



## Original Article

# Effects of Aloe Vera on healing of colonic anastomoses: experimental rat study



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### ABSTRACT

**Background:** Although herbal medicinal products are being used widely throughout the World, beneficial and harmful effects have not been well documented. Our aim was to evaluate the effects of Aloe Vera (AV) on colonic anastomosis healing.

**Material and methods:** 112 albino Wistar rats were randomly assigned into five main groups: preoperative Aloe Vera Group (P), pre- and postoperative Aloe Vera Group (PP), Control Group (C), sham Aloe Vera Group (SA) and Sham Control Group (SC). Groups P, PP, and SA received 1.6 mL/kg per day Aloe Vera by orogastric feeding catheter for 1 month prior to the experiment. Groups P, PP, and C underwent anastomosis of the distal colon, and subgroups ( $n=4$ ) of each were sacrificed on postoperative day 3, 7, 14 and 21. Anastomotic bursting pressure, perianastomotic collagen content and histopathological changes were studied.

**Results:** The SC Group had significantly higher ABP when compared with the SA Group ( $p=0.0002$ ), although hydroxyproline content showed no difference. When ABP was compared between anastomosis groups, it was found significantly lower in Aloe Vera groups on Day 3 (P3 vs. C3,  $p=0.003$  and PP3 vs. C3,  $p=0.007$ ). Hydroxyproline content was significantly lower in Group PP than Group C, also on Day 3 ( $p=0.05$ ). Significant difference was not detected after Day 3 in any of the study parameters.

**Conclusion:** Aloe Vera decreased tissue collagen content in the early postoperative period. It is advisable to call into question the concomitant usage of conventional medicine and the herbal supplements for the surgeons in their clinical practice.

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## Efeitos da *Aloe vera* na cicatrização de anastomoses colônicas: estudo experimental em ratos

### R E S U M O

#### Palavras-chave:

Anastomose intestinal  
Anastomose colônica  
Pressão de ruptura anastomótica  
Nível de hidroxiprolina  
*Aloe vera*  
Cicatrização de feridas

**Fundamentação:** Embora os medicamentos à base de plantas sejam amplamente utilizados no mundo inteiro, seus efeitos (benéficos e prejudiciais) não estão bem documentados. Este estudo teve como objetivo avaliar os efeitos da *Aloe vera* (AV) na cicatrização de anastomoses colônicas.

**Material e métodos:** 112 ratos Wistar albinos foram distribuídos aleatoriamente em cinco grupos principais: AV pré-operatório (P), AV pré e pós-operatório (PP), controle (C), sham AV (SA) e sham controle (SC). Os grupos P, PP e SA receberam AV em uma dose de 1,6 mL/kg por dia por sonda de alimentação orogástrica por 1 mês antes do experimento. Os grupos P, PP e C foram submetidos a anastomose do cólon distal. Subgrupos (n = 4) de cada grupo foram sacrificados no terceiro, sétimo, 14° e 21° dias pós-operatórios. Os seguintes parâmetros foram avaliados: pressão de ruptura anastomótica (PRA), conteúdo de colágeno perianastomótico e alterações histopatológicas.

**Resultados:** O grupo SC apresentou PRA significativamente maior quando comparado ao grupo SA (p = 0,0002), embora o conteúdo de hidroxiprolina não tenha apresentado diferença. Ao comparar a PRA entre os grupos de anastomose, ela foi significativamente menor no terceiro dia nos grupos que usaram AV (P3 vs. C3, p = 0,003 e PP3 vs. C3, p = 0,007). No terceiro dia, o teor de hidroxiprolina foi significativamente menor no grupo PP do que no grupo C (p = 0,05). Após o terceiro dia, não se observou diferença significativa em nenhum dos parâmetros do estudo.

**Conclusão:** O uso de AV diminuiu o conteúdo de colágeno tecidual no período pós-operatório imediato. É aconselhável questionar o uso concomitante da medicina convencional e suplementos fitoterápicos na prática clínica.

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## Introduction

While use of Herbal Medicinal Products (HMPs) have been widely used primarily in healthcare in around 80% of the general population in underdeveloped and developing countries; there was increased global interest in the last 30 years that widened their use throughout the World.<sup>1</sup>

The general opinion of consumers is that these natural source supplements are safe. Most HMPs may not cause direct toxicity in small doses; however, there is always risk for unwanted pharmacodynamic and pharmacokinetic interactions with pharmaceutical medicines.<sup>2,3</sup> Both consumers and clinicians do not have enough knowledge based on medical evidence in terms of possible interactions and effects. Surgeons are increasingly encountering patients who are willing to continue use of HMPs early after surgical operations as a consequence of increasing global interest.<sup>4</sup> Among many general surgery operations, colorectal surgical procedures are of particular importance those have significantly increased morbidity and mortality risk if complications arise. Therefore, understanding potential beneficial and harmful effects of commonly used HMPs would help the surgeons in this regard.

*Aloe Vera* preparations are one of the most popular HMPs. It has been used in wound healing since ancient times, but contradicting results have been reported.<sup>5</sup> The purpose of this study was to evaluate the effects of *Aloe Vera* on colonic

anastomosis healing in albino Wistar rats by assessing anastomotic bursting pressure, hydroxyproline content, and histopathological changes.

## Materials and methods

### Experimental animals

One hundred and twelve Wistar rats, 56 female (F) weighting 175–275 g, and 56 male (M) weighting 275–425 g, were used in this controlled experimental animal study. The rats were housed according to their gender with 12 h light–dark cycles, at a temperature of 21 °C and controlled humidity, and were allowed free access to standard food and water throughout the study including surgical operation and postoperative follow-up in the Experimental Animal Care and Research Laboratory of Uludag University. The study protocol was approved by the Animal Care and Research Committee of Uludag University Medical Faculty (certificate no. 2004/29).

### Study procedures

The rats were randomly assigned into five main groups: preoperative *Aloe Vera* Group (Group P, n = 32), pre- and postoperative *Aloe Vera* Group (Group PP, n = 32), Control Group (Group C: surgery (+), *Aloe Vera* (–), n = 32), sham *Aloe Vera* Group (Group

SA: surgery (–), *Aloe Vera* (+),  $n=8$ ), and Sham Control Group (Group SC: surgery (–), *Aloe Vera* (–),  $n=8$ ). Gender distribution was 1:1 in all groups and subgroups.

*Aloe Vera* at a dose of 1.6 mL/kg in 1 mL (based on the recommended dose in humans [50–100 mL of 96.2% pure *Aloe Vera* 60 kg body weight]; *Aloe Vera* Gel, Forever Living Products, Scottsdale, USA) was given via orogastric gavage once-a-day for one month before the surgery in Groups SA, P and PP; and additionally in Group PP, at the same dosage, during the post-operative follow-up period until sacrificed. *Aloe Vera* gel was stored in the refrigerator as recommended in the instruction manual, except for the feeding time. Group C and Group SC did not receive *Aloe Vera* but fed with standard rat diet for a month.

Groups P, PP and C were undergone surgery and colonic anastomosis was performed as described below. Subgroups of the rats ( $n=4$  in each subgroup) were sacrificed on post-operative day 3, 7, 14 and 21, while rats in Groups SA and SC were sacrificed on day 0 without any surgical procedure for the study measurements of colonic/anastomotic bursting pressure, and collagen (hydroxyproline) content and histopathological examination of the anastomotic wound.

### Colonic anastomosis

Following a one night fasting, rats were anesthetized by intraperitoneal administration of thiopental sodium (40 mg/kg, I.E. Ulagay, Turkey). Under general anesthesia, the abdomen was shaved and cleaned with povidone-iodine. Sterilized equipment was used throughout the whole operation. In order to minimize the risk of anastomotic leakage, transection and anastomosis without resection was preferred. Laparotomy was performed through a midline 4 cm incision. A left colonic segment, 1 cm in length, approximately 3 cm proximal to the peritoneal reflection was transected. The colon was re-anastomosed end-to-end with standard 8 full-thickness knots using 5/0 propylene sutures (Prolene, Ethicon, Edinburgh, UK) in single-layer interrupted fashion. Before the abdomen was closed, 5 mL of 0.9% NaCl was given into the abdomen as fluid resuscitation. The abdominal muscle wall was then closed with 3/0 silk sutures, followed by skin closure with 3/0 silk sutures (Mersil, Ethicon, Cincinnati, USA). After the surgical procedure was completed, rats were labeled and kept in individual cages according to their group and gender.

### Study measurements

Rats were sacrificed with an overdose of ether for study measurements. In rats underwent surgery, the previous abdominal incision was reopened, and the anastomotic site identified and inspected for possible adhesions and leakage. A 4 cm segment of the colon with the anastomosis in the middle was resected. Care was taken not to detach adhesions from the anastomosis, but to dissect the surrounding tissues. In SA and SC Groups, 4 cm of left colonic segment at 1 cm above the peritoneal reflection was resected. The resected specimen was gently irrigated with saline to remove feces and was mounted on a table.

### Anastomotic bursting pressure

Anastomotic bursting pressure is a well-established and frequently applied parameter used to determine the strength and sufficiency of experimental bowel anastomosis. The colon segment was bloated with an infusion pump (Baxter Flo-Gard 6201, IL, USA) at a rate of 2 mL/min with saline. Pressure on the anastomosis line was simultaneously recorded on the computer with AcqKnowledge v.3.5.5 (Biopac Systems Inc., Santa Barbara, CA, USA). The highest pressure value before the perforation was recorded as the explosion pressure. The catheters were removed and the colon segment was completely cleaned from the adherent surrounding tissues, washed with saline. The segment was opened parallel to the long axis and cut into two equal parts from the middle. Sutures of the anastomosis line were removed and placed in a narrow routine tube containing 10% formaldehyde for histopathological evaluation. The other piece was then wrapped in aluminum foil for biochemical examination and placed in a narrow, routine tube and stored at  $-50^{\circ}\text{C}$  in the freezer.

### Hydroxyproline determination

The hydroxyproline content could be measured as an indicator of the amount of collagen in biological tissues. Following the measurement of bursting pressure, the resected piece of the colonic segment was stored at  $-50^{\circ}\text{C}$  until further processing. After unfreezing, the anastomosis segments were prepared to be  $10 \times 5$  mm in size. The tissue was washed with physiological saline solution and dried between two filter papers. The dried tissue weight was measured on a precision scale, and placed in glass tubes. 200  $\mu\text{L}$  saline and 300  $\mu\text{L}$  of 50 mM potassium phosphate buffer was added. Hydrolysis was performed at  $110^{\circ}\text{C}$  with concentrated HCl in a 1:1 ration for 16 h. The extracts were neutralized with 6 N NaOH. Hydroxyproline determination was performed using spectrophotometry (Bausch&Lomb 20, Germany) explained by Bergman and Loxley.<sup>6</sup>

### Histopathology examination

Following the measurement of bursting pressure, the resected piece of the colonic segment was placed in a narrow routine tube containing 10% formaldehyde for histopathological evaluation on the same day of procedure. The specimens were processed for slide examination. The slides, stained with Hematoxylin Eosin (HE), were examined under the light microscope (Olympus BX, Japan) over a high power field ( $100 \times / 400 \times$ ). Examination was done over ten fields per specimen in a random fashion and an average was taken as the final count. Masson trichrome stain was applied for better evaluation of collagen. It included fibroblast count (not including mature fibroblasts), neovascularization, Polymorphonuclear Leucocytes (PML) which were counted semi quantitatively and graded histologically using the 0–4 Ehrlich and Hunt numerical scale (0, no evidence; 1, occasional evidence; 2, light scattering; 3, abundant evidence; 4, confluent cell).<sup>7</sup>

**Table 1 – Anastomotic bursting pressures in study groups.**

	Anastomosing bursting pressure (mmHg)			
	Day 3 Mean (SD)	Day 7 Mean (SD)	Day 14 Mean (SD)	Day 21 Mean (SD)
Group C	30 (6)	147 (6)	197 (5)	189 (7)
Group P	7 (3) <sup>a</sup>	138 (5)	176 (12)	195 (5)
Group PP	9 (5) <sup>b</sup>	134 (5)	179 (11)	190 (8)

Group C: surgery (+), Aloe Vera (-); Group P: surgery (+), preoperative Aloe Vera; Group PP: surgery (+), pre- and postoperative Aloe Vera; SD, Standard Deviation.

<sup>a</sup>  $p=0.003$ .

<sup>b</sup>  $p=0.007$  vs. Group C.

### Statistical analysis

All data were recorded using Statistical Package for the Social Sciences (SPSS) 10 for Windows. Kruskal-Wallis and Mann-Whitney *U* nonparametric statistical methods were conducted to compare the bursting pressure for the groups of experimental animals. The Pearson correlation test was used to find out whether there was a relationship between groups.  $p$ -values  $\leq 0.05$  were considered significant.

### Results

All animals survived until they were sacrificed. There were no surgical or anesthetic complications. No cases of ileus were observed in the laparotomy groups after sacrifice.

The mean anastomotic bursting pressure, hydroxyproline content and histopathological scores according to groups are shown in Tables 1-3, respectively.

#### Anastomotic bursting pressure

Group SC mean (Standard Deviation - SD): 149 (4) mmHg had significantly higher anastomotic bursting pressure when compared with Group SA mean (SD): 121 (4) mmHg ( $p=0.0002$ ). When the bursting pressure compared among the groups undergone anastomosis procedure, statistically significant difference was observed only on Day 3 between Group C and P ( $p=0.003$ ) and between Group C and PP ( $p=0.007$ ). As shown in Table 1, bursting pressures were much lower for both Aloe Vera Groups. Moreover, the anastomotic leak was detected in the Groups of PP (in 4 of 8 rats) and P (in 1 of 8 rats) on Day 3.

On the other hand, although there was no anastomotic leak in Group C, there was one perianastomotic hematoma, and the anastomotic explosion was detected as the lowest value of the pressure for this case.

The difference between groups having anastomosis procedure was not significant on postoperative Day 7, 14 and 21.

#### Hydroxyproline levels

Mean (SD) colonic hydroxyproline level in Group SA and SC was 414 (40) and 360 (94)  $\mu\text{g/g}$  tissue, with no significant difference. The difference of postoperative anastomotic hydroxyproline levels between groups having anastomosis procedure was also not significantly different than each other except Day 3 on which hydroxyproline level in Group PP was significantly lower than Group C ( $p=0.050$ ) (Table 2).

#### Histopathological examination

Histopathological examination on the anastomosis line revealed no statistically significant difference between groups (Table 3).

### Discussion

The Alma-Ata declaration on Primary Health Care (PHC) by the World Health Organization (WHO) in 1978 witnessed a response from several countries to improve their traditional medicine use and regulation of use within the primary health care model. WHO Traditional Medicine Program (WHO-TMP) aimed at the integration of conventional medicine and

**Table 2 – Collagen (hydroxyproline) levels at the anastomosis wound in study groups.**

	Hydroxyproline level ( $\mu\text{g/g}$ tissue)			
	Day 3 Mean (SD)	Day 7 Mean (SD)	Day 14 Mean (SD)	Day 21 Mean (SD)
Group C	291 (30)	249 (36)	364 (30)	325 (34)
Group P	269 (25)	273 (57)	322 (32)	433 (45)
Group PP	253 (44) <sup>a</sup>	280 (41)	308 (27)	437 (42)

Group C: surgery (+), Aloe Vera (-); Group P: surgery (+), preoperative Aloe Vera; Group PP: surgery (+), pre- and postoperative Aloe Vera. SD, Standard Deviation.

<sup>a</sup>  $p=0.050$  vs. Group C.

**Table 3 – Histopathological examination at the anastomosis wound in study groups.**

	Histopathologic evaluation score							
	Polymorphonuclear leucocytes				Neovascularization			
	Day 3	Day 7	Day 14	Day 21	Day 3	Day 7	Day 14	Day 21
Group C	3.0 (2–3)	2.0 (1–4)	2.0 (0–3)	1.5 (0–2)	1.0 (0–1)	3.0 (0–1)	3.0 (2–4)	1.0 (0–2)
Group P	2.0 (1–3)	1.5 (1–3)	2.0 (0–3)	0.0 (0–1)	1.0 (0–1)	3.0 (1–4)	3.0 (2–4)	1.0 (0–2)
Group PP	2.5 (1–4)	3.0 (2–4)	2.0 (0–3)	0.0 (0–1)	0.0 (0–1)	3.5 (3–4)	2.5 (1–4)	1.0 (0–3)
	Fibroblasts				Collagen			
	Day 3	Day 7	Day 14	Day 21	Day 3	Day 7	Day 14	Day 21
	Group C	1.0 (2–3)	3.0 (3–4)	3.0 (2–4)	1.5 (1–3)	1.0 (0–1)	2.0 (2–3)	4.0 (2–4)
Group P	0.0 (0–1)	4.0 (3–4)	3.0 (2–4)	1.5 (0–2)	1.0 (0–2)	3.0 (2–4)	4.0 (3–4)	4.0 (3–4)
Group PP	0.0 (0–2)	3.5 (3–4)	3.0 (2–4)	1.0 (0–3)	0.0 (0–1)	3.0 (2–4)	4.0 (3–4)	4.0 (0)

Data are given as median (minimum–maximum).  
 Group C: surgery (+), Aloe Vera (–); Group P: surgery (+), preoperative Aloe Vera; Group PP: surgery (+), pre- and postoperative Aloe Vera. SD, Standard Deviation.  
 No significant difference was detected between groups; Kruskal–Wallis test.

traditional medicine under the topic of WHO “Health for All”. In this program, first of all, 35,000 plant species had been screened and 39 of them, the most known ones, was planned for integration. Scientific articles about these herbal products including *Aloe Vera* have been reviewed in terms of safety and efficacy, and the results were published in a booklet in 1999. WHO-TMP was intended to enhance the knowledge of both providers and patients in order to ensure the safe and effective use of traditional medicine in daily life.<sup>8</sup>

These products which are called nutritional supplement products, can be used without supervision by Ministry of Health. The US market share of these products was of \$200 million dollars in 1988, and reached \$12.7 billion in 2012.<sup>1</sup> WHO 10 year strategy report highlighted the need for evidence-based research in the field of herbal products in 2013.<sup>9</sup>

As the global use of herbal medicinal products continues to grow and many more new products are introduced into the market, public health issues, and concerns surrounding their safety are also increasingly recognized.<sup>2,3,9</sup> Although some herbal medicines have promising potential and are widely used, many of them remain untested and their use also not monitored. This makes knowledge of their potential adverse effects very limited and identification of the safest and most effective therapies as well as the promotion of their rational use more difficult. It is also common knowledge that the safety of most herbal products is further compromised by lack of suitable quality controls, inadequate labeling, and the absence of appropriate patient information. It has become essential, therefore, to furnish the general public including healthcare professionals with adequate information to facilitate better understanding of the risks associated with the use of these products and to ensure that all medicines are safe and of suitable quality.<sup>4,10</sup>

*Aloe Vera* plant extract studies on wound healing are mixed with some studies reporting positive results including

favorable effects on immune system, collagen synthesis, inflammation,<sup>11–16</sup> and others showing no benefit or potential worsening.<sup>17–21</sup>

Bowel anastomoses are common procedures in both elective and emergency general surgery. Clinically relevant anastomotic leakage rates range between 5% and 15% and result in significant morbidity and mortality up to 30%.<sup>22–24</sup>

Many colorectal cancer patients lean to Complementary and Alternative Medicine (CAM) in order to strengthen the body to cope with conventional treatments in surgery or to prevent recurrence following conventional treatment. In a study conducted in 7 European countries, it was reported that the use of herbal products was 7.7% before diagnosis, and increased to 48.7% after diagnosis.<sup>25</sup>

In the present study, we examined the effects of *Aloe Vera*, which has conflicting results on wound healing, on colonic anastomosis experimentally in rats. Collagen is the major protein component of colonic anastomosis stability and is found in the Extracellular Matrix (ECM) of the submucosal layer of the colon.<sup>26,27</sup> Hydroxyproline (HP), an amino acid formed upon hydrolysis of connective-tissue proteins such as collagen and elastin. In practice, the HP content represents the amount of collagen.<sup>28</sup> Anastomosis healing is a dynamic process that flows along a continuum with collagen production and degradation. In the early period, perianastomotic collagen degradation occurs by Matrix Metalloproteinases (MMP), and Type III immature collagen is produced from active fibroblasts migrating to the wound site. This production is only measurable from Day 3 onwards. The first 7 days are referred to as suture capacities and are correlated with the level of net collagen production and degradation.<sup>29–31</sup> Studies showed that colonic anastomoses reached sufficient tension force from Day 7. Clinically, most of the anastomotic leaks occur in the early postoperative period.<sup>26,29,32,33</sup> Collagen degradation is carried out by MMP, and is normally controlled not to cause leaks.<sup>34</sup>

Cowcat et al.<sup>35</sup> reported that the amount of total collagenase increased significantly on the 3rd day due to interleukin-1 (IL-1). MMP-1 and MMP-13 are the most effective MMPs in colon anastomoses and hydrolyze Type I and III collagen.<sup>27</sup> In a recent study of MMPs, it was reported that MMP-8 played an important role in degradation of collagen Type I, and exhibited synergistic activity with MMP-9, suggesting that this activity may be an anastomotic leak with overexpression.<sup>36</sup>

In the present study, although not statistically significant, HP levels in *Aloe Vera* groups were high. Besides, similar results were observed in histopathological collagen evaluation. The Pearson correlation test revealed no significant relationship between HP amount and bursting pressure ( $p=0.14$ ). On day zero, although the HP amount was greater in SA Group compared to SC, the bursting pressure was significantly lower ( $p=0.0002$ ). This correlation maybe due to collagen types in the tissue. In an experimental study, it was emphasized that not only the quantity, but also the quality of an HP is an important determinant for bursting pressure.<sup>33</sup> Mature Type I collagen significantly increases the bursting pressure, although it contains less HP than Type III collagen.<sup>32</sup> It has been reported that the ratio of Type I/III collagen of the colon is important, and lower in anastomotic leaks.<sup>27</sup> *Aloe Vera* increases the production of Type III collagen.<sup>14,37</sup> These findings may explain lower bursting pressures despite higher HP content.

Another possibility arises from the 3 dimensional structure of the collagen. The collagen which forms cross-links with each other is more elastic and durable. Solubility in both salt and acid environment is reduced (insoluble) in the presence of cross-links, and insoluble collagen has significantly higher bursting pressure.<sup>38</sup>

On the postoperative Day 3, while no leakage was observed in the Control Group, 4 cases in the PP Group, and a case in the P Group was observed. The HP values were decreased significantly in the PP Group in which the administration of *Aloe Vera* continued ( $p=0.05$ ). This decrease in the HP level suggests primarily increased MMP activity. Since *Aloe vera* increases IL-1 release,<sup>39</sup> it is possible that it increases collagenase stimulation. However, Barrantes and Guinea reported that aloin and *Aloe Gel* inhibit collagenase and MMP-8.<sup>40</sup> MMP-8 (neutrophil collagenase) specifically hydrolyses Type 1 collagen,<sup>41</sup> but it has lower affinity for Type III collagen. This also serves with our hypothesis related to high Type III collagen content in the SA control group.

Another possibility to explain low levels of HP in the leaks may be anti-angiogenic effect of *Aloe Vera*.<sup>42</sup> Anti-angiogenic agents reduce HP levels.<sup>43,44</sup> In our experiment, histopathological examination on Day 3 revealed that collagen and neovascularization was lower in *Aloe Vera* groups compared to control group. However, since our findings did not yield statistically significant results, we could not make a judgment. The PML level, an indicator of the inflammatory phase, had not been affected by *Aloe Vera*.

This study has some limitations which have to be pointed out. In our study, we did not evaluate of collagen typing, MMP activities and solubility. Besides, it could be criticized that if the resection anastomosis had been done, there was no need for the sham group. To minimize the effect of the surgical

technique on anastomoses, transection had been preferred. In our experiment we found that *Aloe Vera* increased the risk of leaks early in the postoperative period, contrary to previous findings.<sup>16</sup>

## Conclusion

The variety of alternative/complementary herbal products usage in conjunction with conventional medicinal products is increasing rapidly. The effective doses, interactions with the conventional medicinal products, and the positive or negative effects of these compounds are not fully addressed.

Evidence is much controversial because it is difficult to establish the bioavailability, the major active ingredients, plasma concentration on the basis of the multiplicity of components of the plants in an experimental animal models.

Based on our findings, although we could not correlate the collagen content with the bursting pressure, we observed that *Aloe Vera* extract increased the risk of anastomotic leakage early in postoperative period. Further studies are necessary in order to reveal the etiopathogenesis involving collagen typing, MMP and collagenase activities and solubility ratios. It is advisable to call into question the concomitant usage of conventional medicine and the herbal supplements for the surgeons in their clinical practice.

## Conflicts of interest

The authors declare no conflicts of interest.

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