALLA E. KANDIL, AHMED H. LABIB, HATEM A. ALHADAINY B IN 2014 Studied1 the effect of different irrigants on root dentin microhardness and smear layer removal. In the present study a total of 50 roots were equally divided into two halves to measure dentin microhardness and to evaluate the amount of smear layer. One hundred root halves were divided into five equal groups 20 sample each according to the final irrigants used: Group 1: 2.5% NaOCl, Group 2: 2.5% sodium hypochloride (NaOCl) followed by 7% malic acid (MA), Group 3: 2.5% NaOCl followed by 17% ethylenediamine tetraacetic acid (EDTA), Group 4: 2.5% NaOCl followed by mixture of tetracycline, acid and detergent (MTAD) and Group 5: saline. Ten root halves from each group were prepared to measure dentin microhardness at baseline measurement and after treatment to determine the change in microhardness, while the remains 10 root halves were prepared for scanning electron microscope to evaluate the amount of smear in the coronal, middle and apical thirds.Data were analyzed using one-way ANOVA and Student's t-test for microhardness and Kruskul–Wallis and Mann–Whitney for smear layer. Malic acid showed the greatest significant reduction in dentin microhardness ($P < 0.05$), followed by EDTA, MTAD, NaOCl and saline (control). EDTA, malic acid and MTAD efficiently removed smear layer, respectively, in the coronal and middle thirds of root canal. However, in the apical region, malic acid showed more efficient removal of the smear layer than the other irrigants.Authors concluded Malic acid showed the greatest significant reduction in dentin microhardness ($P < 0.05$), followed by EDTA, MTAD, NaOCl and saline (control).

NAVEEN CHHABRA, HITESH GYANANI, AND LAXMIKANT KAMATAGI IN 2015 studied the effectiveness of the combination of two natural extracts in varying ratios for removal of smear layer either alone or supplemented with sonic agitation.In the present study a total Fifty extracted single-rooted teeth were collected, disinfected and decoronated below the cementoenamel junction to obtain standardized root length of 10 mm.4 Root canals were instrumented using rotary files at working length 1 mm short of the apex. Specimens were divided into six groups according to the irrigation protocol as follows: Group A – Distilled water, Group B – 17% ethylenediaminetetraacetic acid, Group C – Herbal extracts in 1:1 ratio, Group D – Herbal extracts in 1:1 supplemented with sonic agitation, Group E – Herbal extracts in 2:1 ratio, Group F – Herbal extracts in 2:1 ratio supplemented with sonic agitation.5 Specimens were longitudinally sectioned and evaluated under scanning electron microscope for smear layer removal efficacy. Obtained scores were statistically analyzed using one-way analysis of variance and post-hoc test. Among all, Group B showed the best results followed by Group F. Remaining other

groups showed inferior outcome ($P < 0.05$). And the Authors concluded that the combination of two extracts in 2:1 ratio was slightly better than 1:1 ratio and the smear layer removal efficacy was further improved when accompanied with sonic agitation. 5

Eick et al (1970)6 were the first who identified the smear layer using scanning electron microscope (SEM) and found that smear layer is made from different size of particles ranging from <0.5 to 15 μm. The presence of smear layer on instrumented root canals was first reported by McComb and Smith7 in (1975). They showed that this layer is made of remnants of dentin, odontoblastic processes, necrotic or viable pulp tissues, and bacteria. Lester and Boyde (1977) reported that smear layer is a mineralized collagen matrix made up of entrapment of organic matter within inorganic dentin. 7

Sen BH,Wesselink PR, Türkün M (1995) discussed that smear layer should be removed or not from the instrumented root canals, are still controversial. It has been shown that, this layer is not a complete barrier to bacteria and it delays but does not abolish the action of endodontic disinfectants. Endodontic smear layer also acts as a physical barrier interfering with adhesion and penetration of sealers into dentinal tubules. In turn, it may affect the sealing efficiency of root canal obturation. When it is not removed, the durability of the apical and coronal seal should be evaluated over a long period. If smear layer is to be removed, EDTA and NaOCl solutions have been shown to be effective, among various irrigation solutions and techniques, including ultrasonics, that have been tested. Once this layer is removed, it should be borne in mind that there is a risk of reinfecting dentinal tubules if the seal fails. They also emphasized that, further studies are needed to establish the clinical importance of the absence or presence of smear layer.

Violich DR, Chandler NP (2010) overviewed on the articles on the smear layer, focusing on its relevance to endodontics. The PubMed database was used initially; the reference list for smear layer featured 1277 articles, and for both smear layer dentin and smear layer root canal revealed 1455 publications. Smear layer endodontics disclosed 408 papers. Data obtained suggests that smear layer removal should enhance canal disinfection. They concluded that if smear is to be removed, the method of choice seems to be the alternate use of EDTA and sodium hypochlorite solutions. Conflict remains regarding the removal of the smear layer before filling root canals, with investigations required to determine the role of the smear layer in the outcomes of root canal treatment.

Li, D., Jiang, S., Yin, X., Chang, J.W.W., Ke, J. and Zhang, C. (2015)10 conducted study to use high-resolution micro-computed tomography (micro-CT) and scanning electron microscopy (SEM) to compare the efficacy of four irrigation techniques (needle, ultrasonic, Endoactivator, and photon-induced photoacoustic streaming (PIPS)) in removing calcium hydroxide from the root canal and isthmus of maxillary premolars. PIPS and ultrasonic irrigation more effectively removed calcium hydroxide from the main canal and isthmus in maxillary premolars than did Endoactivator or needle irrigation. 11,13

Researchers have different opinion regarding the importance of removing or leaving this smear layer. Some investigators advocated the importance of maintaining the smear layer after canal preparation, and some studies provide strong evidence to prove that smear layer acts as a seal to the dentinal tubules and minimizes bacterial and its toxin from invasion by altering dentinal permeability.12,13 Pashley (1985)14 reported that the presence of a smear layer may limit bacteria present in the infected canal to enter the dentinal tubules in case of inadequate canal disinfection or recontamination of the canal between treatment sessions. However, a study by Williams and Goldman (1985)15 reported that this layer cannot act as a complete barrier and its presence could only delay bacterial invasion.

Madison and Krell (1984)16 using a chelating agent, ethylenediaminetetraacetic acid (EDTA) solution, found no difference in the leakage properties regardless of the presence of smear layer.

However, a major disadvantage of these studies is that the experiments did not mimic the clinical condition and were undertaken using cross-sectional root models or dentin discs. This limitation was overcome by a study of Drake et al (1994)17 and they suggested that smear layer formed during mechanical instrumentation can prevent bacterial colonization of root canals as it limits bacterial penetration into dentinal tubules.

Some authors advocated the significance of removing the smear layer since it contains necrotic tissue, bacteria, and its by-products. Smear layer can act as a reservoir for further microbial irritants and may serve as a substrate for microorganisms to survive, multiply, and then proliferate deeply inside the dentinal tubules18,19,20 ,23 Brännström (1984)21 advocated that these microorganisms inside the dentinal tubules can easily be destroyed once the smear layer is removed. In addition, the smear layer can minimize the ability of disinfecting agents to penetrate the dentinal tubules. Other studies showed that it can also minimize the ability of intracanal medicaments to penetrate deeply. Therefore, smear layer can delay but did not completely eliminate the effect of disinfectant agent or intracanal medicament. 22

The advantages and disadvantages of smear layer removal are still controversial. The need and the importance of smear layer removal are connected to the root content (live or necrotic pulp). De Deus (2011)23 found that in case of treating vital teeth where there is no contamination and the aseptic chain is maintained, removal of the smear layer may not be required. However, if treatment of a necrotic tooth is due, the smear layer will become infected, and the clinician should consider the importance of its removal.24

Limitations of the study : The influence of various variables suggests necessity of further studies on larger number of samples under strictly controlled experimental conditions. As this is vitro study this cant be totally correlated with vivo environment. Large size sample required.

CONCLUSION:

Irrespective of technique used, EDTA as compared to NEW HERBAL FORMULATION was more efficiently removed in coronal and middle third as compared to apical third of the root canals.

Conflict of Interest:

All the authors associated with present manuscript declared no potential conflicts of interest with respect to research, authorship or publication of this article.

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Clinical and Pathological Impact of Fatty Liver Hemorrhagic Syndrome in Caged Hens Farms in Diyala Province

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ABSTRACT

The aim of present study was to show the Fatty liver hemorrhagic syndrome (FLHS) in different areas of Diyala province and its effects on the eggs production. A total of 250 layer hens from different areas of Diyala Province (44) weeks suffering from pale appearance, anorexia, low weight and low egg production without any other signs refers to an outbreak of known infectious diseases were enrolled. Post mortem signs show enlargement of livers in the most cases with fatty appearance. The microscopic study appears the fatty change in hepatic section. The weights of layer hens, blood parameters, lesions score were studied. The results showed higher incidence of case in 11.6% the oldest layer hens in compare with 0.8% the youngest and low weights of affected farms at 31 and 64 wks of age. Birds of Farm 3 had a significantly higher BW than that recommended for Isa brown. No significant increase of all blood parameters measured at 73 wks of age in Farm 1. No significant changes in blood cell profile of hens at different flocks/ages at Farm 1. FLHS cause a significant economic loss by dropping of egg production and mortality increases.

Keywords: Fatty Liver Hemorrhagic Syndrome, Isa brown, Hens, Liver hemorrhage, Hormonal factors

Introduction

Fatty liver hemorrhagic syndrome is a metabolic condition that occurs in commercial layers and is frequently the major cause of death in high producing laying flocks. FLHS is characterized by excessive fat in the liver and hemorrhage from a ruptured liver. The syndrome occurs in caged laying hens, primarily in birds that are in positive energy balance (Polin and Wolford, 1977). However other factors have also been implicated as potential contributory elements to the occurrence of FLHS (Thomson et al., 2003). The condition is easy to recognize at necropsy with hens having excess abdominal and liver fat, hemorrhages and hematomas of various size in the liver (Fig. 1-1 A and B), and in many cases large blood clots in the abdominal cavity (Fig. 1-1 C). Outbreaks occur sporadically in commercial flocks (Squires and Leeson, 1988), and 3-5% of the affected flocks die from the condition. Ugochukwu (1983), Weitzenburger et al. (2005) and Shini et al. (2006) have reported higher mortality (6-20%) due to FLHS. The decrease in egg production and increase in mortality associated with FLHS have implications for the welfare of hens and cause considerable economic losses to egg producers.

The etiology of this syndrome is still poorly understood and the occurrence underappreciated. Since the 1990s, the main factors that have been involved in the etiology of the FLHS include: a) “Nutritional factors (e.g. consumption of high energy diets Intake of high-energy diets that allows caged hens to consume energy in excess of the requirements for maintenance and egg production, results in a positive energy balance and increased hepatic fat deposition. The fact that FLHS can be experimentally induced through force-feeding and/or oestrogen administration indicates that the condition might be caused by a surfeit of energy rather than being specific to an excess of any nutrient such as fat or carbohydrate”. “Butler (1975) suggests that excess fat in the liver arises mainly from increased lipogenesis rather than

from dietary lipids. Several studies have indicated that high energy maize or wheat diets produce higher incidences of FLHS (Haghighi and Polin, 1982). Branton et al. (1995) observed a high incidence of FLHS in hens that consumed diets containing chelated minerals".

b) Hormonal factors

Oestrogens influence the lipid synthesis which is required for yolk deposition. Polin and Wolford (1977) indicated that the liver hemorrhage score was markedly increased when excess energy intake was combined with exogenous oestrogen treatment. The possibility of a hormonal imbalance has been suggested by the observation of greatly elevated serum calcium and cholesterol in chickens from flocks with FLHS (Harms et al., 1972; Miles and Harms, 1981).

c) Environmental temperatures (i.e. heat and cold stress)"

Exposure to cold or heat induces stress and influences lipid metabolism in the fowl (Annison, 1983). The injection of adrenocorticotropic hormone (ACTH) also produces this response (Jaussi et al., 1962). However, most investigators have shown that increased lipogenesis may occur partly due to an excessive intake of carbohydrate during hot weather (Couch, 1956; Pearson and Butler, 1978); while, Jensen et al. (1976) observed more.

Material and Methods

Hens

Layer hens white Hy-Line and Isa brown (250) purchased in different regions in Diyala. The average number of birds per producer per year ranged from less than 10,000 (2 producers); 20,000 to 190,000 (8 producers); and 900,000 birds (1 producer), with the number of sheds ranging from 1 to 16. Only two producers use controlled environment sheds, others have their sheds naturally controlled/ventilated. Eight of the producers use cages housing 5-6 birds/cage, and 4 use cages housing 3 birds /cage. All of producers use cages conforming to the welfare code. Five of producers use Hy-Line brown layers, 2 use Isa brown, 2 use HI-SEX birds and 1 uses both Hy-Line and Isa brown to operate farms. Seven of the producers used farm-mixed feed and only 4 used commercial feed. The mortality rate of flocks ranged from 2% to 11% and the average rate of production for laying cycle ranged from 70% to 89%. None of the producers know the causes of mortalities in their flocks, and only 3 use veterinary laboratories to determine causes of bird mortality; while, all used lighting programs for laying flocks. Only 1 of the producers was aware of FLHS being sporadically observed in their flocks (dead birds).

Blood samples

The blood collected from jugular vein and collected in clean anticoagulant tubes to send to the laboratory to evaluate the hematological parameters for cases.

Histopathology

Necropsy applied for the cases and samples of livers collected to be fix in 10% formaldehyde solution for prepare to routine histopathology.

Statistical analysis

All collected data were analysed using the Chi-square test (x2) in the SPSS Program (Al-Gharban, 2017).

Results

Results were showed higher incidence of FLHS in 11.6% the oldest layer hens in compare with 0.8% the youngest. The mortality rates increased with age ($P<0.05$), although there very low differences in mortalities between flocks of similar ages. The results indicate that for Farm 1 at the 29, 54 and 73 wks

of age (end of April) the mortality rate was 2, 4.8 and 11.6%, respectively. At 72 wks, Farm 2 (shed/flock 1) mortality rate (cumulative) was 7.4% of the initial flock, and at 31, 49 and 64 wks of age the mortality rate (cumulative) for Farm 3 (shed 1, flocks 1, 2, and 3) was 0.8, 2.5 and 4.8%, respectively". As indicated in the methodology only 30-50% of dead birds were necropsied. The results indicate that 42% of birds necropsied from Farm 1 showed clinical signs of FLHS, while for Farm 2 only 28% of dead birds have had FLHS, and for Farm 3, 34% died due to this condition. Interestingly, the results showed that of birds that died in Farm 1, between the ages 37 to 54 wks more than 50% demonstrated FLHS. The average BW of those dead birds was 2008±107 g. The average of BW of birds that died in Farm 2 and 3 was 1821±78 and 1954±92 g, respectively".

Performance parameters

Collected layer hens from Farms 1, 2 and 3 at three sampling points: February, March, and April 2020. For breeder's recommendations at peak of production (32 wks) BW was increased with age. At 32 and 72 wks of age birds of Farm 1 and 2 had a BW comparable with that recommended by the breeder. At 31 and 64 wks of age, birds of Farm 3 had a significantly higher BW than that recommended for Isa brown".

Body weights

Body weight of birds in both feed restricted groups (E2-treated and non-treated group) decreased starting first week post-treatment (Table 1), but this decrease was not significant ($P>0.05$). The decrease was more pronounced ($P<0.01$) on the second week of treatment, and continued to remain at this level (without recovering) even 1 week after the treatments was interrupted".

Table (1): Body weight of birds in feed restricted groups (E2-treated and non-treated group)

Parameter	Farm 33	Isa brown 1	Farm 2	Farm 12	Hy-Line 1
BW (g)					
32 wks	1985	1985 (1975 at	2117	1872 gm	1980 gm
72 wks	2163	64 wks)	gm	2128 gm	2250 gm

Table (2): Hen Day Production and Mortality rate of enrolled farms

Parameter	Farm 33	Isa brown 1	Farm 2	Farm 12	Hy-Line 1
Hen Day Production		94.3			
(%)	91	75 (79.7 at 64	74%	94.3	94
32 wks	85	wks)		77.4	72
72 wks					
Mortality					
Cumulative (%)	0.8	1.2	7.4	2.0	0.8
32 wks	4.8	5.8 (4.9 at 64		11.0	4.0
72 wks		wks)			

Blood parameters

All Blood parameters in layer hens affected by FLHS were taken and analyzed at the end of each month (February, March, and April 2020). Data presented here are calculated as an average of 40 birds per shed/age for Farm 1, 18 birds per shed/age for Farm 2, and 27 birds per shed/age for Farm 3 at each sampling point/time. Although, there was a slight increase of all parameters measured at 73 wks of age in Farm 1, this was no significant ($P>0.05$). No significant changes were found in blood cell profile (RBCs, HGB, and HCT) in hens at different flocks/ages at this Farm.

Table (3): Blood parameters in layer hens affected by FLHS

Age (wk)	RBC (x106/L)	HGB (g/L)	HGB (g/L)
21	23.8	132	29.5
25	2.47	134	30.3
29	2.61	138	31.2
46	2.47	132	30.8
50	2.45	135	30.6
54	2.41	126	30.5
65	2.59	132	32.0
69	2.52	139	31.7
73	2.69	146	34.3

Table (4): Blood physiological parameter of hens with FLHS

Age (wk)	Cholesterol (Mmol/L)	Triglyceride (Mmol/L)	GGT (U/L)	Protein (g/L)	Glucose (Ml/L)
12.1	43.7	33.7	12.3	2.5	12.1
14.8	50.7	32.7	11.4	2.7	14.8
15.1	48.7	35.0	11.1	2.3	15.1
12.9	56.7	32.3	19.9	2.4	12.9
14.8	58.3	31.7	23.5	2.8	14.8
15.9	55.0	34.7	19.9	2.5	15.9
12.7	53.7	41.7	20.8	2.5	12.7
14.5	55.3	43.7	23.3	2.6	14.5
15.1	54.0	46.0	24.6	2.3	15.1

Liver weights (g) fat content (%)

Data were recorded during the whole experimental period. Number of birds sacrificed from each treatment at each sampling point was 6. At the end of experimental period all birds were sacrificed and undergone post-mortem examination there were no significant differences between not-treated and oil-treated groups therefore data are pooled and presented together. The dyed layers of livers showed (during experimental period) were pale, swollen and friable with different grades of hemorrhages and haematomas on both surfaces (dorsal and ventral) and/or in the edges of both lobes. In advances cases (hemorrhage score 4 or 5) liver tissue was ruptured and large blood coagulations was found inside the abdominal cavity".

Liver

Lesion Scores

Lesion scored as mentioned by (Ginns et al., 2000) by post mortem examination of liver grossly and microscopically.

Organ	Score	Description of lesions
Liver	0	No lesion.
	1	Mild lesion.
	2	Haemorrhages and fatty liver

Table (5): Liver lesion scores of hens with FLHS

Farm 33	Isa brown 1	Farm 2	Farm 12	Hy-Line 1
0-1	0-1	1	1-2	1-2

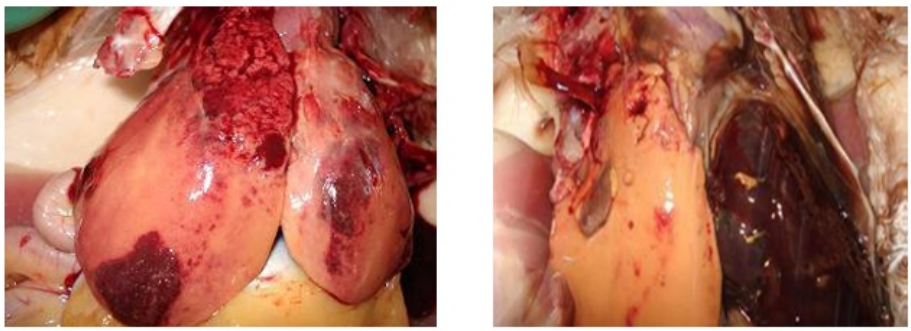

Figure (1): Liver lesion of score 2

Table (6): Birds diagnosed with FLHS, liver weight to BW ratio, fat content and mortality %

No. of birds 2	Birds diagnosed with FLHS (5)	Liver weight to BW ratio (g/100g)	Liver weights (g) fat content (%)	Mortality (%)
32	18.75 6.25	2.07	38.6±5.1 25.2±2.8	0
32	6.25	2.19	39.2±4.8 23.8±2.2	0
16	87.5 12.5	2.91	53.0±6.0 51.4±5.3	18.75
16	68.75 18.75 12.5	2.82	47.4±5.5 43.6±3.8	6.25

Liver histopathology examination

Oestradiol treatment resulted in an increased infiltration of hepatocytes and liver tissue with fat and fat vacuoles (Fig. 4-10). Histologically, all livers had significant slight and moderate lipid accumulation in livers, however, E2-treated birds demonstrated severe fat deposition and large vacuoles containing fat and distending hepatocytes. In addition to fat deposition, histological sections of E2-treated birds indicated focal inflammatory (heterophilic and/or lymphocytic/ mononuclear) infiltration, hemorrhage and congestion of sinusoids, demonstrating an increased incidence of inflammation and hemorrhage. Massive lipid infiltration, diffuse inflammatory infiltration and congestion were observed especially in the liver parenchyma of birds that macroscopically demonstrated severe lesions of FLHS.

Discussion

In this project a questionnaire followed by an epidemiological survey were used to determine the occurrence of FLHS in caged laying hens in Diyala. The results demonstrate that FLHS is present in caged birds in Diyala. The questionnaire provided important data on hen management practices, and also suggests that most egg producers are not aware of FLHS, but the presence of FLHS was confirmed in the epidemiological study. Post-mortem examination conducted in 3 farms with 7 flocks of different ages indicated that 234 birds (or 36%) of all birds necropsied (597) had FLHS. This indicates that FLHS is the most significant cause of death of laying hens kept in cages. It also confirms our previous observations with a small flock of caged hens at UQ Gatton (Shini et al. 2006) where we found that FLHS was the main cause of death (74% of birds necropsied) in a flock indicating a 6% cumulative mortality rate. The results are also in agreement with previous overseas studies which have shown a high mortality rate (5-20%) due to FLHS in healthy flocks.

“Death from FLHS occurs only in extreme cases following massive liver hemorrhage (Squires and Lesson, 1988). Therefore, it is likely that a significant number of hens within a flock are also suffering from subacute and chronic FLHS that may cause a drop in egg production but little increase in mortality

(Julian, 2005). These hens may exhibit reproductive dysfunction (Chen et al. 2006), due to chronic liver tissue damage and an impairment of the transport of triglycerides, phospholipids, and cholesterol from the liver to the ovary (Walzem, 1996), resulting in decreased yolk formation and egg production. Our data showed that most deaths occurred in heavier hens over 40 wks of age".

Moreover, the examination of livers from hens euthanised systematically indicated that more than 50% of hens had focal hemorrhages or haematomas, while 10% of hens euthanised showed focal necrosis and signs of previous subcapsular hemorrhage. Together, the acute and chronic form of the disease suggests that FLHS is a significant source of lost in egg production and confirms our prediction that FLHS is a neglected disease of significant economic importance.

The results of this study also confirm our previous observations that laying hens, in multi-tier cages and in a controlled environment shed, are most at risk of developing FLHS. To our knowledge, we are the first to show the effect of a thermoneutral environmental temperature on the occurrence of FLHS in caged hens. Previous studies that examined the effect of temperature on the occurrence of FLHS were conducted 30 years ago, when controlled environment sheds were not widely used in the industry. In these studies increased mortality due to FLHS was found at temperature extremes. In our study heavier birds in a flock were more likely to have the condition than the lighter birds, particularly in a controlled environment shed. Birds are maintained in a thermoneutral zone and have lower energy requirements. Both factors (lack of activity and controlled environmental temperature) contribute to increased BW and increased hepatic lipid deposition.

From the first part of the study, it was concluded that FLHS is present in caged flocks in diyala, and the age of the flock and housing conditions influence the incidence of this metabolic disorder.

Induction of FLHS in the laying hens was investigated to study its pathogenesis and establish the role of oestrogen in the production of FLHS, showing that birds with a higher feed and energy intake are more predisposed to the occurrence of FLHS.

Oestrogen-induced hens from feed restricted group also developed FLHS, although with a lower frequency, Body weights and egg production of hens that were restricted to feed was slightly impacted.

In laying hens hepatic lipogenesis is increased dramatically by oestrogen in order to meet the demand for vitellogenesis (Hansen and Walzem, 1993). Although the main products of de novo hepatic lipogenesis are triglycerides, the liver is also the major site of cholesterol and phospholipid synthesis. These lipids along with protein are the main components of lipoproteins. It is well known that, because de novo fatty acid synthesis in birds takes place mainly in the liver (Annison, 1983), adipose tissue growth and subsequent extrahepatic fattening depend on the availability of plasma triglycerides, which are transported as components of lipoproteins (Hermier, 1997). Many factors, e.g. external (nutritional and environmental factors) and internal (hormones and other mediators) may affect lipid metabolism and disturb metabolic, endocrine and immune interactions resulting in hepatic pathology.

Fatty liver occurs in birds when the increase in lipogenesis exceeds the capacity of synthesis and secretion of lipoproteins (Hermier, 1997). Studies in mammals have demonstrated that fat accumulated in the liver and abdominal cavity constitutes an interesting tissue that communicates with other tissues of the body including hepatocytes via adipokines, lipid factors, and lipoprotein particles (Tilg and Moschen, 2008). One of the first organs to be affected when adipose tissue becomes dysfunctional and inflamed is the liver (Attie and Scherer, 2008).

An extremely severe case of a fatty liver will causes an inflammation of the liver cells (steatohepatitis). In chickens, there is a lack of information on the role of a fatty liver in metabolic, endocrine and immune responses.

In this study, elevated leukocyte numbers and fibrinogen levels were highly altered in oestrogen induced birds and slightly altered in natural cases of FLHS (in birds monitored for 52 wks). As in mammals, in

birds the elevation of these parameters demonstrates increased systemic inflammation and tissue repair. Overall, it appears that in addition to the metabolic state of the bird, inflammatory and immune responses might have been involved in the pathogenesis of FLHS. This was also supported from histological data.

Conclusions

FLHS is present in caged layer hens in Diyala and impacts hen health and welfare. Significant economic losses to producers occur because egg production drops and mortality increases. The results demonstrated that FLHS is a major of hen mortality which has the following implications for the industry.

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Bilateral, Elongated Styloid Process in A Dry Skull; its Clinical Implications: A Case Report

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ABSTRACT

The styloid process of temporal bone is a slender, elongated, conical bony projection that lies anteromedial to the mastoid process. Its length normally varies from 2-3 cm, and a styloid process longer than 3 cm is found in 4 to 7% of the population. During the routine osteological study of the skull, we came across a skull with bilateral elongated styloid process. The length of the process on the right side was 5.3cm and on the left 5.2cm and the thickness around the base was 1cm bilaterally. The elongated styloid process is recognised as one of the causes of pain in the cranio-cervical region and is one of the causes of Eagle's syndrome. Eagle syndrome, or elongated styloid process syndrome, is associated with such symptoms as chronic facial and neck pain, dysphagia, tinnitus, referred pain in the ear, glossopharyngeal neuralgia, orbital pain, and radiating pain in the maxillary regions, which worsen when the head rotates or the tonsillar fossa region is palpated. Awareness about an elongated styloid process is important for otolaryngologists, radiologists, surgeons and dentists.

Key words: Styloid process, Eagle syndrome, Elongated.

Introduction

An elongated styloid process may compress on the vital neurovascular structures that are in its close vicinity and give rise to symptoms related to the compression of nerves (trigeminal,, facial, glossopharyngeal Vagus and accessory nerve) or, symptoms of compression of carotid arteries, which were first described by an Otolaryngologist Watt Eagle[1]. (1937).

The exact cause of an elongated styloid process is not yet determined, however the most accepted hypothesis is the ossification of the stylohyoid ligament that attaches the styloid process to the lesser cornu of hyoid bone, which may occur as a result of a congenital variation, local reactive metaplasia after local trauma, calcification, or the ageing process.

Its length normally varies from 2-3 cm. There is no consensus, regarding the maximum limit of length of the styloid process; some researchers have reported the maximum upper limit to be 3 cm [2] [3]. However, some other reports proposed that the SP should be considered elongated when its length exceeded 4.5cm [4] [5].

The length of the styloid process has been studied by several researchers in dry skulls, in cadavers, in computed tomography and in radiographs [6] [7][8] [9] [10][11].

Although approximately 4%-7% of the population is thought to have an elongated styloid process, only a small percentage (between 4 and 10.3%) of this group is thought to be symptomatic [12][13]. The typical patient with an elongated styloid process is a female between the ages of 30 and 50 years. There is a 3:1 female predominance in ES [14].

Some researchers have shown that SPE was not the only reason of the symptoms and signs, and they suggested that it's the increased medial or anterior angulation that makes the elongated styloid process the sole cause of Eagle's syndrome [15][16].

Case Finding

During the routine osteological study of the skull, we came across a skull with bilateral elongated styloid process. The length of the process on the right side was 5.3cm and on the left 5.2cm and the thickness around the base was 1cm bilaterally.

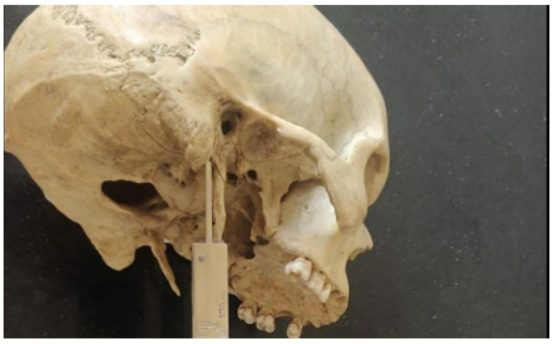

Figure 1: Abnormally large styloid process on right side

Figure 2: Abnormally large styloid process on left side.

Discussion

Eagle described two distinct syndromes related to an elongated styloid process :1. Classic Syndrome- which included symptoms of pain in head and neck, dysphagia, Otalgia, facial pain when turning the head, foreign body sensation in throat. It is associated in most cases with tonsillectomy that may have been performed many years earlier. Several researchers have reported cases where patients presented with symptoms of classic syndrome and have added some more symptoms such as tinnitus, radiating pain into the orbit or maxillary region to the clinical presentation of the classical syndrome.

The second type, the carotid artery syndrome, usually is not associated with tonsillectomy. The carotid artery syndrome is caused by impingement. It includes pain along the distribution of the carotid artery, due to irritation of the sympathetic plexus around the carotid artery. Cases of elongated styloid process associated with symptoms of Horner's syndrome, transient ischaemic attacks and sudden death attributed to compression of both carotid sinuses have been reported [17] [18] [19].

The diagnosis of ES must be based on a good medical history, physical examination and confirmed by radiological investigations. It should be possible to feel an elongated styloid process by careful intraoral palpation, placing the index finger in the tonsillar fossa and applying gentle pressure [20]. If pain is reproduced by palpation and either referred to the ear, face, or head, the diagnosis of an elongated styloid process is very likely. A styloid process of normal length is usually not palpable. Injection of local anaesthetic into tonsillar fossa relieves pain and can be used as a diagnostic tool[21].
The factors such as variability in the clinical presentation, non-specific symptoms, an elongated styloid process is often asymptomatic, and scant knowledge about this clinical entity makes the diagnosis often challenging for the clinician and very often the diagnosis is delayed.

Conclusion

Symptoms related to an elongated styloid process can be confused or mistaken for many other conditions that must be excluded. Otolaryngologists, neurologists and dental surgeons should be aware of the existence and incidence of this clinical entity, which can be confused or mistaken for many other conditions, which can be excluded by proper and detailed history, physical examination and radiological investigations. The symptoms related to Eagle's syndrome can be confused with those attributed to a wide variety of facial neuralgias or oral, dental and temporomandibular diseases. Awareness about an elongated styloid process is important for otolaryngologists, radiologists ,surgeons and dentists.

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Histological Comparison of White Mouse Liver Dosed Experimentally with Escherichia Coli O157:H7 and Treated with a Drug and Antibiotic

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A B S T R A C T

The current study is conducted to detect the pathogenicity of E. coli O157: H7 and know the effects of its experimental infection in the histological structure of the liver of male mice aged (8-12) weeks. In addition, it attempts to determine the therapeutic effect of an approved drug in the treatment of bacteria and compare it with the antibiotic whose effectiveness is tested in vitro. The antibiotic sensitivity test results show that the bacteria are sensitive to Trimethoprim-Sulfamethoxazole, Metronidazole, Gentamicin, Amikacin, Tetracycline, Azithromycin, Ceftriaxone and Ciprofloxacin, and resistant to Amoxicillin/clavulanic acid and Ampicillin. The most sensitive antibiotic is Ciprofloxacin, followed by TrimethoprimSulfamethoxazole. Since the latter is an approved and widely used antibiotic, it is chosen with Ciprofloxacin to determine its therapeutic effect against the bacteria under study. Animals experimentally infected with this bacteria show different degrees of clinical signs represented by lethargy, recluse, loss of appetite, increase in respiratory rate and heart beat rate different degrees of diarrhea appearing in a number of them. In others, different forms of paralysis appear, either paralysis of the hind feet or complete immobilityy, in addition to the occurrence of a number of deaths in mice infected experimentally with the infectious dose of bacteria. As for the results of the histological examination, they are represented in hypertrophy and sometimes hyperplasia in the hepatocytes, loss of chromatin of the nuclei in a number of them with expansion of blood sinusoids, congestion of blood vessels as well as hemorrhage and inflammatory cellular infiltration. These symptoms are more severe in the half lethal dose group (LD-50). As for the groups treated with the two aforementioned antibiotics, it is noted that they play an effective role in stopping diarrhea with mice somewhat regaining their activity. However, they still had suffered from various tissue lesions represented by necrosis, vacuolation, degeneration, inflammatory cellular infiltration, congestion and hemorrhage.

1- Introduction

Escherichia coli belongs to the Enterobacteriaceae, and is an endemic normal flora of the large intestine in humans and other mammals (Sejal and Leonard, 2015). This harmless bacterium usually becomes a highly adaptive pathogen, capable of causing various diseases in healthy individuals, especially those suffering from immunodeficiency, by obtaining a mixture of mobile genetic elements (Li et al., 2019).

Enterohemorrhagic Escherichia coli is regarded as the leading cause of outbreaks of diarrheal diseases, hemolytic uremic syndrome (HUS), and hemorrhagic colitis (HC) in humans and animals (Tse et al., 2018). The O157:H7 serotype is a common pathogen between humans and animals, transmitted through food and is responsible for most cases of enterohaemorrhagic diarrhea in humans (Dulo, 2014).

The mechanism followed by this bacteria in causing pathogenicity is not fully understood, but the virulence factors that it possesses have a major role in the occurrence of the disease, the most important of which is the Shiga toxins. The bacteria that produce Shiga toxin are called Shiga Toxin E. coli

(STEC). It causes damage to the intestinal vascular lining, and this effect has been observed in people with hemorrhagic colitis and hemolytic uremic syndrome (Fatima and Aziz, 2019). It also has the ability to resist the acidic environment, produce the hemolysin enzyme, possesses fimbriae that adhere to the epithelial cells of the urinary system, as well as flagella that make it able to adhere to the cells lining the intestine. In addition, it leads to the formation of adhesion lesions and damage to the intestinal villi, which leads to a decrease in the absorption capacity of the intestinal mucosa and thus an imbalance in the ion balance leading to the occurrence of diarrhea. It is obvious to notice severe tissue damage and lesions in the liver, as it is the organ in which many substances entering the body are metabolized and detoxified in one way or another. Mescher (2016) states that the liver is the organ responsible for detoxification. Through this organ, the body releases the largest possible amount of toxic substances by breaking down the unwanted substance, or through interactions associated with the formation of other compounds that help the body release and excrete it through the kidneys with urine. Approximately (75%) of the blood incoming to the liver comes from the gastrointestinal tracts and the spleen via the portal vein. This blood brings with it the absorbed substances in a concentrated manner, and the liver enzymes work to detoxify some of the substances contained in it (Feng et al., 2014).

2- Materials and methods

2.1 chemicals and culture agars: the traditional MacConkey agar is used to isolate the bacteria. The SMAC agar is used to confirm its diagnosis, while the HiCrome agar is regarded as one of the selective media for E.coli O157:H7 as EMB agar. The chemicals formalin, ethanol, xylene, paraffin, Hematoxylin, Eosin, and D.P.X are used in the preparation of histological sections of the liver.

2.2 Bacterium Isolate

The diagnosed and ready-to-use E.coli O157:H7 isolate is used. Despite this, some culture laboratory tests are performed to confirm the validity of its diagnosis. Its type is also determined by biochemical tests using the VITEK® 2 Compact device. The Antibiotic susceptibility testing is carried out using the modified Bauer-Kirby method (Bauer et al., 1966) approved by the World Health Organization.

2.3 Determination of the half lethal dose (LD-50), the infectious dose, and the bacterial count The half lethal dose and the infectious dose are determined according to the Reed- Muench method (1938). As for the bacterial count, the Pour plate method is used for its determination.

2.4 The Experimental Design

Forty-eight male mice of the Balb/c strain, aged (8-12) weeks, weighing between (22-28) gm, are used. They are randomly distributed into six groups of (8) mice per group, as follows:

1- The first group (the control group): a group of mice treated with a physiological solution at a rate of 1 ml for one time per day.

2- The second group: a group of mice treated with the half lethal dose (LD-50) of E.coliO157:H7 at a concentration of 9×102 cell/ml.

3- The third group: a group of mice treated with the infectious dose of bacteria at a concentration of 5×105 cell/ml.

4- The fourth group: thae group of mice treated with the antibiotic TrimethoprimSulfamethoxazole by1 ml per day at a concentration of 102.88 mg / kg according to Nair and Jacob (2016).

5- The fifth group: a group of mice treated with the antibiotic Ciprofloxacin by 1 ml at a concentration of 102.88 mg / kg according to Nair and Jacob (2016).

6- The sixth group: a group of mice treated with the two antibiotics TrimethoprimSulfamethoxazole and

Ciprofloxacin together at a concentration of 102.88 mg/kg each.
After that, glass slides are made using the section method of the liver tissue by adopting the Luna (1968) method, which includes: fixation, washing, dehydration, clearing, infiltration , embedding, trimming and sectioning, staining, and mounting. Finally, the tissue glass slides are examined and photographed under a light microscope at different magnification powers.

3- Results and Discussion

3.1 Confirming the diagnosis of E.coli O157:H7

The diagnosis of the bacteria under study with its type is confirmed based on bacterial isolation on traditional and selective culture media agars, and biochemical tests using the Vitek device as illustrated in Figures (3-1), (3-2), (3-3), (3-3), (3-4) and Table (3-1).

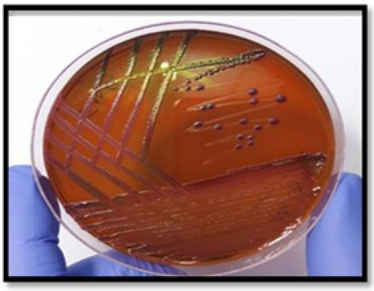

Figure (3-2): *E.coli* on EMB agar

Figure (3-1): *E.coli* on MacConkey agar

Figure (3-4): *E.coli* O157:H on SMAC agar

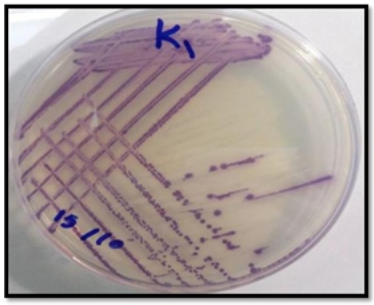

Figure (3-3):*E.coli* O157:H7 on HiCrome agar

Table (3-1): Biochemical diagnosis using the Vitek device

Identification Information	**Card:**	6N		**Lot Number:**	2411419403		**Expires:**	Oct 15, 2021 13:00 CDT									
	Status:	Final		**Analysis Time:**	10.00 hours		**Completed:**	Apr 2, 2021 12:23 CDT									
Organism Origin	VITEK 2																
Selected Organism	98% Probability Escherichia coli O157 Biomumber: 0405611140567251 Confidence: Excellent identification																
Analysis Organisms and Tests to Separate:																	
Analysis Messages: Confirm by serological tests Highly pathogenic organism																	
Contraindicating Typical Biopattern(s) Escherichia coli O157 SAC(80),																	
Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	+	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	+	62	ELLM	+	64	ILATa	-			

Our results agree with Al-Taie (2020) in his study of isolating and diagnosing E.coliO157:H7. They appear as pink colonies on the MacConkey agar since they ferment lactose sugar, while characterized by the phenomenon of metallic luster on EMB agar. Our results also agree with Yadav et.al (2018) in the appearance of typical colonies of these colorless bacteria as they do not ferment the sorbitol sugar on SMAC agar, while the pink-purple colonies indicate E.coli O157:H7. In a study conducted by Klaif et. al (2019), they have found that the HiCrome agar of Escherichia coli bacteria of the O157:H7 serotype is

useful for the diagnosis of this bacteria.

3.2 Testing the sensitivity of E.coli O157:H7 to antibiotics

A sensitivity test is conducted towards (10) types of different antibiotics. The results show that the bacteria are sensitive to the antibiotics Trimethoprim-Sulfamethoxazole, Metronidazole, Gentamicin Amikacin, Azithromycin, Ceftriaxone, Ciprofloxacin and Tetracycline, and are resistant to Amoxicillin/clavulanic acid and Ampicillin. It is to be noted that the bacteria are more sensitive to the antibiotic Ciprofloxacin, followed by the antibiotic Trimethoprim-Sulfamethoxazole, as shown in Figures (3-5) and (3-6).

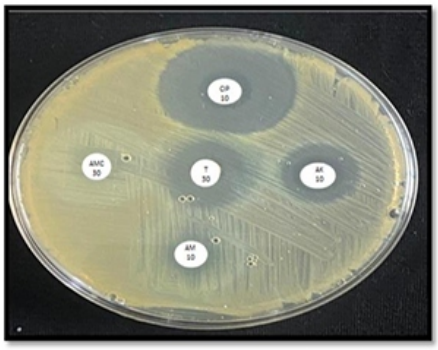

Figure (3-6): Bacterial sensitivity to Amikacin, Tetracycline and Ciprofloxacin and their resistance to Amoxicillin/clavulanic acid and Ampicillin

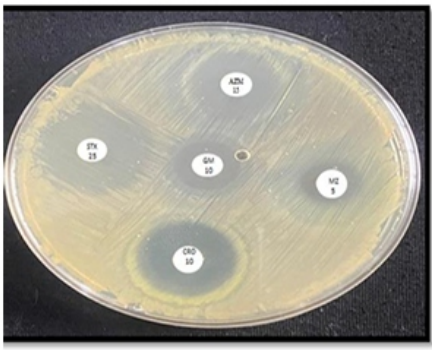

Figure (3-5): Bacterial sensitivity to the antibiotics Trimethoprim-Sulfamethoxazole,Metronidazole, Gentamicin, Azithromycin and Ceftriaxone

Our results agree with what is reached by Tawfiq (2006) in his study on coliform bacteria isolated in hospitals in Saudi Arabia in that their resistance to ampicillin was high. The results by Ali (2012) supported this, with the exception of the results regarding the resistance of the bacteria to the antibiotics Trimethoprim and Amoxicillin/ clavulanic acid in which our results do not agree with his. Also, our results do not agree with Nguyen et. al.(2005) who reached the same result. As for our results regarding the sensitivity of E.coli to Ciprofloxacin and Trimethoprim, they agree with the results of Mahdi (2019).

3.3 The Histological Examination

The histopathological examination of the first group of mice shows the normal structure of the visceral tissue of the liver, which is composed of hepatic cords or sheets formed by polygonal hepatic epithelial cells. The distinctive shape of the hepatocytes is radially arranged around the central vein, separated by bloody spaces representing sinusoids containing phagocytic Kupffer cells, as in Figure (3-7). As for the second group, the results reveal pathological changes represented by acute necrosis in a number of hepatocytes, vacuolation of their cytoplasm, loss of its chromatin material and thickening of the nuclei of a number of others. In addition, there is congestion of the central vein and inflammatory cellular infiltration around it with an increase in the number and size of Kupffer cells as in Figures (3-8) and (3-9). Changes are also observed in the portal triad, represented by severe hyperemia in the branch of the portal vein, with thickening and fibrosis of its walls, hemolysis in the branch of the hepatic artery, and hyperplasia of the lining of the bile duct, as shown in Figure (3-10). The histological sections of the third group show acute necrosis of the hepatocytes, thickening of the nuclei, vacuolation of parts of the tissue, cellular debris, with inflammatory cellular infiltration, as well as an increase in the number of Kupffer cells. Moreover, there is severe damage to the wall of the central vein which leads to severe hemorrhage and the formation of an inflammatory exudate containing red blood cells, as shown in Figures (3-11) and

(3-12). The histological changes of the fourth group are represented by vacuolar degeneration of a number of hepatocytes and necrosis of others, vacuolation of the cytoplasm, hypertrophy in some Kupffer cells. There is also diffuse congestion in the sinusoids, the central vein and the branch of the portal vein, with inflammatory cellular infiltration around the blood vessels and the bile cannula branch as in Figures (3-13), (3-14), (3-15).

The results of the fifth group also indicate necrosis in a number of hepatocytes, with hypertrophy of Kupffer cells, cellular infiltration around the two branches of the hepatic artery, the bile duct, and the two branches of the lymphatic vessel. In addition, there is the occurrence of thrombosis in the hepatic portal vein branch, hemolysis in the central vein with partial damage in its wall, and the presence of diffuse bleeding as in Figures (3-16), (3-17), (3-18). As for the sixth group, the results show the persistence of pathological changes after treatment with both antibiotics and the absence of signs of improvement in mice. This is represented by necrosis, vacuolation, loss of chromatin material for a number of nuclei, thickening of the nuclear membrane of a number of nuclei, hypertrophy in a number of hepatocytes, increase in the number and size of Kupffer cells, expansion of the blood sinusoids and their congestion with blood, and the presence of a thrombus in the central vein. As shown in Figures (3-19), (3-20), (3-21).

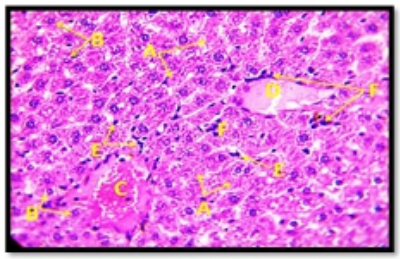

Figure (3-8): A microscopic image of a section in the liver of a mouse from the second group, in which necrosis is observed in a number of hepatocytes (A), loss of chromatin in the nuclei of a number of others (B), congestion and hemolysis in a central vein (C), hemolysis in another central vein (D), hyperplasia and hypertrophy of Kupffer cells in blood sinusoids (E) inflammation cells infiltration around and near a central vein (F). (H&E, X40).

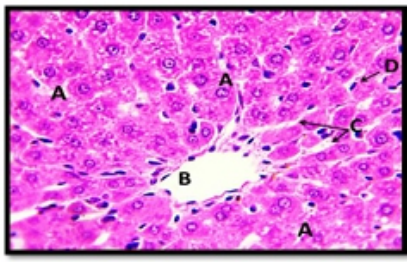

Figure (3-7): A microscopic image of a sectior in the liver of a mouse from the first group, ir which hepatocytes are arranged radially (A) around the central vein (B) separated by sinusoids (C) containing Kupffer cells (D). (H&E, X40).

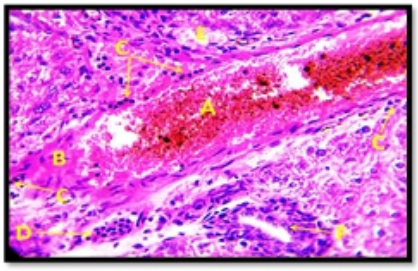

Figure (3-10): A microscopic image of a section in the liver of a mouse from the second group, in which severe hyperemia is observed in the portal venule branch (A), thickness and fibrosis of its walls (B), infiltration of inflammatory cells around it (C), focal infiltration near it (D), hemolysis in the branch of the hepatic artery (E), hyperplasia of the bile ductule (F). (H&E, X40).

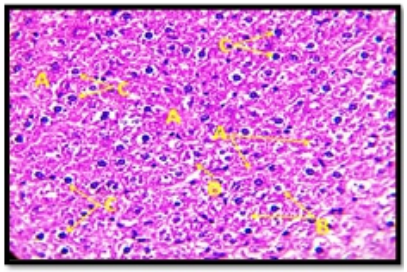

Figure (3-9): A microscopic image of a sectior in the liver of a mouse from the second group in which the observance of necrosis of a number of hepatocytes (A) vacuolation of the cytoplasm of a number of others (B), and pyknosis of the nuclei throughout the histological section (C). (H&E, X40).

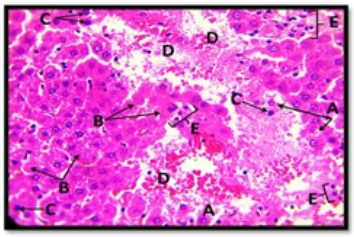

Figure (3-12): A microscopic image of a section in the liver of a mouse from the third group, in which degeneration (A) and necrosis of a number of hepatocytes (B) are noted, pyknosis of the nuclei in others (C), and severe hemorrhage (D), inflammatory cytokine infiltration (E). (H&E, X40).

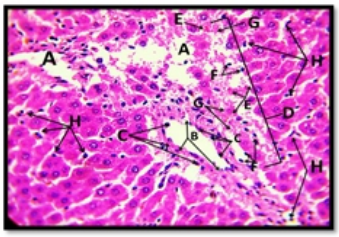

Figure (3-11): A microscopic image of a section in the liver of a mouse from the third group, in which acute necrosis of the hepatocytes is noted leading to the vacuolation of parts of the tissue (A), severe damage to the wall of the central vein (B), the infiltration of inflammatory cells around it (C), inflammatory exudate (D) containing erythrocytes (E), lymphocytic infiltration (F), debris (G), increase in number and size of Kupffer cells (H). (H&E, X40).

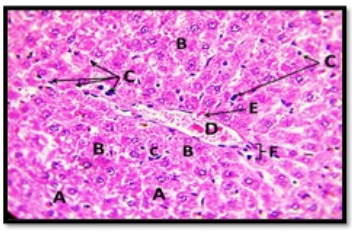

Figure (3-14): A microscopic image of a section of the liver of a mouse from the fourth group, in which degeneration is observed in a number of hepatocytes with vacuolation of the cytoplasm (A), acute necrosis in a number of others (B), hypertrophy in a number of Kupffer cells (C), hyperemia of the central vein (D) partial damage to its wall (E) and near inflammatory cellular infiltration (F). (H&E, X40).

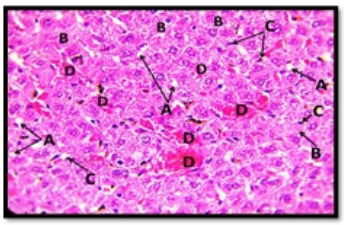

Figure (3-13): A microscopic image of a section in the liver of a mouse from the fourth group, in which vacuolar degeneration is observed in a number of hepatocytes (A), necrosis in a number of others (B), hypertrophy in some Kupffer cells (C), diffused blood congestion in sinusoids (D). (H&E, X40).

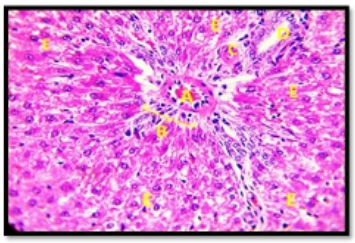

Figure (3-16): Microscopic image of a section of the liver of a fifth group mouse, in which thrombus is observed in the portal vein branch (A) with inflammatory cellular infiltration around it (B) and around each of the hepatic artery branch (C) and the bile duct branch (D), vacuolation in the cytoplasm of most hepatocytes (E). (H&E, X40).

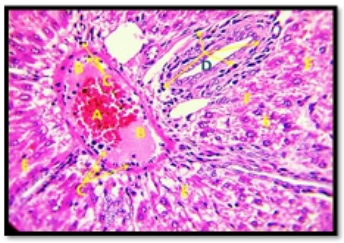

Figure (3-15): Microscopic image of a section of the liver of a mouse from the fourth group, in which congestion (A) and hemolysis are observed in the portal vein branch (B) with infiltration of inflammatory cells at its periphery (C), in addition to their infiltration around the bile duct branch (D), degeneration (E) and necrosis of hepatocytes (F). (H&E, X40).

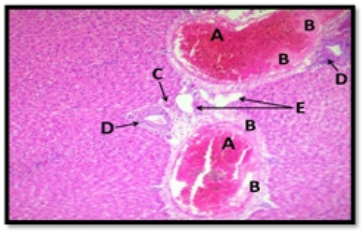

Figure (3-18): Microscopic image of a section in the liver of a mouse from the fifth group, in which acute blood congestion is observed in the two branches of the portal vein (A) partial hemolysis in them (B), inflammatory cellular infiltration around each of the branches of the hepatic artery (C). Two ductal bile branches (D) and two lymphatic vessels (E). (H&E, X4).

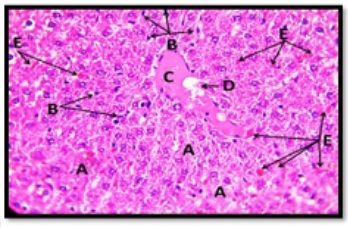

Figure (3-17): A microscopic image of a section of the liver of a mouse from the fifth group, in which necrosis is observed in a number of hepatocytes (A), hypertrophy in some Kupffer cells (B), hemolysis in the central vein (C) partial damage In its wall (D), hemorrhage spread throughout the histological section (E). (H&E, X40).

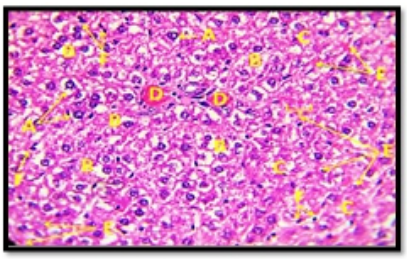

Figure (3-20): A microscopic image of a section in the liver of a mouse from the sixth group, in which an hypertrophy of some hepatocytes (A), a general vacuolation in the cytoplasm of cells (B), necrosis in a number of others (C), two dilated Sinusoids and their blood congested (D), hemorrhage spread throughout the histological section (E), Kupffer cell hypertrophy (F). (H&E, X40).

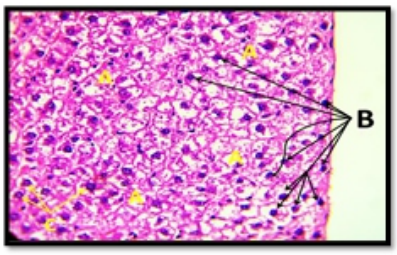

Figure (3-19): A microscopic image of a section in the liver of a mouse from the sixth group, in which the cytoplasm of hepatocytes vacuolates throughout the histological section (A), nuclei pyknosis of a number of cells (B), and the nuclear envelope thickens in a number of others (C). (H&E, X40).

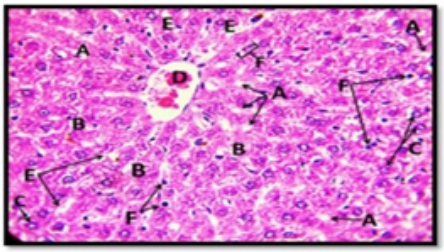

Figure (3-21): Microscopic image of a section in the liver of a mouse from the sixth group, in which vacuolar degeneration is observed in a number of hepatocytes (A), necrosis in a number of others (B), loss of chromatin in a number of nuclei (C), central venous thrombosis (D), dilatation of some sinusoids (E), increased number and size of Kupffer cells (F). (H&E, X40).

The occurrence of histological lesions is attributed to the action of endotoxins that are part of the components of the wall of Gram-negative bacteria. the arrival of E.coli O157:H7 to the liver tissue leads to the occurrence of an inflammatory defense response by the tissue (Rubin and Reisner, 2009). The cause of the gathering of defensive cells is due to the decomposition of the hepatocytes, which leads to the liberation of substances that have ability to chemically attract the devoured defensive cells in order to get rid of them, which in turn causes the death and decomposition of more cells. This is consistent with what Mahdi (2019) has reported in that damaged hepatocytes release compounds such as prostaglandin E1 that have the ability to chemically attract the neutrophils. He has also indicated that neutrophils migrate to the inflamed tissue and secrete a chemo-attractant to attract more of them. In addition, the proteins released as a result of cell damage are subject to partial degradation, which leads to making the proteins chemically attractive to the defensive cells. Moreover, damage to the walls of blood vessels leads to hemorrhage and proliferation of red blood cells in the parenchyma of the liver tissue (Kumar, 2016).

The occurrence of congestion in the blood vessels of the liver tissue is attributed to the local reduction of venous blood flow as a result of occlusion in the blood vessels supplying the part in which the congestion has occurred. This may be due to the presence of large amounts of lipopolysaccharide, which leads to impairment of liver function by damaging the hepatic vascular tissue, and may lead to blockage of blood vessels and thus a decrease in the amount of blood supplied to the tissue and then tissue death (AL-Jobory et al., 2018). The infiltration of lymphocytes in the liver tissue is one of the tissue's defenses against the causative agent. As for the increase in the number of Kupffer cells, it is only a result of inflammation of the liver tissue due to bacteria or their toxins. Our results agree with what has been found by Khalifa et al. (2005), who indicate that E.coli O157:H7 causes severe histopathological lesions

in the liver due to the direct effects of the bacteria and their toxins on hepatocytes. It also agrees with the findings of Chabek (2010), who obtained the same results when administering mice with E.coli O157:H7. These results state that the pathological changes are represented by the presence of degenerative changes resulting from the fact that the germ has the ability to produce some substances that cause tissue changes. For example, the possession of this bacteria of lipopolysaccharides, which is characterized by its direct action on the tissues of the host or its effect on the immune system and the liberation of inflammatory mediators, as well as the possession of virulence factors, the most important of which are Shiga toxins that work synergistically in causing histological changes that result from the inflammatory response of the tissue against the causative agent. Polysaccharides induce genes encoding Hypoxia Inducible Factor (HIF-1), which negatively affects tissue (Peyssonaunx et al., 2007).

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