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## Antiulcer effect of *Persea americana* seed against alcohol-induced peptic ulcer in guinea pig

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### Abstract

Peptic ulcer is a widespread disease caused by a overproduction of hydrochloric acid stimulated by *Helicobacter pylori* and certain irritating substances like alcohol and antiinflammatory drugs. Modern anti-ulcer medicines are available, but poor communities often use accessible herbal remedies, including *Persea americana*. This study aimed to evaluate, in guinea pigs, the protective and curative effects of *Persea americana* seed extracts against HCl/ethanol-induced ulcers. The endpoints consisted of macroscopic and histopathological features translated in terms of antiulcer index, protection and healing percentages. Oral administrations of extracts 100 mg/kg one day before inducing ulcer protected 50% of exposed animals compared with 37.5% by omeprazole (20mg/kg) and 25% by ranitidine (50mg/kg); while, a 7-days treatment of induced ulcers healed 72.92% of animals, compared with 88.9% by the reference drugs. The extract contained alkaloids, phenolic compounds, flavonoids, sterols/triterpenes, and reducing acids. The findings back the observations made by other researchers and support the use of avocado seeds.

**Keywords:** *Persea americana*, avocado seed, peptic ulcer, efficacy

### Introduction

Peptic ulcer is the most common disease of the gastrointestinal tract, affecting approximately 10% of the world's population [1, 2]. It results from an imbalance between the defense factors and the factors of aggression. The stomach is exposed continually to a wide range of endogenous and exogenous substances that can cause epithelial damage [3, 5]. The known endogenous factors of aggression include hydrochloric acid, pepsin, and reactive oxygen species. The exogenous agents consist of *Helicobacter Pylori* infection, unhealthy diet, tobacco, excessive alcohol consumption, stress, and excessive ingestion of nonsteroidal anti-inflammatory drugs. These ulcerogenic substances cause a more or less extensive loss of material from the digestive wall, which reaches the muscle layer, hence the appearance of a gastric ulcer [3, 6]. That overcomes the primary local protections by the mucus mat, the blood flows through the vessels of *lamina propria*, and the constant renewal of cells. The balance between deep multiplication and flaking on the surface that ensures the integrity of the cell barrier is broken off.

The standard practical treatments of ulcers consist of proton pump inhibitors, antihistamine-H<sub>2</sub>, mucus producers and antibiotherapy against *H.pylori*. These medications are costly for poor people in developing countries, and also have a wide range of side effects [7]. To date, the general interest in phytotherapy has continued to evolve, as it represents an exciting therapeutic alternative at a lower cost. *Persea americana* Mill (*P. americana*) of the Lauraceae family, also called the avocado tree, is one of the plants used. The species is a large tree (15 to 18 meters), supporting dense, fleshy fruits that are spherical or oval and each coating a large seed [8]. The avocado seed has several therapeutic uses in folk medicine, including gastralgia, gastric ulcer, diabetes, hypertension, anemia, etc. [8, 9]. In the Democratic Republic of Congo (DRC), information gathered from traditional healers mention that, for against gastralgia, the patient takes a decoction of 10 seeds crushed and boiled in 2 liters of water for 10 minutes (1-glass in the morning and 1-glass in the evening for 3-days); for hypertension and palpitation, a decoction of 1-handful of root powder and 1- a handful of fresh leaves in 2 liters of water boiled for 10 minutes is administered (1 glass twice a day until relief); against anemia, the remedy consists of a decoction of two handfuls of fresh leaves in 2 liters of water boiled for 10 minutes (1 glass in the morning and 1-glass in the evening for a period of 3 to 4 days). HIV/AIDs people also take an infusion of 1 tablespoon of almond powder in 1 liter of hot water (one glass twice a day over a few days) to boost immunity [10].

The present study aimed at validating the protective and curative effects of the phytochemicals present in aqueous seed extracts from local *P. americana* species using guinea pigs with alcohol-induced ulcers.

## Materials and Methods

### Plant material

The seeds of *P. americana* were exuded from the fruit, washed with water, cut into small pieces, dried in the shade for four weeks, then ground into small particles. The resulting powder was stored protected from light until analysis.

### Preparation of seed extracts

Boil 100 g of dry seed powder in 1000 ml of distilled water for 15 minutes, then filter and evaporate on a hot plate. Complete drying in an oven at 45-55°C and then store in a desiccator. For administration to the animals, prepare a 1% aqueous solution.

### Chemical composition

The identification tests were carried out on the aqueous extract using common identification reagents to detect the main groups of secondary metabolites<sup>[11]</sup>.

### Preparation of ulcerative solution

The ulcerative solution consisted of a mixture of ethanol (60%), concentrated hydrochloric acid (1.7%), and distilled water (38.3%).

### Animals

Male guinea pigs (*Cavia porcellus* species), around 3 to 5 months old and weighing between 200 and 400 g, were accommodated under normal conditions at the animal boundary of the pharmacy department of the University Official of Bukavu (UOB). The number of animals was kept to 4 per group to avoid unnecessary loss.

### Experimental protocol

As shown in Table 1, the animals were partitioned into different groups comprising of negative control, positive control, protective test, and curative test groups. In G1, the animals received only water and not the ulcerative solution. In G2-3, the animals received ulcerative solution and not a medication. In G4-6, the animals received the extract 1 day before the ulcerative mixture. In G7-9, the animals received medications for seven days after induction of ulcers. Each group contained four animals<sup>[12, 13]</sup>.

**Table 1:** Protocol followed for ulcer induction, protection, and healing effect

Treatment P.O. gavage	Negative Control			Protective Effect			Healing Effect		
	G1	G2	G3	G4	G5	G6	G7	G8	G9
Distilled Water 10ml/kg/day in 3 days	√								
Ethanol-HCl 10ml/kg/day in 1-3 days		√	√	√	√	√	√	√	√
Medication before ulcer induction				√	√	√			
Medication after ulcer induction							√	√	√
Extract 100mg/kg before and after ulcer				√			√		
Ranitidine 50mg/kg before and after ulcer					√			√	
Omeprazole 20mg/kg before and after ulcer						√			√

The animals were sacrificed at six hours after the last treatment, and then stomachs were removed, opened along the greater-curvature, washed, and rinsed under a jet of water before being spread in a petri dish. Using a 10 x magnifying lens, we examined the gastric mucosa to count the ulceration spots (irritation, hemorrhagic spots, or furrows). Histological study of the stomach tissue was performed according to the standard laboratory procedures at the pathological laboratory of the General Hospital of Panzi<sup>[14]</sup>. The macroscopic count of ulcers (hemorrhagic spots) and the microscopic severity of the lesions made it possible to calculate the evaluation endpoints, including:

Score (S) : 0=no ulcer spot; 1=1-2 spots; 2=3-4 spots; 3=more than 4 spots or furrows;

U =number of animals with score >0 per group; IU (Index of Ulceration) = mean score x U/N where N=number of animals per group; PU (Percentage of Ulceration) = (IU × 100) /3;

PP (Percentage of Protective)=[PU(positive control)-PU(test)]/[PU positive control];

PH (Percentage Healing)=[PU(positive control)-PU(test)]/[PU positive control].

### Statistical analysis

Data were expressed as mean±SD. Comparisons were performed by one way ANOVA with Bonferroni post hoc test.

## Results

### Chemical composition

Table 2 shows the presence of alkaloids, anthocyanins, phenolic compounds, flavonoids, and reducing sugars, but quinones and coumarins in the operating conditions.

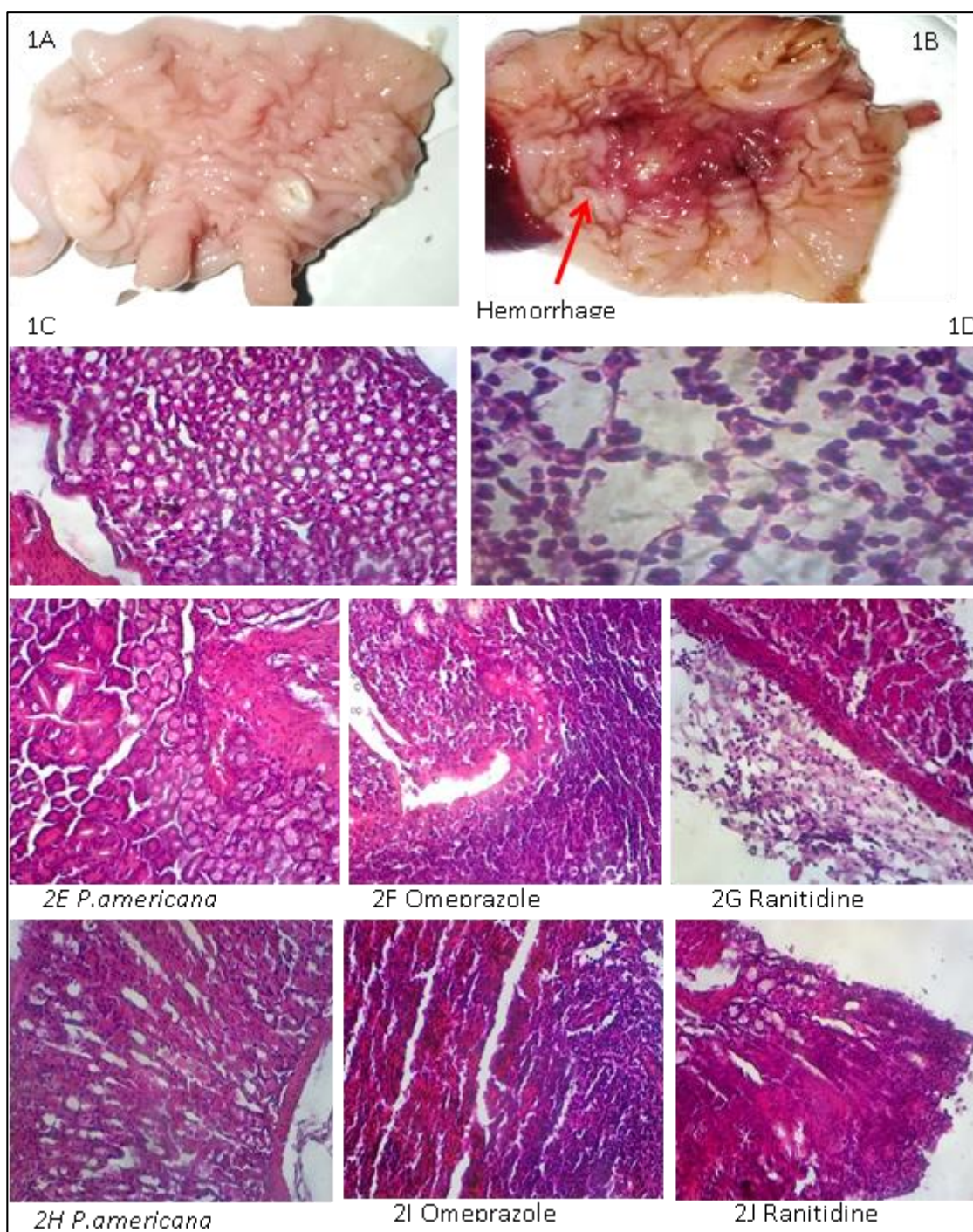
**Table 2:** Preliminary phytochemical screening of *P. americana* seed aqueous extract

Second metabolites	Reaction / Reagents	Result
Alkaloids	Dragendorff	+
Anthocyanins	Butanol + HCl concentrated	+
Phenolic compounds	K <sub>3</sub> Fe(CN) <sub>6</sub> 1% + FeCl <sub>3</sub> 2%	+
Flavonoids	HCl conc + Mg + alcohol isoamylic (Shinoda)	+
Sterols and triterpenes	Libermann-Buchards	+
Reducing sugars	Fehling's test	+
Quinones	Borntraeger	-
Coumarins	NH <sub>4</sub> OH 10%	-

**Antiulcer capacity of *P. americana* seed extract**

**Figure 1** shows macroscopic and microscopic pictures of the stomachs. Visual examination with magnified lens showed intact gastric mucosa in normal animals (1A) and hemorrhagic furrows (erythematous plate) in ulcerated animals (1B). The same, microscopic inspection of normal gastric mucosa (1C) showed sections of the gastric wall with no notable histological lesion, while the microscopic inspection of ulcerated gastric mucosa (1D) showed abundant mixed inflammatory lymphocytic infiltrate and edema. The microscopic histological architecture in animals medicated for one day before induction of ulcers (protection capacity) showed mucous membrane gastritis with mild lymphocytic

inflammatory infiltrate (mild chronic gastritis) for plant extracts (2E), gastric mucosa site of an abundant lymphocyte inflammatory infiltrate forming foci lymphoid follicles (severe chronic gastritis) for omeprazole (2F), and gastric mucosa with moderate inflammatory lymphocytic infiltrate and edema (moderate chronic gastritis) for ranitidine (2G). The microscopic histological architecture in animals medicated during 7 days after induction of ulcers shows sections of a gastric wall without notable histological lesions for plant extract (2H), and parts of the gastric wall with less striking histological lesions for omeprazole (2I) and Ranitidine (2J).



**Fig 1:** Macroscopic and microscopic structures of gastric mucosa

Macroscopic view of normal gastric mucosa (1A) and ulcerated gastric mucosa (1B); Microscopic view of normal gastric mucosa (1C) and ulcerated gastric mucosa (1D); Microscopic gastric mucosa in protected animals with extract (2E), omeprazole (2F), and ranitidine (2G); Microscopic view of healing capacity with plant extract(2H), omeprazole (2I) and ranitidine (2J).

Table 3 shows the ulcerative effect of the HCl-alcohol mixture used. As shown, the ulcerative solution induced up to 6 ulceration spots in the positive control (untreated animals), giving PU of 20.8%, 66.7, and 75% in groups exposed for 1, 2, and 3 days, respectively.

Table 4 shows the protective and healing capacity of the plant extract in comparison with the reference drugs. As shown, the administration of the *P. americana* extract and reference drugs one day before administration of ethanol-HCl in two days resulted in a decrease of percentage of ulceration compared with the untreated control group. The PU was 50% for control group, 37.5% for Ranitidine, 31.2% for Omeprazole, and 25% for *P. americana*, giving the PP of 0%, 25%, 37.6%, and 50% respectively. The healing capacity of *P. americana* was the highest (97.23%), compared with ranitidine (88.9%), and omeprazole (88.9%). The differences were statistically significant ( $p < 0.001$ ) in comparison to the untreated group.

**Table 3:** Ulcerative potency of Ethanol-HCl-Water on the guinea pig stomach

Group	Animals				Treatment outcomes						
	A1	A2	A3	A4	Indicators						
Positive	Q(S)	Q(S)	Q(S)	Q(S)	N	U	U/N	Mean-S	±SDV	IU	PU%
1 day	6(3)	3(2)	0(0)	0(0)	4	2	0.5	1.25	1.50	0.63	20.8
2 days	5(3)	2(1)	3(2)	3(2)	4	4	1.0	2	0.82	2	66.7
3 days	5(3)	3(2)	4(2)	3(2)	4	4	1.0	2.25	0.50	2.25	75.5
Negative	0(0)	0(0)	0(0)	0(0)	4	0	0	0	0	0	0

Legend: Q=number of ulcer spots; S=score; U=Number of ulcerated animals; Index of ulceration; PU=percentage of ulcerated animals. Positive: animals given HCL-alcohol during 1 day, 2 days or 3 days; negative (unexposed).

**Table 4:** Protective and healing potential of *P. americana* and reference drugs

Treatment	Animals				Treatment outcomes						
	A1	A2	A3	A4	Protection indicators						
	S	S	S	S	N	U	U/N	Mean-S	IU	PU%	PP%
P. Americana	0	1	2	1	4	3	0.75	1.00	0.75	25.0	50.0
Omeprazole	0	2	2	1	4	3	0.75	1.25	0.94	31.2	37.6
Ranitidine	0	3	2	1	4	3	0.75	1.50	1.13	37.5	25.0
Positive control	2	1	1	2	4	4	1	1.50	1.50	50.0	0.0
Treatment	Animals				Healing indicators						
	S	S	S	S	N	U	U/N	Mean-S	IU	PU%	PH%
P. Americana	1	0	0	0	4	1	0.25	0.25	0.06	2.08	97.2
Omeprazole	1	0	0	1	4	2	0.50	0.50	0.25	8.33	88.9
Ranitidine	1	1	0	0	4	2	0.50	0.50	0.25	8.33	88.9
Positive control	3	2	2	2	4	4	1	2.25	2.25	75.00	0.0

Legend: A1-A4: animals used; Qotation(Q) score(S); index of ulceration (IU); percentage of ulceration(PU); Healing percentage(PH); Protection: one dose of medication 2 hours before 1-day ulcer inducing; Healing: 7 days of medication after 3-days ulcer inducing.

## Discussion

The current study has been conducted to validate the capacity of the phytochemicals present in *P. americana* seed extracts to prevent and heal alcohol-induced ulcers in an animal model.

The chemical screening showed the presence of alkaloids, anthocyanins, phenolic compounds, flavonoids, and reducing sugars, consistent with other published data. Athaydes *et al.* [9] revealed, by spectrometric method, the presence of (-)-epicatechin, (+)-catechin, and other important phenolic compounds (caffeoylquinic acid, flavonoids, phenylpropanoids and tannins). The study by Yasir *et al.* [15] had revealed the presence of saponins, alkaloids, and

terpenoids in both seed and leaf extracts, whereas the total alkaloid profile was higher in the seed extract than in the leaf extract. Other studies reported the presence of peptone, beta-galactoside, glycosylated abscisic acid, alkaloids, cellulose, polygalactose, polyuronids, cytochrome *P*-450, volatile oils, fat, protein, and minerals in avocado almond [16]. In addition, a high content (34.8%) of dietary fiber was found [17] in seed extracts.

For the antiulcer effect, the mixture of alcohol-HCl (10ml/kg) we used showed the capacity to induce ulcer in 75% of animals after 3 days of administration. One day dose of 100mg/kg of *P. americana* extract given before administration of the ethanol-HCl reduced gastric ulceration in 50% of animals compared to 25% by ranitidine (50mg/kg) and 31.2% by omeprazole (20mg/kg). The capacity of *P. americana* to cure alcohol-induced ulcers was 75%, compared to ranitidine (88.9%), and omeprazole (88.9%). The findings support what has been found by others. Athaydes *et al.* [9] reported 92% of protection using ethylacetate fractions of avocado seeds in ulcer induced with indomethacin; the extracts were effective in mitigating oxidative stress through a decrease in the oxidized product levels (reduction of 90% in lipid peroxidation in plasma) and increase of superoxide dismutase enzyme activity (4.25-fold compared to the indomethacin group). Oyemitan *et al.* [17] found that the extract decreased lipid peroxidation and restored total thiols in the gastric wall of mice that had been treated with ethanol.

The mechanisms of the antiulcer effect of various plants have been reviewed. The study by Batista *et al.*, [18] elucidated the associated antiulcer mechanisms of action of *Syngonanthus arthrotrichus* and *Syngonanthus bisulcatus*. When administered to rats submitted to ethanol-induced gastric lesions, the extracts (100 mg/kg, p.o.) increased the somatostatin serum levels, while the gastrin serum levels were proportionally decreased. In chronic gastric ulcers, a single daily oral dose (100 mg/kg) for 14 consecutive days accelerated ulcer healing to an extent similar to that seen with an equal dose of cimetidine. The pre-treatment of mice with N-ethylmaleimide or N-nitro-L-arginine abolished the protective activity, which indicates that antioxidant compounds and nitric oxide synthase activity are involved in the gastroprotective. It is likely that *P. americana* would act by similar mechanisms.

Besides its antiulcer effect, several other biological activities of the avocado seed have been reported. Ezejiofor *et al.* [19] investigated the hypoglycaemic and tissue-protective effects of hot aqueous *Persea americana* seed extracts on alloxan-induced albino rats. The results showed that the extract possessed a significant hypoglycaemic ( $P < 0.05$ ) effect and reversed the histopathological damage that occurred in alloxan-induced diabetic rats, comparable to the effects glibenclamide. The cardiovascular effects of *P. americana* Mill aqueous leaf extract have been investigated in some experimental animal paradigms. The results by Owolabi *et al.* [20] showed that the seed extract produced significant vasorelaxation on isolated rat aorta and that the effect is dependent on the synthesis or release of endothelium-derived relaxing factors (EDRFs) as well as the release of prostanoid. The extract also reduced vasoconstriction probably by inhibiting Ca<sup>2+</sup> influx through calcium channels. The hypotensive (antihypertensive) effect of the plant extract was examined in healthy normotensive and hypertensive Dahl salt-sensitive rats *in vivo*. *P. americana* aqueous leaf extract (25-800 mg/ml) produced concentration-dependent, significant ( $p < 0.05-0.001$ ), negative inotropic and negative

chronotropic effects on guinea pig isolated electrically driven left and spontaneously beating right atrial muscle preparations, respectively [21]. Oyemitan *et al.* [17], investigated the neuron-pharmacological effects and acute toxicity profile of the ethanolic dried seed extract in mice. They found that the extract, at 250 to 1000 mg/kg, caused significant ( $p < 0.01$ ) dose-dependently reduction in rearing and locomotor activity, signifying central nervous system depression; significantly lowered average rectal temperature (hypothermic effect); shortened onset and increased total sleeping time of ketamine (100 mg/kg, i.p) suggesting sedative activity; reduced mortality due to pentylene tetrazole, picrotoxin, and strychnine, and blocked hind limb tonic extension on the electro-shock, conveying evidence of anticonvulsant activity; increased reaction time on the hot plate and inhibited acetic acid-induced writhings, indicating analgesic potential. Oboh *et al.* [16] report that Avocado pear (*Persea americana* Mill.) leaves and seeds are used in traditional medicine for the management of Alzheimer disease (AD). The effect of *P. americana* leaf and seed aqueous extracts on some enzymes linked with AD (acetylcholinesterase [AChE] and butyrylcholinesterase [BChE] activities) and their antioxidant potentials *in vitro* has been demonstrated [22]. The extracts inhibited AChE and BChE activities and prooxidant-induced TBARS production in a dose-dependent manner.

Additionally, several studies have focused on the evaluation of the anti-parasitic effects and toxicity of the fruit and leaves. The hexane and methanol extracts from avocado seeds were toxic against *Artemia salina* (LC50 values of 2.37 and 24.13 mg/ml), *Aedes aegypti* larvae (LC50 16.7 mg/ml and 8.87 mg/ml). The extracts tested were also active against some yeast strains tested *in vitro*, including *Candida* spp, *Cryptococcus neoformans* and *Malassezia pachydermatis*, respectively (MIC from 0.08 to 156 mg/ml) [23]. Another study evaluated the antifungal activity of *P. americana* extract on *Candida albicans* biofilm and its cytotoxicity in macrophage culture (RAW 264.7). The MIC of the extract was 6.25 mg/mL, and with 12.5 mg/mL there was elimination of 100% of planktonic cultures. Regarding the biofilms, a significant reduction ( $P < 0.001$ ) of the biofilm at concentrations of 50 ( $0.580 \pm 0.209 \log_{10}$ ), 100 ( $0.998 \pm 0.508 \log_{10}$ ), and 200 mg/mL ( $1.093 \pm 0.462 \log_{10}$ ) was observed [24].

Acute and sub-acute toxicity profiles in rats were conducted to determine the oral median lethal dose (LD<sub>50</sub>) and other gross toxicological manifestations on acute basis [25]. Following 2.5 g/kg (p.o) per day of the extract for 28 consecutive days, the LD<sub>50</sub> could not be determined after a maximum dose of 10 g/kg. Sub-acute treatment with the extract neither affected whole body weight nor organ-to-body weight ratios but significantly increased the fluid intake ( $P < 0.0001$ ). Haematological parameters and the levels of ALT, AST, albumin and creatinine were not significantly altered. However, the concentration of total proteins was significantly increased in the treated group. Another study examining acute toxicity and genotoxicity obtained oral LD50 close to 1200 mg/kg body weight with ethanolic seed extract [26], while showing no genotoxic activity in the micronucleus test *in vivo* by induction of micronuclei in blood polychromatic erythrocytes of BALB/c mice [26].

## Conclusion

Previous studies and our findings support the use of avocado seeds in traditional medicine to manage a variety of diseases. The antiulcer healing potential is satisfactory compared to the standard antisecretory medications. Polyphenolic and

flavonoid derivatives may be responsible for the healing activity. The avocado seed represents 13–18% of the fruit, and it is a byproduct generally not utilized most of times. We also think that valorisation of the seed waste may solve a preoccupant ecological problem. The plant deserves further in-depth pharmaceutical studies for a development of improved dosage forms.

## Ethics

The study protocol and procedures, approved by the Research Review Committee and Ethical Review Committee to the Official University of Bukavu, complied with the guidelines laid down in the Declaration of Helsinki and were done in accordance with the International Guiding Principles for Biomedical Research Involving Animals (CIOMS, ICLAS, 2012).

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## Conflict of interest

No conflict

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