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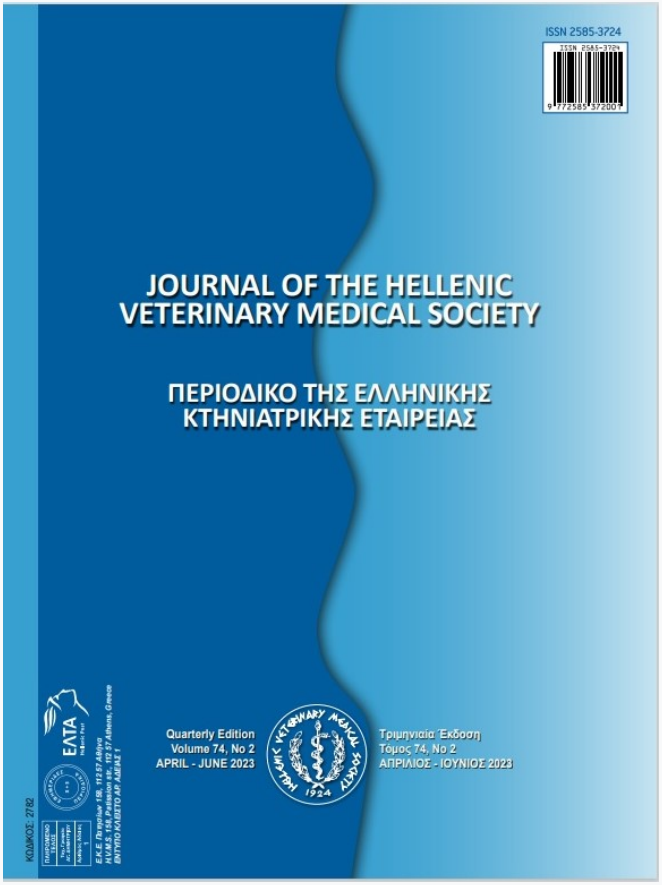

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Research article
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Physiological and Blood Response in Goat Kids Disbudding by Subcutaneous Injection of Eugenol and Cautery

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ABSTRACT: This study aimed to compare the pain in five-day-old goat kids disbudding by eugenol and cautery method. Eugenol is the main factor in a novel disbudding method named clove oil. This study evaluated serum cortisol levels and clinical changes compare disbudding by eugenol and cautery. A total of 25 Raieni (Cashmere) goat kids aged 5 days old were randomly allocated to 1-5 treatment groups (n=5). To differentiate the pain caused by disbudding with the stress of restraint and blood sampling in the results, the animals in both methods of disbudding were divided into different groups according to the evaluated parameters; groups 1,2 cautery-disbudded(550°C,3-6s), and groups 3-5 SC eugenol(0.1cc) administration- disbudded. The cortisol serum level immediately after disbudding in group 2 was 7.94±0.02 µg/dL, vs. group 4, 6.34±0.03 (mean ± SE), and over time, the values were in favor of the eugenol group, despite being insignificant up to 240 and 480 minutes after defoliation. The respiratory rate in group 3 was 77±1.13 vs. group 1, 108±0.67 (mean ± SE), and pulse rate in group 3 was 239.4±1.13 vs. group 1, 284±1.53; rectal temperature showed no significant difference among groups 1&3. In group 5, there was no significant difference between before and after cell blood count and blood enzymes analysis, which could be an argument for the lack of effect of eugenol on vital organs. The results showed less stress and pain level in eugenol-disbudded groups compared to cautery-disbudded in goat kids (P≤0.05). Analysis of specific blood serum enzymes represented no side effect on vital organs in eugenol injection. Our data suggest that eugenol may be a suitable alternative to old and outdated or painful routine disbudding methods. The difference in our findings with similar studies can be due to the variation in sampling methodology, analysis and measurement accuracy. However, more research with wide aspect on pain reliability, availability, economy, and behavioral is needed to assess whether eugenol can replace other methods.

Keywords: Cautery; cortisol; eugenol; goat kid; disbudding

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INTRODUCTION

Disbudding is a standard procedure to reduce animal itself, other farm animals, and farmhand injuries, as well as reducing the space required (Ajuda et al., 2020; Van den Brom et al., 2020). Thermal disbudding like cautery is a routine and at the same times the painful practice used for dairy goat kids disbudding (Thompson et al., 2005). However, although farmers are responsible for minimizing the pain of animals, for economic reasons or lack of knowledge of effective methods of pain moderation, this process is usually performed without effective pain relief (Wagmann et al., 2018; Hempstead et al., 2017; Oehme 1988). Except for the painful method, misuse and placement of the cautery over time will cause severe burns in the area and due to the young age of the goat kids at the time of use of this method and not complete formation of the frontal bone, the possibility of meningitis following this procedure is very high (Fubini and Ducharme, 2016). Topical agents used in other farm animals such as potassium hydroxide, sodium hydroxide, calcium hydroxide, and calcium chloride either lack the desired effect in goat kids or create more severe disadvantages like damages to structures adjacent to the frontal horns, eye, ear, frontal bone and even brain. The other complication of disbudding by these techniques is the horn buds' regrowth (Koger, 1976). It also prevents the production of hornless goats from genetic manipulation because of the association of intersex recessive genes with those of the hornless (Schibler et al., 2000). Consequently, novel alternatives should be investigated to improve goat kids' welfare.

Stress and pain stimulate the sympathetic and parasympathetic nerves, and subsequently result in changes in clinical parameters such as body temperature, pulse rate, and respiratory rate (TPR) (Molony and Kent, 1997) which is usually absent in the similar studies and not much attention is paid to these changes together with cortisol. In addition to physiological and behavioral changes measurement (Giannetto et al., 2017; Fazio et al., 2016) in animals to measure pain, serum cortisol levels can also be used. Cortisol is the main glucocorticoid hormone that is released in response to pain or stress. Cortisol level in the serum can be measured for the hypothalamic-pituitary-adrenal axis (HPA) stimulation, which is activated in the painful conditions (Molony and Kent, 1997; Hechter and Pincus, 1954). Due to the response of serum cortisol levels to other endogenous and exogenous stimuli such as sexual cycle, restrain, blood sampling, as well as circadian, interpretation of the results of its measurement requires high accuracy and should be along with other measured parameters (Ajuda et al., 2020; Endo et al., 2018).

One of the novel methods that has been introduced is clove oil subcutaneously (SC) injection (Molaei et al., 2015). Studies on the chemical structure of clove essential oil have shown that 72-95% is made up of eugenol (EG), so it is a probable effective factor in disbudding by the clove essential oil (Abbasi et al., 2018; Jaganathan and Supriyanto, 2012). EG as a chemical component (4-allyl-2-methoxyphenol–C10H12O2) is found in essential oil taken from some herbal species like Syzygium aromaticum (clove), Cinnamomum verum, and Pimenta racemose (Carrasco et al., 2008; Pavithra, 1981). Anti-inflammatory, anesthetic, and anti-oxidant effects of EG have been well documented in the previous studies (Hernández-Sánchez et al., 2012; Alma et al., 2007; San-Myint and Abu-Bakar,1995). Inhibition of cyclooxygenase type II enzyme causes anti-inflammatory activity of EG, and selective binding to capsaicin receptor makes anti-nociceptive effects of EG (Hernández-Sánchez et al., 2012; Ohkubo and Shibata, 1997). Also, some studies have shown that EG have anti-bacterial and anti-inflammatory effects (Ali et al., 2005; Di Pasqua et al., 2006). EG has been used as a core in folk medicine in ancient civilizations. It has also been used in oral hygiene, food flavor, perfume production, anesthetic productions in fish, and dental analgesia (Di Pasqua et al., 2006).

Our hypothesis was that goat kids disbudding by EG as a novel procedure might be less painful than other traditional routine method in lack of any analgesic or anesthetic agent. The present study was conducted to investigate the pain and hematological effects of disbudding by EG. So, the cortisol serum level, cell blood count (CBC), blood biochemical parameters, and some vital organ enzymes in the goat kids were compared following two methods of disbudding: eugenol and cautery.

MATERIALS AND METHODS

This study was carried out on 25, five-day-old, male and healthy Raieni (Cashmere) goat kids. The animals used in this study were acquired from the domestic animal farm of Shahid Bahonar University of Kerman. All kids received colostrum at birth and to minimize potential stress, they were not separated from their dam or other kids. Kids were kept in the

research farm in standard kids pen with clean and dry bedding during the study (March to April). They feed with their mother's milks two times in the day under the farm routine standards plan. Kids had access to fresh water in a pond attached to the pen walls. The temperature ranged between 10-25°C (day and night) and the humidity was 21% in the barn. The animals randomize divided kids were ear tagging and the number of all 25 registered.

To separate the stress effect of blood sampling (needle puncture and restraining) with disbudding, as well as to avoid hypovolemic shock due to intermittent blood sampling in goat kids, the animals in both methods of disbudding were randomly allocated into different five equal groups (n=5) according to the evaluated parameters. To randomize the goats in different treatment groups, the ear tag numbers of all healthy goat kids aged 5 days were entered into Excel software ver. 2016. The numbers obtained from the herd were separated by a double-blind randomization method. Every five numbers were placed in groups of 1 to 5, respectively.

In 1 and 2 groups, disbudding was done by electrical cautery (HORN'UP ®), specifically designed for goat kids. The circular part of the device was placed on the goat kid's horn bud for 3 to 6 seconds after the preparation, the device light was turned off which showed the device heat has arrived at 550°C. At the end of desired time, the horny bud along with the surrounding skin, the size of a circular area of the device head, is removed by pressing the device on the place. In group 3 to 5, disbudding was performed by SC injection of 0.1 ml EG (Table 1). Subcutaneous administration of eugenol was performed by an insulin syringe in the horn bud region. For this purpose, a 0.5-inch 30-gauge needle was used. The needle was the penetrated lateral aspect and guided under the horn bud at a 45° angle.

Table 1: The names of the groups involved in the study based on the parameters measured.

	Clinical Parameters (TPR)	Serum Cortisol Level	CBC & Blood Chemistry
Cautery groups	1	2	-
Eugenol groups	3	4	5

(n=5)

At the end of the study, all the goat kids were clinically examined by a farm veterinarian and they were returned to their flock.

Clinical Evaluation: Same as the above reason, the TPR evaluation (Molony and Kent, 1997) groups were isolated from serum cortisol level evaluation groups to separate stress effect of clinical evaluation and disbudding. In 1 and 3 groups, the goat kids were kept in their special box; the body temperature was measured by a digital thermometer was located in kids rectal, and the pulse and respiratory rate were measured by a stethoscope (Riester Duplex 4200, Germany) and chest movement observation in 15s for 4 continuous time (Hempstead et al., 2020; Nfor et al., 2016). All parameters evaluated before disbudding (baseline), immediately after disbudding, and every two hours until 8hr, and 24hr following disbudding by cautery or EG administration.

Serum Cortisol Level Evaluation: Serum cortisol level was measured in 2mL blood samples that were collected by jugular vein catheterization, before (baseline), immediately after disbudding, and 30, 60, 120, 240, 480min post disbudding in 2 and 4 groups. The jugular vein catheterization (G-22 or 24) was placed 12hr before disbudding and fixed in position until the end of sampling time. For this aim, the animals were kept in goat box while sampling was performed. The blood samples were transmitted to micro tubes and they were centrifuged at 4000 rpm for 10 min, and then, separated serums were stored at −80°C until analysis. Serum cortisol level measured by enzyme-linked immunosorbent assay (ELISA) commercial cortisol kit (Monobind, USA) in commercial veterinary laboratory. The operators were blind to the treatment groups.

CBC, Blood Chemical, and Vital Organ Serum Enzyme Evaluation: In group 5, blood samples (5.5 mL) were taken from jugular vein, 24hr before (baseline) and 24hr after EG administration without catheterization. The blood samples were poured into two different tubes (2.5 mL in the CBC tube with EDTA and 3 mL in the serum separator tube) (FL medical, Italy) to test CBC and blood enzymes. The CBC test was done by commercial auto analyzer. To perform blood chemistry test, the blood samples were centrifuged at 4000 rpm for 10 min, and then, the serum was separated and stored at −20°C until analysis. The enzymes included aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transpeptidase (GGT), succinate dehydrogenase (SDH), and alkaline phosphatase (ALP) as the liver enzymes, and

creatine phosphokinase (CPK) as the muscles or heart muscles enzyme. All these enzymatic tests were performed using enzymatic colorimetric method (Pars Azmoon commercial kits, Iran) and biochemistry autoanalyzer (Alpha Classic, Sanjesh Co., Iran). Other biochemical parameters were total protein (TP), blood urea nitrogen (BUN), and creatinine (Cr, Jaffe method; Pars Azmoon commercial kits, Iran, and biochemical auto analyzer; Alpha Classic, Sanjesh Co., Iran). Both analyses were done using standard method in commercial veterinary laboratory. The operators were blind to the treatment group, too.

All data were presented expressed as mean ± standard error. Statistical analysis was performed using repeated measures analysis of variance (RMANOVA). For running the statistical analysis, we considered treatment as the fixed and time as the random effects. Statistical model equation: Predicted response= Intercept+groupi+timej+timej×groupi.

Before doing the analysis, we checked the data for normality using the Kolmogorov-Smirnov test. Also, data were checked for the presence of outliers. As the data were normal, no transformation was needed. Statistically significant level was considered at P≤0.05 and statistical analysis was done using SPSS software (SPSS Inc. Released 2011. SPSS for Windows, Version 20, IBM, SPSS Inc.). The power analysis was done by Stata Ver. 14.2

All procedures were approved by the Animal Ethics Committee of Shahid Bahonar University of Kerman (Ethical code: IR.UK.REC.1398.001).

RESULTS

1. Clinical Evaluation

1.1. Body Temperature: There was a time and disbudding interaction in rectal temperature (Figure 1). There was no significant difference in rectal temperature between group 1 and 3 except at 2hr after disbudding (p=0.03, power=0.95). Also, no statistically significant evidence was detected between time sequence and the counted amount of body temperature in each group.

1.2. Pulse Rate: The statistical difference, between groups 1 and 3 at 6hr (p=0.027, power=062) and 24hr (p=0.045, power=0.54) was detected, also the weak statistical evidence (p=0.07) was seen immediately after disbudding. There was no relationship between experimental period time and pulse rate in each group (Figure 2), even with an average increase of 41 pulses rate/min, no statistically significant increase was observed in group 1.

1.3. Respiratory Rate: respiratory rate changed from baseline to immediately after disbudding in group 1 with average inverse of 31 rate/min, we had poor statistically change (p=0.06) and strong statistically change (p=0.01, power=0.43) (Figure 3).

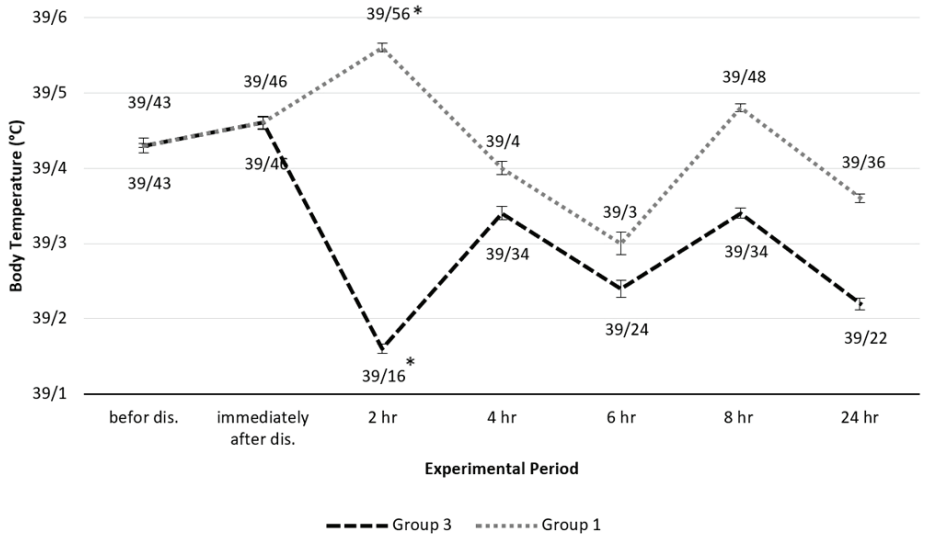

Figure 1: Body temperature (°C; mean ± SE) of goat kid's changes in group 1 and 3 (cautery) compared to those in baseline 2hr after disbudding.

* significant difference in results between different groups (p≤0.05)

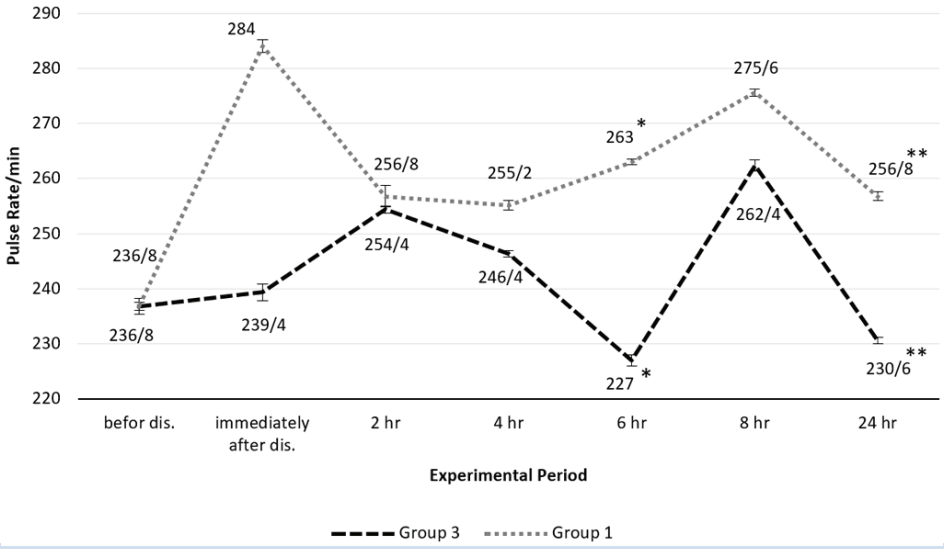

Figure 2: Changes in pulse rate (n/min; mean ± SE) following disbudding by cautery or SC injection of eugenol (0.1 mL) in groups 1 and 3, respectively.

*, ** significant difference in results between different groups (p≤0.05)

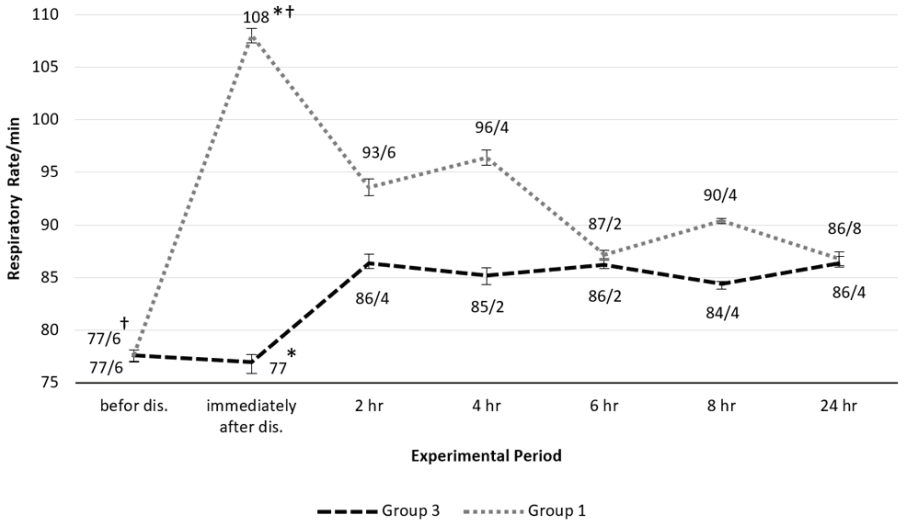

Figure 3: Effect of disbudding on respiratory rate (n/min; mean ± SE) using cautery or SC injection of eugenol (0.1 mL) in groups 1 and 3, respectively.

*, † significant difference in results between different groups (p≤0.05)

2. Serum Cortisol Level Evaluation: In groups 2 and 4, between immediately and 30,60,120min after disbudding, the effect of time on changes in serum cortisol levels in each group was significant (in chronological order, p-value in group 2 = 0.014, 0.041, 0.07, and in group 4 = 0.02, 0.000, 0.001). Also, there was a reasonable downward trend due to pain post disbudding reduction, and the results approached the baseline scale (before disbudding) over time. The time interaction was measured for cortisol level (Figure 4). There was a difference between baseline and immediately after disbudding in groups 2 and 4 (p<0.000). Also, significant differences were observed between the two groups immediately, (p=0.004, power=0.93), 240min (p=0.026, power=0.56) and 480min (p=0.065, power=0.40) after disbudding.

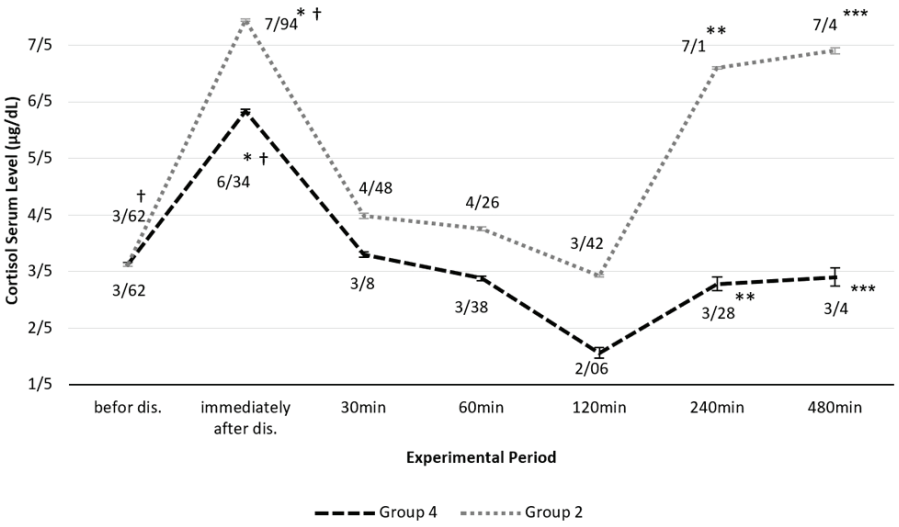

Figure 4: Serum cortisol level (mg/dL; mean ± SE) before (baseline) and immediately after disbudding, also, over 30, 60, 120, 240, and 480 min; by cautery or SC injection of eugenol (0.1 mL) in groups 2 and 4, respectively.

*, **, ***, † significant difference in results between different groups ($p \leq 0.05$)

Tabel 2: CBC and biochemical evaluation of goat kids disbudded by the SC injection of eogenol (0.1 mL; group 5)

CBC Panel	Group 5			normal range[1]	Biochemical Panel	Group 5			normal range
	24h before	24h after				24h before	24h after		
RBC* (×10⁶/µL)	6.22±0.021	7.326±0.01	↑	8.0-18.0	BUN (mg/dL)	42.4±0.63	30.6±0.17	↓	
Hb (g/dL)	7.66±0.03	8.52±0.03	↑	8.0-12.0	Cr. (mg/dL)	0.538±0.00	0.496±0.00	↓	
HCT* (%)	20.62±0.09	24.6±0.09	↑	22-38	TP (g/dL)	5/988±0.01	5.758±0.01	↓	
MCV (fL)	34.36±0.05	33.06±0.04	↓	16-25	ALT (U/L)	18.00±0.06	19.00±0.06	↓	15.3-52.3
MCH* (g/dL)	12.3±0.01	11.9±0.01	↓	5.2-8.0	AST (U/L) *	68.00±0.38	91.80±0.45	↑	167-513
MCHC* (g/dL)	37.2±0.05	35.08±0.02	↓	30.0-36.0	GGT (U/L) *	117.60±1.42	76.00±0.92	↓	20-56
WBC* 1000/Cumm	9.424±0.13	8.474±0.04	↓	4-13	ALP (U/L)	124.68±11.5	164.62±10.8	↑	93-387
PLT* 1000/µL	162.2±15.3	66.6±10.3	↓	100-300	CPK (U/L)	96.40±2.15	83.80±1.11	↓	0.8-8.9
					SDH (U/L)	15.5±0.07	16.1±0.04	↑	14-23.6

(*) show significant difference ($p<0.05$) in comparison with 24hour before, and after disbudding by EG injection.
(↓) increase or (↑) decrease in measurement parameters.
1- Ettinger, 2017
Red Blood Cell (RBC), Hemoglobulin (Hb), Hematocrit (HCT), Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Cell (WBC), Platelets (PLT), Blood Urea Nitrogen (BUN), Creatinine (Cr), Total Protein (TP), Transaminase (ALT), Aspartate Transaminase (AST), Alanine Gamma-Glutamyl Transpeptidase (GGT), Phosphatase (ALP), Creatine Phosphokinase (CPK), Succinate Alkaline Dehydrogenase (SDH)

3. CBC, Blood Chemical, and Vital Organ Serum Enzyme Evaluation: The results of the CBC test in group 5 showed a decrease in the number of red blood cells, hemoglobin, hematocrit percentage, and platelet compared to those obtained 24hr before and after disbudding by EG (Table 2; $P>0.05$). White blood cell count was not significantly different 24hr after the injection of EG.

DISCUSSION

Interpretation of physiological parameters along with behavioral symptoms during the disbudding or other painful procedure can help to achieve better results. But due to the difference between animal species seems it's impossible to suggest the same pattern for all under various painful procedures. However, such studies about the routine procedures will increase the animal welfare by reducing the pain, which

itself promises a better future in animal husbandry.

This evaluation was done through the measurement of serum cortisol level and TPR to determine the pain and stress levels in disbudding by cautery compared to the EG injection over time. Analysis of the cortisol level in disbudding by cautery showed more pain inducing than EG administration in goat kids for 30 min after disbudding. This result was also supported by the TPR measurement, in other words, at early hours after disbudding by cautery, more physiological changes occurred due to stress compared to our new disbudding alternative method. A similar study did not report that cautery is more painful than clove oil injection (Hempstead et al., 2018a). However, they introduced the clove oil method as an alternative based on the low rate of tissue damage compared to the cautery. Based on the experience of the authors, the lack of significant difference in the pain level in disbudding by cautery and clove oil in the above study may was due to the variation in the type of blood sampling and the use of clove oil instead of pure eugenol. We believe, blood sampling by needle puncher in each time during the study, could induce more stress and pain, also, restrain the kid during the sampling could make the situation worse. In the present study, the results of serum cortisol levels showed an overlap with the clinical examinations results and we found the significant deference between cautery and eugenol disbudding pain amount. In previous studies, the requirement to concomitant use of analgesic and anesthetic compounds during the disbudding of goats and calves has been proven to be a reason for the pain in the old disbudding or dehorning methods used in these studies, especially cautery (Hempstead et al., 2018b; Vickers et al., 2005).

Furthermore, the increase of approximately 3µg/dL blood cortisol in groups 4 and 2 (Figure 4) combined with an increase in the body physiological activity in groups 1 and 3 (Figure 1-3) indicates a physiological response to the pain and stress stimulus, however, this increase in the groups disbudded by EG was less than those disbudded by the cautery, indicating low levels of stress and pain in EG injection.

Pain and stress through sympathetic effects increase changes in the heart and respiratory system and reduce peripheral blood flow. Stress and pain also affect the appetite and nutrition of animals, and subsequently, can result in weight loss in food animals (Morton and Griffiths, 1985). The reaction of both sympathetic and HPA axis under stress conditions is well-known (Schaefer et al., 2001). In the meantime, the response of the sympathetic axis to the stress stimulus is very rapid and it releases the catecholamines into the blood serum. Cortisol serum level has been used to measure pain and stress in ventricular resection surgery, sterilization, and pain induced by electric shock (Mellor and Murray, 1989a; Mellor and Murray, 1989b, Jephcott et al, 1987; Pearson and Mellor, 1975). Cortisol levels change throughout the day, so, intermittent blood sampling should be used to evaluate this parameter more accurately (Carrasco et al., 2008). To determine the effects of stress and pain on body, in addition to cortisol level, measurement of vital parameters (TPR) would be also helpful.

EG, as the main ingredient of clove oil, has anti-inflammatory and analgesic effects that are routinely used in dentistry (0.1% solution). Therefore, it can be concluded that the reduction in pain following eugenol injection may be a result. However, in some studies, this analgesia has not been clearly shown, or in some cases, the amount of pain caused, has been calculated as the same with cautery (Hempstead et al., 2018a; Kozam and Mantel, 1978). According to our results, the amount of pain due to EG injection was significantly less than the cautery, especially immediately after injection, and 240 - 480 minutes later. As our study was intended to compare the values of serum cortisol levels in the two groups of eugenol and cautery with the baseline values, perhaps if there was an intact goat kids' group as sham for cortisol measurements, we could better show the difference in pain level between these two methods. The reason of pain caused by eugenol injection, which is clearly observed in the results of both our clinical and serum methods, is probably caused by the type of use (injection by needle) and also the volume of fluid entering the subcutaneous area. The phenolics structures can also cause local irritation.

Mc Meekan et al., (1998), compared cortisol serum levels in dehorning of cattle by local anesthetics alone or with non-steroidal anti-inflammatory drugs (NSAIDs). They found that cortisol serum level in group that received local anesthetics and NSAID was lower than those that received local anesthetics alone. This study clearly showed that the disbudding is a completely painful operation and analgesic requirement to relief the pain and the stress is inevitable.

In a similar study conducted by Stilwell et al. (2010), similar results about disbudding of cattle by cautery were obtained. Also, it was revealed that

Xylazine as a sedative cannot prevent the increase of cortisol serum levels following dehorning of cattle by cautery, while there was no considerable difference in the cortisol level between the animals received Xylazine and those received no Xylazine, therefore, it had no acceptable effects on the pain reduction in cattle dehorned by cautery. Although in another study by Stilwell et al. (2012), Carprofen was able to keep the cortisol serum level low by controlling the pain following cautery dehorning of cattle. Stilwell et al. (2009) showed that the application of a local anesthetic method with NSAIDs in cautery dehorning of cattle, could reduce the pain during and after dehorning.

Based on previous studies, the method of using caustic paste disbudding in calves and goat kids is more painful than other routine methods. In this method, the serum cortisol level was in high range compared with other groups done by cautery and clove oil (194 nmol/L by caustic paste vs. 54 nmol/L by clove oil). Also, the behavioral changes related to stress level were clearly observed and showed similar overlap with changes in serum cortisol levels. However, the use of analgesics such as alpha 2 agonists (Xylazine) in calves can reverse the situation in favor of the caustic paste method, which can be due to the lack of behavioral symptoms similar to those in animals under painful procedures (Hempstead et al., 2018a).

Bengtsson et al., (1996) and Hempstead et al. (2018), both were of the opinion that the use of cryosurgery for disbudding is more painful than the cautery. Although, according to the authors, the interpretation of behavioral patterns in different species may have conflicting results, so measurements of the serum cortisol levels can help provide a reasoned rationale (Hempstead et al., 2018b; Bengtsson et al., 1996).

Body temperature changes can be an important parameter in measuring the pain or inflammation (Hempstead et al., 2020). Temperature changes seen in the results of the current study, along with others, show a low level of pain in the method of dehorning by EG. Although it is possible, due to the low number of animals in each group because of ethical reasons, as well as individual variation in the tested herd, the values obtained are not statistically significant differences.

The increasing number of white blood cells in blood samples of group 5, 24hr after disbudding by the EG injection compared to 24hr before it, was not indicative of leukocytosis because it was limited to the normal range of white blood cells. The increase of platelet count was in normal range and it may have been due to the EG injection in horn buds.

Since EG leads to hepatic metastasis, based on some studies, the systemic use of this compound may cause toxic hepatitis. Thompson et al. (1991) investigated the toxicity and metabolism of EG with the concentration of 1 mM in the rat hepatocyte cell line. They revealed that EG is metabolized in hepatocytes and Quinone-Methide is a probable cause of toxic hepatitis by EG (Thompson et al., 1991). However, in this study, the results of serum enzymes measurement, especially AST, SDH, and GGT, showed that toxic hepatitis was not induced due to the SC injection of 0.1 ml EG in horn buds. Although AST increased 24hr after the EG injection due to hemolysis or liver injury, but since the SDH was within normal range, the possibility of hepatitis could be ruled out.

This study, like other similar ones, has some limitations. The first thing we faced was the limitation in determining the number of samples, which is the reason why some data are not significant and therefore affected the power of the study. This restriction was due to the considering similar articles and also ethical reasons which due to the novelty of the method and the impossibility of presenting strong evidence, the Ethics Committee did not allow us to use a larger sample obtained in the calculations.

Regarding the research on pain and stress, because of the sensitivity of results and some doubts, it is better to use several parameters related to pain, which in our study due to lack of access to the necessary Behavioral expertise as well as not being included in study's objectives, only measuring serum cortisol levels and clinical symptoms (TPR) considered sufficient, which is strongly recommended for future studies that other blood parameters such as glucose and behavioral changes should be used in conjunction with other available methods.

The study has one limitation. The power of the study to show the differences in some study times was low. This low power could be related to the low sample size. The calculated power varied between 40% to 95%. Due to ethical considerations, the ethics committee that approved the study protocol did not permit to include of more animals in each group.

CONCLUSIONS

In general, disbudding by EG compared with cau-

tery, induces less stress and pain in the goat kids and has no harmful effect on the other vital organs of the body. Therefore, this method can be used or suggested as a suitable alternative. However, to determine the presence or absence of pain and its exact extent in future studies, it is suggested to use behavioral measurements and even electrophysiology for better understand.

CONFLICT OF INTEREST

The authors decelerate there was no competing interest in their research.

ABBREVIATION

TPR: temperature, pulse rate, and respiratory rate, HPA: the hypothalamic-pituitary-adrenal axis, SC: subcutaneously injection, EG: eugenol, CBC: cell blood count, ELISA: enzyme-linked immunosorbent assay, AST: aspartate transaminase, ALT: alanine transaminase, GGT: gamma-glutamyl transpeptidase, SDH: succinate dehydrogenase, ALP: alkaline phosphatase, CPK: creatine phosphokinase, TP: total protein, BUN: blood urea nitrogen, Cr: creatinine, NSAIDs: non-steroidal anti-inflammatory.

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