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ORIGINAL ARTICLE

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The optimal Holmium laser settings for disintegration of cystine and calcium oxalate monohydrate stones: *In vitro* study

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ABSTRACT

Objective: Disintegrating cystine and calcium oxalate monohydrate stones present a formidable challenge owing to their hardness and distinct composition. This study aimed to establish optimal laser settings for these hard stones lithotripsy.

Patients and Methods: Cystine and calcium oxalate monohydrate stones were extracted from two patients. Two experiments were conducted in vitro by utilizing a 272 µm laser fiber with variable settings to disintegrate the cystine and calcium oxalate monohydrate stones. In the first experiment, energy was adjustable while frequency was constant, whereas the second experiment involved constant energy with adjustable frequency on each type of stone and each experiment was repeated three times to ensure robustness and reliability.

Results: Our findings indicated that for cystine stones, use of higher total power with high energy and low frequency proved to be effective. Conversely, for calcium oxalate monohydrate stones, settings involving higher total power with low energy and high frequency demonstrated superior efficacy and safety.

Conclusion: Holmium (Ho: YAG) laser settings with higher total power, high energy, and low frequency effectively disintegrate cystine stones despite increased heat, which was measured by a thermometer with a thermocouple. For calcium oxalate monohydrate stones, higher total power, high frequency, and low energy settings are recommended and safe.

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Lithotripsy; laser setting; hard stone; cystine; calcium oxalate

Introduction

In modern endourology, the laser is essential in urolithiasis [1]. Holmium: YAG (Ho: YAG) Laser has become the most common type of laser used in lithotripsy regarding its effectiveness and safety [1].

Many factors play a role in lithotripsy performance, including pulse modulation and peak power [2]. Peak power refers to most of the energy of each pulse that is instantly distributed [3].

The main target of lithotripsy is to completely disintegrate the stone without leaving as many residual stone fragments as possible [4]. The techniques by which the laser disintegrates stones include fragmentation (high energy, low frequency, short pulse) and dusting (low energy, high frequency, long pulse) [5].

Cystine stones are uncommon, constituting 1% to 2% of adult urinary stones and up to 10% in pediatric cases [6]. Cystine stones are more likely to require surgical intervention because they are typically hard and difficult to remove using shock wave lithotripsy [7]. So, laser lithotripsy is the mainstay of the management of these stones [8]. The studies reporting the disintegration of cystine stones by standard laser settings are lacking [9].

Although calcium oxalate monohydrate stones are prevalent, there are no international guidelines for standard laser settings for disintegration [10]. Therefore, this preliminary in vitro study was designed to determine the optimal laser settings for disintegrating these types of hard stones.

Aim of the study

Optimization of laser settings to be standardized for hard stones as cystine and calcium oxalate monohydrate stones disintegrating.

Methods

This experimental observational preliminary in vitro study was conducted on NaN Invalid Date at Menoufia University Hospital. After approval of the ethics committee of the faculty of medicine of Menoufia University, Following the procurement of written consent from a diagnosed cystinuria patient with a pre-operative Computerized Tomography scan (CT) the stone Hounsfield Unit (HFU) was 1258, a substantial stone was surgically extracted. The stone, displaying a yellowish-waxy appearance, weighed 31.5

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gm and measured 4.5 cm in its most significant dimension. Approximately 520 mg of the stone was meticulously isolated, including the shell and central core fragments. This isolated fragment underwent quantitative mineral analysis utilizing Crystallography optical techniques and Infrared Spectroscopy, conclusively confirming its composition as 100% L Cystine (Figure 1a).

Another large stone fragment (478 mg) was extracted via percutaneous nephrolithotomy after patient consent. The HFU of the stone in the preoperative CT scan was 1187. The fragments underwent mineral analysis, confirming them as calcium oxalate monohydrate stones (Figure 1b).

Each cystine stone and the calcium oxalate stone were fragmented manually with High-precision Abrasive Blades and 30 small fragments of equal sizes were isolated from each stone; each fragment was 1 cm in greatest dimension and 1.2 gm in weight, which were quantified by High Precision Laboratory Weighing Scale (Joanlab ® electronic scale).

Ten cystine stone fragments were individually placed in ten test tubes containing 3 ml of saline (NaCl 0.9%) at ambient temperature (25.2°C). The tubes were randomly labeled EXP.Ac1 - Ac5 for procedure A and labeled Bc1-Bc5 for experiment B. Similarly, ten calcium oxalate stone fragments were placed in ten separate test tubes with 3 ml saline at the same temperature (25.2°C). These tubes were labeled EXP. Ao1 - Ao5 and EXP.Bo1 - Bo5 for the experiments A and B, respectively.

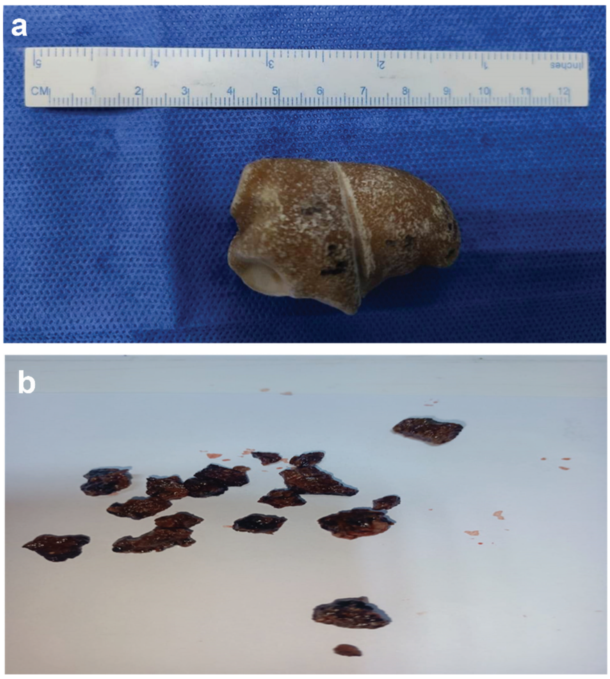

Figure 1. (a) the cystine stone is yellow-waxy in appearance and asymmetrical in shape. (b) the calcium oxalate fragments are black in color.

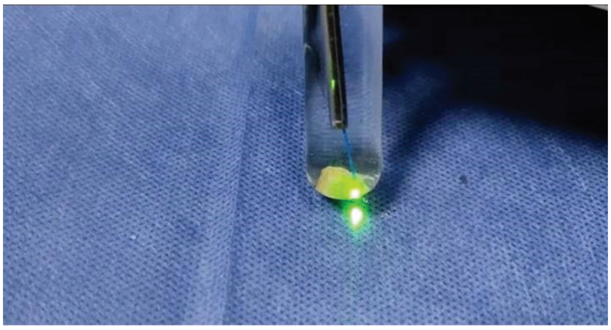

Figure 2. Lithotripsy was done in a test tube with semi-rigid ureteroscopy.

A digital thermometer (Vee Gee Scientific ® company) with a thermocouple (MICC ® company) is attached to each test tube to monitor the saline temperature continuously during lithotripsy. The thermocouple connected to a thermometer was immersed in saline. We ensured precise thermometer probe placement at the center of the saline, away from the test tube wall. To enhance accuracy, insulation, a foam insulator was applied to the upper part of the thermometer probe above the saline level, minimizing heat exchange with the surroundings for reliable temperature measurements.

A single operator fragmented the stones at room temperature using laser fiber (flexifib-Wierderverwendbare Laser fiber®) with an optical core of 272 μm and a semi-rigid ureteroscope (Karl Storz.). The laser fiber tip was positioned 5 mm beyond the distal opening of the ureteroscope and placed in direct contact for fragmentation with the stone (Figure 2).

Each test time was fixed at 5 min. Time out was estimated from pedal activation of the laser generator (using Sphinx litho® set 30 W) generator.

In Experiment A, stones in labeled test tubes (EXP. Ac1 - Ac5 and Ao1 - Ao5) were subjected to varying laser energy levels: 0.7, 1, 1.5, 1.7, and 2 Joules, respectively, while maintaining a fixed frequency of 15 HZ (Table 1).

In Experiment B, stones in test tubes are labeled EXP. Bc1 - Bc5 and Bo1 - Bo5 were exposed to a fixed energy of 1.1 Joules, with frequencies gradually increasing to 12, 15, 20, 23, and 27 HZ, respectively (Table 1). Both experiments employed a long pulse width in pulse mode.

Experimental temperature

Each experiment temperature was recorded by the digital thermometer and recorded from the initiation of lithotripsy until the end of the process; the temperature was recorded throughout the disintegration process.

Fiber end Burnback

A particular caliper (digital one) was utilized to determine the laser fiber burn back by measuring the

Table 1. Experimental A and B laser settings for both types of stones.

EXP.	Energy (Joule)	Frequency (HZ)	Power (Watt)	Pulse mode
Ac1,Ao1	0.7	15	10.5	Long
Ac2,Ao2	1.0	15	15.0	Long
Ac3,Ao3	1.5	15	22.5	Long
Ac4,Ao4	1.7	15	25.5	Long
Ac5,Ao5	2.0	15	30.0	Long
B1,Bo1	1.1	12	13.2	Long
B2,Bo2	1.1	15	16.5	Long
B3,Bo3	1.1	20	22.0	Long
B4,Bo4	1.1	23	25.3	Long
B5,Bo5	1.1	27	29.7	Long

EXP: experiment Ac:test tube with cystine stone, Ao:test tube with calcium oxalate stone, Bc:test tube with cystine stone,Bo:test tube with calcium oxalate stone.

distance from the blue jacket covering to the fiber end before and after each process. The fiber tip burnback was measured in millimeters after each stone disintegration.

The ablation size of the stone and residual stone fragments

For accurate measurements prior to and after the procedure, the stones underwent drying by placing them in a gauze sieve and dehydrating them in a dry environment for 24 h before their weight measurement.

The ablated size of the stone and the residual fragments were assessed and measured in mm Figure 3. Then, the ablation percentage was calculated according to the following equation:

$$Ablation(\%) = \frac{Intial\ stone\ weight\ (mg) - weight\ after\ disentigration\ (mg)}{intial\ stone\ weight\ (mg)} \times 100.$$

Occurrence of light flashes and charring

As the flashlight and charring may occur accidentally during stone disintegration, the light flashes and charring of the stone fragments or test tube charring were observed in each experiment. Experiments A and B were repeated three times with the same parameters and the remaining stone fragments to avoid technical errors.

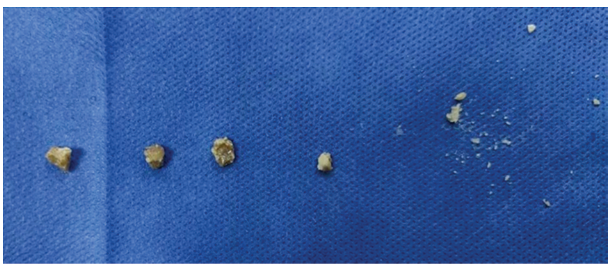

Figure 3. Residual fragments of cystine stones after each experiment Ac.

Statistical analysis

Data analysis utilized SPSS (Statistical Package for the Social Sciences) version 26. Categorical variables were described via absolute frequencies and compared using chi-square and Fisher exact tests where appropriate. Ordinal data between groups were compared using the chi-square test. The Shapiro–Wilk test verified assumptions for parametric tests. Quantitative data between groups were compared using independent sample t-tests. Pearson correlation coefficients gauged the correlation strength and direction between continuous variables. The significance threshold was set at $p < 0.05$, with highly significant differences indicated by $p \leq 0.001$.

Results

The total power used in experiment A, in which the energy was rising gradually with fixed frequency, was 20.7 ± 7.89 Watt, and the total power in experiment B, in which the frequency was increasing gradually with fixed energy, was 21.34 ± 6.63 Watt and there no significant difference between two approaches regarding the total power ($p = 0.893$).

The ablation volume and residual fragments

A comparison of approaches A and B concerning cystine stones demonstrated a significant disparity in ablation volume percentage. Which was in Experiment A (95.23 ± 1.43%), markedly surpassing Experiment B (92.2 ± 1.47%). This divergence suggests that elevating energy while constant frequency (Experiment Ac) led to a superior stone-free rate in cystine stones compared to increasing frequency with constant energy (Experiment Bc), despite similar total power utilization (Table 2).

Regarding the calcium oxalate monohydrate stones, a statistically significant difference was observed between the two experimental conditions.

The ablation volume percentage in Experiment Bo (95.85 ± 0.77%) surpassed that of Experiment Ao (93.16 ± 1.34%). This outcome highlights that, in the

Table 2. Comparison between two experiments for cystine stones concerning settings and ablation volume.

	Exp.Ac Mean ± SD	EXp.Bc Mean ± SD	t	P
Power (Watt)	20.7 ± 7.89	21.34 ± 6.63	−0.139	0.893
Energy (J)	1.38 ± 0.53	1.1 ± 0	1.19	0.3
Frequency (Hz)	15 ± 0	19.4 ± 6.03	−1.633	0.178
Stone residual mass (mg)	52.27 ± 17.17	93.6 ± 17.67	−3.297	0.011*
Ablation volume percentage (%)	95.23 ± 1.43	92.2 ± 1.47	3.297	0.011*

t independent sample t test *$p < 0.05$ is statistically significant.

Table 3. Comparison between two experiments for calcium oxalate monohydrate stones concerning ablation volume percentage and residual stone mass.

	Exp.Ao Mean ± SD	EXp.Bo Mean ± SD	t	P
Power (Watt)	20.7 ± 7.89	21.34 ± 6.63	−0.139	0.893
Energy (J)	1.38 ± 0.53	1.1 ± 0	1.19	0.3
Frequency (Hz)	15 ± 0	19.4 ± 6.03	−1.633	0.178
Stone residual mass (mg)	82.12 ± 16.04	49.83 ± 9.1	3.907	0.005*
Ablation volume percentage (%)	93.16 ± 1.34	95.85 ± 0.77	−3.907	0.005*

t independent sample t test *$p < 0.05$ is statistically significant.

case of calcium oxalate monohydrate stones, augmenting the frequency while maintaining constant energy (Experiment Bo) results in a higher ablation volume percentage compared to scenarios where energy is increased while maintaining a constant frequency (Experiment Ao) with the same total power (Table 3).

By comparing the residual stone mass between the two types of stones in both experiments, there was a significant difference between the cystine and calcium oxalate stones.

In experiment A, the residual mass of calcium oxalate stone (82.12 ± 16.04 mg) was significantly higher than cystine residual mass (52.27 ± 17.17 mg) (p 0.046).

In experiment B, the residual stone mass in cystine stone was (93.6 ± 17.67 mg) significantly higher than the calcium oxalate stone residual mass, which was (49.83 ± 9.19 mg) ($p < 0.001$) Figure 4.

In both experiments, the correlation coefficients were relatively high, indicating a robust relationship between stone ablation volume and total power. The p-values were below the conventional significance level of 0.05, further supporting the statistical significance of the correlations (Table 4).

These findings suggest that increasing the total power used in the laser disintegration of stones will likely result in larger stone ablation volumes, indicating a potential strategy for optimizing stone disintegration procedures.

The experiments temperature

Initial temperature: 25.20 ± 0.22°C. No significant initial temperature differences were found between experiments (A) and (B) for cystine and calcium oxalate monohydrate stones in the 5 min.

Comparing (Ac) and (Bc) for cystine stones revealed significant temperature differences at the first and second minutes (higher in Ac) with p-values of 0.003

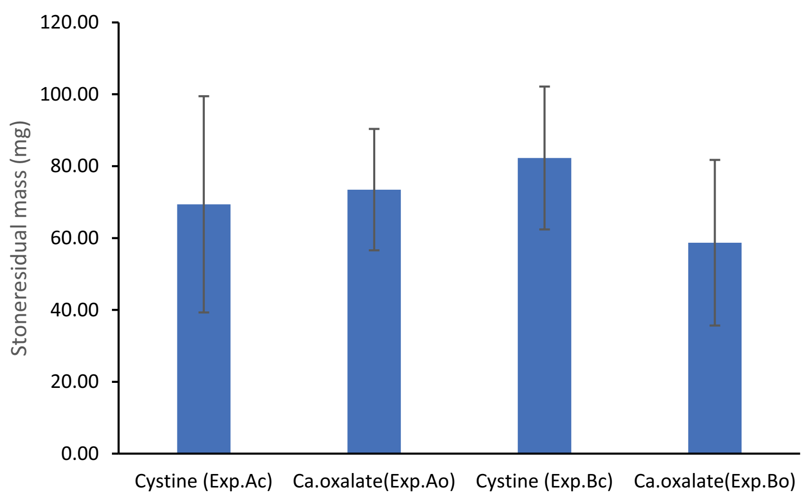

Figure 4. Simple bar chart showing stone-free mass of different types within each experiment.

Table 4. Correlation between stone ablation volume (%) and total power in each experiment.

	A				B			
	Ac		Ao		Bc		Bo	
	R	P	r	P	r	P	r	p
Total Power	0.98	0.003	0.94	0.017	0.94	0.014	0.932	0.0188

Table 5. The temperature of two approaches with cystine stone.

	Exp.Ac Mean ± SD	Exp.Bc	t	P
Power	20.7 ± 7.89			
Energy	1.38 ± 0.53			
Temperature 1st minute	42.73 ± 6.65	31.06 ± 1.43	5.392	0.003*
Temperature 2nd minute	45.75 ± 6.16	36.32 ± 1.88	3.204	0.013*
Temperature 3rd minute	54.1 ± 11.54	42.44 ± 4.09	0.984	0.354
Temperature 4th minute	67.0 ± 13.41	50.04 ± 7.62	1.677	0.132
Temperature 5th minute	83.78 ± 11.26	63.04 ± 13.71	0.78	0.461

t independent sample t test $*p < 0.05$ is statistically significant.

Table 6. Temperature of two approaches of calcium oxalate stones.

	Exp.Ao Mean ± SD	Exp.Bo	t	P
Power (Watt)	20.7 ± 7.89	21.34 ± 6.63		
Energy (Joule)	1.38 ± 0.53	1.1 ± 0		
Temperature 1st minute	40.06 ± 3.47	31.08 ± 1.34	3.877	0.036*
Temperature 2nd minute	42.88 ± 3.87	36.32 ± 2.44	3.296	0.011*
Temperature 3rd minute	47.76 ± 8.95	43.46 ± 3.89	2.13	0.087
Temperature 4th minute	52.0 ± 7.41	46.02 ± 2.95	2.458	0.039*
Temperature 5th minute	70.7 ± 13.91	63.46 ± 13.79	2.613	0.031*

t independent sample t test $*p < 0.05$ is statistically significant.

and 0.013, respectively. No significant differences emerged in the last 3 min (Table 5).

Calcium oxalate stones exhibited significant temperature differences between experiments Ao and Bo at the first, second, fourth, and fifth minutes, with higher temperatures in Experiment Ao (Table 6).

Overall, the gradual increase of energy with constant frequency led to a more pronounced temperature rise compared to the gradual increase of frequency with constant energy for both stone types.

Fiber end burnback

The fiber end burnback occurred with cystine stone at experiment Ac4 and Ac5, which was 0.17 mm with experiment Ac4 and 0.22 mm with experiment Ac5. In contrast, with calcium oxalate monohydrate stone, fiber end burn back occurred in EXP. Ao5, which was 0.19 mm. In experiment B, no fiber end burnback occurred in either type of stone.

Flashlights and charring

Flashlights were observed in EXP.Ac3's last minute and EXP.Ac4 and EXP.Ac5's final 2 min for cystine stones and in EXP.Ao5's last 2 min for calcium oxalate stones. In contrast, no flashes were observed in experiment (B). No test tubes exhibited charring during the experiment.

Discussion

There are different laser settings for stone disintegration. Fragmentation, during which the operator uses a high pulse energy and low frequency, leads to the fragmentation of stone into large particles followed by active stone fragments retrieval. This technique is usually used with hard stones like calcium oxalate monohydrate [11].

Dusting is achieved by using low pulse energy with high frequency to create tiny fragments left in situ for spontaneous passage [12,13]. Stone composition is one of the main predictors of lithotripsy efficacy [14,15]. However, stone composition is not considered in pre-operative planning items [16], and there are no international lithotripsy guidelines for hard stones [10].

Treatment of cystine and calcium oxalate monohydrate stones is challenging due to the high risk of rapid recurrence in the presence of residual fragments [8]. Although cystine stones are known as hard stones due to the double sulfur bonds between cystine atoms, the laser is very effective for both dusting and fragmenting these stones [17].

Until now, no specific laser settings have been recognized to be used for disintegrating this type of stone. However, Lorenzo Ruggera et al. documented a 71% stone-free rate after using laser disintegration for urinary cystine stones [17].

Our preliminary study was carried out on two types of hard and most challenging stones, the cystine and calcium oxalate monohydrate stones to determine the optimal laser settings for disintegrating these stones.

In the first experiment, the operator used a Holmium laser with a fixed frequency at 15 Hz and gradually increased the energy, while the other experiment fixed the energy at 1.1 joule and gradually increased the frequency with caution to make the total power between two experiments as comparable as possible.

The study revealed that using laser settings with higher energy and lower frequency with higher total power in cystine stone is more effective than using the same total power with higher frequency and low energy. While in calcium oxalate monohydrate stone, the use of lower energy and higher frequency is more effective than the settings of lower frequency and higher energy of the same total power.

Also, our study showed the temperature was significantly higher in experiment Ao than Bo with calcium oxalate monohydrate, which means that the increase of the energy at the expense of the frequency produces more heat that reflects the thermal injury to urothelium in vivo disintegration.

The study revealed that the increase of the total power is more effective in the stone volume ablation percentage but is associated with a greater rise in temperature and fiber tip burnback. These results are common sense and were expected.

These findings align with the research conducted by Chen, Shushang, et al. [18], where a comparison between High- and Low-power Holmium Laser lithotripsy in multi-tract percutaneous nephrolithotomy demonstrated that employing high-power Ho:YAG laser lithotripsy significantly reduces operative time without a concurrent rise in intraoperative complications.

Even though the evidence for the effectiveness of dusting or fragmenting hard stones with the Ho:YAG laser system is limited [19], an in-vitro study focused on the outcomes of the dusting technique on calcium oxalate monohydrate stones. The study used phantom stones designed to mimic calcium oxalate monohydrate stones, and the results indicated that a pulse energy of 0.5 J, long pulse width, and a frequency of 70 Hz are the optimal laser settings for efficient dusting. This was achieved using the high-power 120 W Ho:YAG laser in combination with a laser 200-μm fiber [20]. These results are in context with our results which used long pulse mode and high frequency in disintegration of calcium oxalate monohydrate stones, but the maximum total power in our experiment was 30 W.

One of the main challenges in our study was the irregular shape of the stone with different dimensions, so accurate cutting of 30 small fragments manually was essential.

The limitation of our study is that the lithotripsy was done by the human operator, not a 3D automated positioning system to control the fiber end, which is commonly used in such in vitro studies; this was done to simulate actual lithotripsy in patients. Also, we did not use continuous saline irrigation in the test tube, which resulted in higher energy in test tubes than expected. A notable limitation of this study resides in the small sample size ($n = 2$). The limited sample size of only two patients in this study poses a challenge in making definitive conclusions regarding the safety of laser settings. Moreover, conclusions cannot be easily generalized because this study included only two stones.

Also, in this study, we used a fixed long pulse mode only while to be more standardized, other pulse modes must be considered as the study carried by Rezakahn Khajeh et al. [21], which was in vitro study that assessed the impact of pulse modes on dusting of calcium oxalate monohydrate stones.

In general, this study paves the way to more well-designed in vitro and in vivo studies to determine the best laser setting to properly disintegrate hard stones like calcium oxalate monohydrate and cystine stones.

Conclusion

Holmium (Ho: YAG) laser settings characterized by higher total power, including high energy and low frequency, prove highly effective for the disintegration of cystine stones despite the notable increase in heat production compared to settings involving high frequency and low energy. Conversely, the recommendation favors utilizing higher total power with high frequency and low energy settings for calcium oxalate monohydrate stones. These settings demonstrate effectiveness and enhance safety by maintaining lower heat production, making them a preferable choice over the same power involving high energy and low frequency.

Disclosure statement

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Informed consent

Written consent was taken from the two patients before stone extraction, and the consent included that the stones would be involved in the experimental study. Also, ethical committee approval was issued from the faculty of medicine at Menoufia University.

Research involving human participants or animals

This article does not contain any studies with human participants or animals performed by any of the authors (while the stones of the study was taken from human patients after their consent).

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