



The Effect of Ethanol Extract of Avocado Leaf (*Persea Americana* Mill.) on Reduction of Gastric Acid in Aspirin Induced Rats

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Abstract

One of the most prevalent dangerous illnesses is arthritis. The pathophysiology of gastritis is an imbalance of aggressive and protective factors. Nonsteroidal anti-inflammatory drugs are known as aggressive agents for the development of gastritis. Gastritis can be managed using avocado leaves. Finding out how an avocado leaf ethanol extract changed the amount of stomach acid in aspirin-treated rats was the aim of the investigation. Secondary metabolite content and characterisation were examined in the avocado leaf ethanol extract. Gastric acid reduction was performed by examining gastric acid concentration, number of ulcers, ulcer index, and pH in aspirin-induced rats. Ethanol extract of avocado leaves contains alkaloids, flavonoids, saponins, steroids, tannins and glycosides. Characterization obtained moisture content of 12.60%, total ash content of 1.34% and acid insoluble ash content of 0.17%. Acid concentration in the administration of avocado leaf ethanol extract 400 mg/KgBW is 16.00 ± 2.65 significantly different from the negative control 27.67 ± 2.52 . The number of ulcers on the administration of avocado leaf ethanol extract 400 mg/KgBW 1.67 ± 2.08 is significantly different from the negative control 5.33 ± 0.58 . When avocado leaf ethanol extract 400 mg/kgBW is administered, the ulcer index (0.003 ± 0.005) differs significantly from the negative control (0.014 ± 0.013). pH in the administration of avocado leaf ethanol extract 400 mg / kgBW 5.53 ± 0.57 is significantly different from the negative control 2.57 ± 0.12 . The ethanol extract of avocado leaf 400 mg/kgBW has a reducing gastric acid effect.

Introduction

The mucosal and submucosal layers of the stomach become inflamed when someone has gastritis. Its clinical presentations can range from mild to severe, with dyspepsia being the most common symptom and severe symptoms including bleeding, perforation, and peptic ulcers. There are two ways that anti-inflammatory drug use might harm the stomach lining: locally and systemically. Being lipophilic and acidic causes damage locally, while a major reduction in prostaglandin synthesis causes damage systemically.

A non-steroidal anti-inflammatory medication of the salicylate class containing an acetyl ester group is aspirin. Aspirin is the common name for the chemical compound acetylsalicylic acid, which is a derivative of salicylic acid (Auyang, 2004; Kowalski, 2019). While some salicylates taken orally, the majority of their absorption occurs in the upper small intestine and occurs quickly in the stomach. The rate of absorption is contingent upon several factors, including the length of stomach emptying, the pH of the mucosal surface, and the rate of tablet breakdown and dissolution (Acharya et al., 2018). The liver is the primary organ where aspirin is absorbed

as intact hydrolyzed salicylic acid (Sankaranarayanan et al., 2020; Kaur & Singh, 2022). Although salicylates are biotransformed in a variety of tissues, liver microsomes and mitochondria are the primary sites of action. Salicylates are metabolites that are primarily eliminated through the kidneys, with perspiration and bile also playing a role (Nagaich & Chaudhary, 2023). Prostaglandins block.

Cimetidine reduces the secretion of stomach acid via blocking the release of histamine through H₂ receptor inhibition, as well as by inhibiting intracellular cyclase adenylate, parietal cell proton pump hormone and Na-K T_pase. When treating gastrointestinal dysfunction, the histamine-2 receptor antagonists pharmacological class is used and shows no appreciable difference in efficacy from the PPI treatment group (Wulansari et al., 2023).

Conventional therapy for stomach wounds often results in relapses since most drugs have adverse effects and are not very effective. Certain medicinal plants have shown promise in treating digestive issues when utilized in traditional medicine (Adefisayo et al., 2017).

Avocado leaves are high in fiber, unsaturated fatty acids, phosphorus, magnesium, calcium, sodium, potassium, triterpenoids, flavonoids, monoterpenoids, and sesquiterpenoids, according to Arukwe et al. (2012). Avocado leaves also contain a wide range of other qualities, such as being anti-inflammatory and histamine secretion inhibitors, according to Oluwole et al. (2011) research.

In rats administered aspirin, the purpose of this study is to ascertain how avocado leaf ethanol extract lowers stomach acid.

Methods

This research employs an experimental design, examining the link between a free variable and a bound variable. Sampling, plant identification, simplisia generation, plant extracts, characterization, phytochemical screening, stomach acid reduction test in vivo, and the research includes statistical analysis utilizing One Way ANOVA (Analysis of Variance) and the Tukey test. The SPSS application (26.0). The study was carried out at the Universitas Sumatera Utara Faculty of Pharmacy's Phytochemistry and Pharmacology Laboratory.

Tool

Measuring glasses, sample filters, sealed beans, pH meters (ATC), dryers, beaker glasses, petri cups, porcelain cups, filing paper, porcelain crosses, animal cages, scratches, passageways, dryer cabinets, analytical balancers, water carbonizer, rotary evaporator, and sput are among the instruments used.

Material

Aqueous distillate, aspirin, Na CMC, formalin 10%, ethanol 96%, potassium iodide, NaOH 0.01 N, indicator of phenolftalein, and Cimetidine 200 mg tablets (PT. Kimia Farma) are the materials utilized in the study.

Sample Collection

Subjective sampling is taking a sample without comparing it to the same plants at different locations. The avocado leaves are from the Ronggur Nihuta section of Samosir's Sabungan Village. The avocado leaves are trimmed, separated from the trees, and then wet weighted after being cleaned under running water.

Identification

At Universitas Sumatera Utara's FMIPA Herbarium Laboratory, samples of avocado leaves (*Persea americana* Mill.) were identified.

Simplisia of Avocado Leaf

Fresh, spotless avocado leaves were dried for 14 days at 30–40°C in a dryer cabinet. The leaves were then powdered after drying.

The Avocado Leaf Ethanol Extract

A 96% ethanol solvent was used throughout the maceration process to prepare the extract. Add five liters of 96% ethanol to 500 grams of simplisia powder in a tightly covered container. Soak for six hours, stirring periodically, and then for eighteen hours. Filter using filter paper and a filtering patch (macerate I). To obtain macerate II, repeat the extraction procedure with 2.5 liters of ethanol and a 96% ethanol. After combining the two macerates, evaporate them at 40°C in a rotary evaporator until a thick extract is produced.

Phytochemical Screening

Alkaloids, flavonoids, tannins, saponins, glycosides, and steroids/triterpenoid are all examined as part of phytochemical screening (Depkes RI, 1995).

Characterisation

Characteristics of Avocado Leaf Simplisia

Avocado leaf simplicity is characterized by many analyses, including water content, water soluble essence, ethanol solubility test, total ash test, and acid insoluble ash test.

Avocado Leaf Extract Characterization

Typical assays on avocado leaf simplicity include water levels, total ash levels, and acid insoluble ash levels.

Tests on Animals

Two-month-old wistar rats weighing 160–180 grams were the test animals.

Tests of Adaptation in Animals

For a total of fourteen days, the test animals were housed in cages with three groups **per** cage in the laboratory. They received husk and pellets to eat.

CMC Suspension 0.5%

Weigh out 0.5 grams of Na CMC, then fill the container with 20 parts hot water, spread the Na CMC over it, and let it soak for 15 minutes or until it becomes translucent. Stir to make it all even, then dilute with water.

The Ethanol Extract of Avocado Leaf Suspension

There are three dosages of avocado leaf ethanol extract available: 100, 200, and 400 mg/kgBW. 100 mg, 200 mg, and 400 mg, in that order, were weighed. After placing each extract into a loop and adding a slightly reduced 0.5% CMC suspension, all while stirring to ensure homogeneity, the extract is then placed into a 10 ml container and the volume is increased up to the reference line.

Suspension of Aspirin

The aspirin equivalent of 400 mg should be weighed, added to a batch, ground to a uniform consistency, placed into a 10 ml grate, and its volume should be added to the mark line.

Suspension of Cimetidine

Cimetidine 200 mg tablets were combined with 0.5% crushed Na CMC and mixed thoroughly before being placed in a 10 ml scrape and the volume was added to the mark line.

Agreement on Health Research Ethics

The test was conducted following approval of the Health Research Ethics Recommendation by Universitas Sumatera Utara's FMIPA Animal Research Ethics Committee (number 0525/KEPH-FMIPA/2023).

Stomach Acid Examination

Rats were given water but no food during a 24-hour fast. The aspirin dosage and the dosage of each treatment were determined by weighing the rats' bodies. Following an oral induction dose of 400 mg/kgBW aspirin, the rats underwent another 24-hour fast. According to the division of groups, each group of rats received the following treatments once a day: the ethanol extract of avocado leaf group 100 mg/kgBW, the ethanol extract of avocado leaf group 200 mg/kgBW, the ethanol extract of avocado leaf group 400 mg/kgBW, and the negative control group (CMC Na). Eight days were dedicated to the treatment. Three rats were removed from each group after two, four, six, and eight days of therapy in order to measure the pH, the concentration of gastric acid, the number of ulcers, and the ulcer index.

Gastric Induction

Aspirin acts as the gastric inducer. All animal groups received 400 mg/KgBW as a single oral dosage of the medication following a 24-hour fast prior to induction.

Measurement of acid concentration and pH

A pH meter is used to measure the pH of the gastric liquid that is collected after it has been centrifuged for 10 minutes three thousand revolutions per minute. utilizing the signal of the phenolphthalein reaction, a titration using NaOH 0.01 N is used to measure the acid content (mEq/L). After inserting NaOH 0.01 N into the buret, 1 milliliter of the stomach liquid was pipetted, placed in an Erlenmeyer flask, and titrated until a purple-red hue was achieved. The alkaline volume utilized was then recorded (Adefisayo et al., 2017).

$$\text{Gastric concentration} = \frac{\text{Volume NaOH (ml)} \times 0.01 \text{ N} \times 100}{0.1}$$

The Number of Ulcers

The rats' stomachs were cut by making a small incision in the middle of their abdomens after they had been washed with a 10% formalin solution. After that, the rats were killed. Gastric tissue is isolated and all gastric fluids are separated. After the stomach's contents are evacuated, a 0.9% NaCl solution is used to cleanse it. Macroscopic observations are made visually by counting the number and size of formed ulcers while extending the isolated stomach organs group after two, four, six, and eight days of therapy in order to measure the pH, the concentration of gastric acid, the number of ulcers, and the ulcer index.

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The Index of Ulcers

The area of the ulcer in relation to the stomach area is called the ulcer index examination. The number of ulcers in each stomach is used to calculate the index of ulcers.

Analyzing Data

Using the One Way ANOVA approach in the SPSS program version 26.0, the concentration, volume, number of ulcers, ulcer index, and pH of the rats' stomach fluid were measured for the study. If differences are detected, perform the Tukey test to ascertain the real differences between the treatments. When a p significance value of less than 0.05 is discovered, it is deemed significant.

Result and Discussion

Identification

The avocado leaf plant, with number 1276/MEDA/2023, is a member of the *Persea americana* Mill species, according to the outcomes of the plant identification procedure carried out at the Universitas Sumatera Utara's Medanense Herbarium Laboratory (MEDA), Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA).

Characteristic of Avocado Leaf

Table 1 show the characteristics of avocado leaves that meet the standards of Farmakopea Herbal, including the content of water, water-soluble essence, ethanol-soluble essence, and acid-insoluble ash.

Table 1. The analysis of avocado leaf characteristics

Characterization	Results	The Herbal Pharmacopea's required
Water content	6.66%	$\leq 10\%$
Water soluble essence content	17.62%	$\geq 14.6\%$
Solubility of essence in ethanol	15.66%	$\geq 13.5\%$
Total ash content	2.72%	$\leq 4.2\%$
The ash content is insoluble in acid	0.45%	$\leq 1.1\%$

The water content, 6.66%, met the criteria of less than 10% based on the results. This water level controls the extract's stability and prevents bacteria from growing quickly.

The water soluble essence contain was determined using a water solvent and ethanol, and the result was a 17.62% greater water soluble essence contain than a 13.5% soluble essence contain in ethanol, which indicated that the soluble compounds in water were more, while the solute concentrations in ethanol showed a soluble composition in a polar solvent.

The Characteristics of Ethanol Extract of Avocado Leaf

After 650 grams of avocado leaf simplisia powder were maserified in 96% ethanol solvent and applied in a rotary evaporator at $\pm 40^\circ\text{C}$, 189 g of thickness extract (rendement of 29.07%) were produced. The yield is calculated by comparing the weight of the sample utilized with the

metabolite result that was obtained during the extraction process. If the yield is greater than 10%, it is considered good.

The ethanol extract characterisation results of avocado leaf are displayed in Table 2, along with the water content, total ash content, and acid-insoluble ash levels that meet Hebal Pharmacopea criteria.

Table 2. The characteristics of avocado leaf ethanol extract

Characterization	Results	The Herbal Pharmacopea's required
Water content	12.60%	≤ 14.0%
Total ash content	1.34%	≤ 5.0%
The ash content is insoluble in acid	0.17%	≤ 1.0%

Phytochemical screening of avocado leaf Ethanol extract

The following table displays the findings of the simplisia phytochemical screening as well as the qualitative ethanol extract of avocado leaf, which indicate the presence of alkaloids, flavonoids, steroid/triterpenoids, saponins, tannins, and glycosides:

Table 3. Phytochemical screening of avocado leaves

Secondary Metabolite	Result
Alkaloids	+
Flavonoids	+
Glycosides	+
Saponins	+
Tannin	+
Steroids/Triterpenoids	+

(+) : contain compound groups

Gastric Acid Concentration

An examination of gastric acid concentration carried out for 8 days can be seen in Table 4 and Figure 1 below:

Table 4. Gastric acid concentration

Group	Average gastric acid concentration (mEq/L) ± SD			
	2 days	4 days	6 Days	8 Days
Negative Control (CMC Na)	33.33 ± 1.53	31.33 ± 2.08	29.00 ± 2.00	27.67 ± 2.52
Positive Control (Cimetidine 200 mg)	19.33 ± 1.53	17.67 ± 2.52	15.67 ± 1.53	13.33 ± 2.08
Avocado Leaf Ethanol Extract 100 mg/kgBW	29.00 ± 1.00	27.00 ± 1.00	25.33 ± 2.52	22.67 ± 0.58
Avocado Leaf Ethanol Extract 200 mg/kgBW	26.00 ± 1.00	23.33 ± 1.53	20.33 ± 1.53	18.67 ± 1.53
Avocado Leaf Ethanol Extract 400 mg/kgBW	22.67 ± 2.08	20.00 ± 1.00	18.00 ± 2.00	16.00 ± 2.65

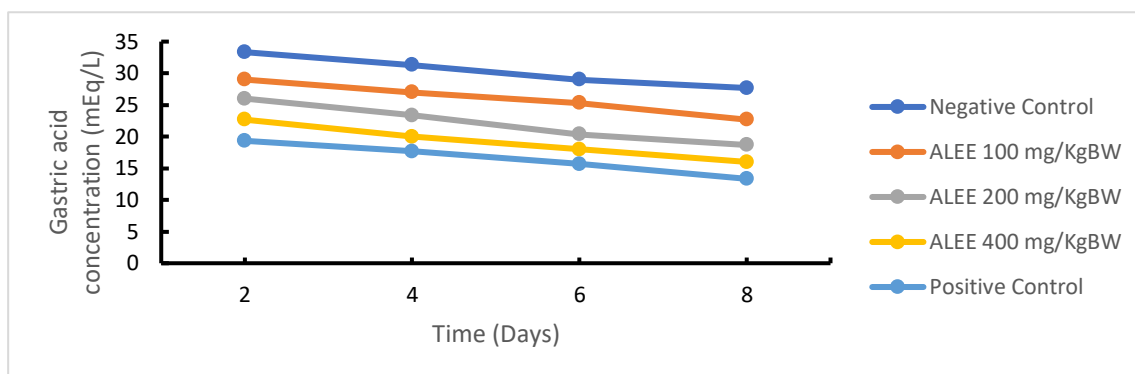


Figure 1. Gastric acid concentration

Table 4 and Figure 1 demonstrate that following an 8-day treatment, the average acid concentration in the avocado leaf 400 mg/kgBW group treated with ethanol extract is 16.00 ± 2.65 . This is significantly different from the negative group (27.67 ± 2.52) but not significantly different from the 13.33 ± 2.08 positive control group. When given 400 mg/kgBW of avocado leaf ethanol extract, the content of stomach acid decreases; this effect is similar to that of cimetidine, another H₂ receptor antagonist.

The Quantity of Ulcers

Examination of the quantity of ulcers in the stomach of rats carried out for 8 days obtained data that can be seen in Table 5 and Figure 2 below:

Table 5. The number of ulcers

Group	Average the quantity of ulcers \pm SD			
	2 days	4 days	6 Days	8 Days
Negative Control (CMC Na)	12.67 ± 3.21	9.00 ± 4.36	7.33 ± 2.52	5.33 ± 0.58
Positive Control (Cimetidine 200 mg)	4.67 ± 1.15	3.00 ± 1.00	1.67 ± 1.53	0.67 ± 1.15
Avocado Leaf Ethanol Extract 100 mg/kgBW	8.33 ± 2.08	7.33 ± 2.08	6.00 ± 3.61	3.00 ± 1.73
Avocado Leaf Ethanol Extract 200 mg/kgBW	6.33 ± 2.31	4.33 ± 1.53	2.67 ± 2.08	2.00 ± 1.00
Avocado Leaf Ethanol Extract 400 mg/kgBW	5.00 ± 1.73	3.67 ± 4.04	2.00 ± 1.73	1.67 ± 2.08

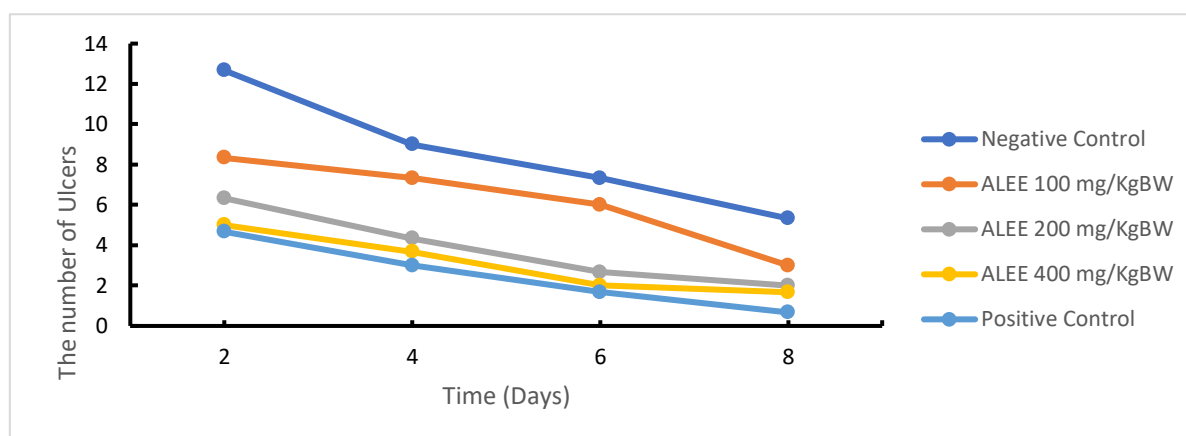


Figure 2. The Number of Ulcers

The group that received 400 mg/kgBW of avocado leaf ethanol extract had an average of 1.67 ± 2.08 ulcers, which differed substantially from 5.33 ± 0.58 ulcers in the negative control group but not significantly from 0.67 ± 1.15 ulcers in the positive control group. Table 5 and Figure 2 present these findings. When 400 mg/kgBW of avocado leaf ethanol extract was given, the number of ulcers decreased, indicating a possible improvement in gastritis.

Index of Ulcers

Data gathered from an 8-day analyzing the ulcer index within the rats' stomachs are displayed in Table 6 and Figure 3 below:

Table 6. Ulcer index

Group	Average Ulcer Index \pm SD			
	2 days	4 days	6 Days	8 Days
Negative Control (CMC Na)	0.041 ± 0.02	0.042 ± 0.01	0.03 ± 0.04	0.014 ± 0.013
Positive Control (Cimetidine 200 mg)	0.01 ± 0.01	0.01 ± 0.01	0.003 ± 0.003	0.0003 ± 0.0005
Avocado Leaf Ethanol Extract 100 mg/kgBW	0.01 ± 0.004	0.01 ± 0.01	0.01 ± 0.01	0.005 ± 0.008
Avocado Leaf Ethanol Extract 200 mg/kgBW	0.01 ± 0.01	0.01 ± 0.01	0.005 ± 0.005	0.004 ± 0.003
Avocado Leaf Ethanol Extract 400 mg/kgBW	0.01 ± 0.01	0.01 ± 0.004	0.004 ± 0.004	0.003 ± 0.005

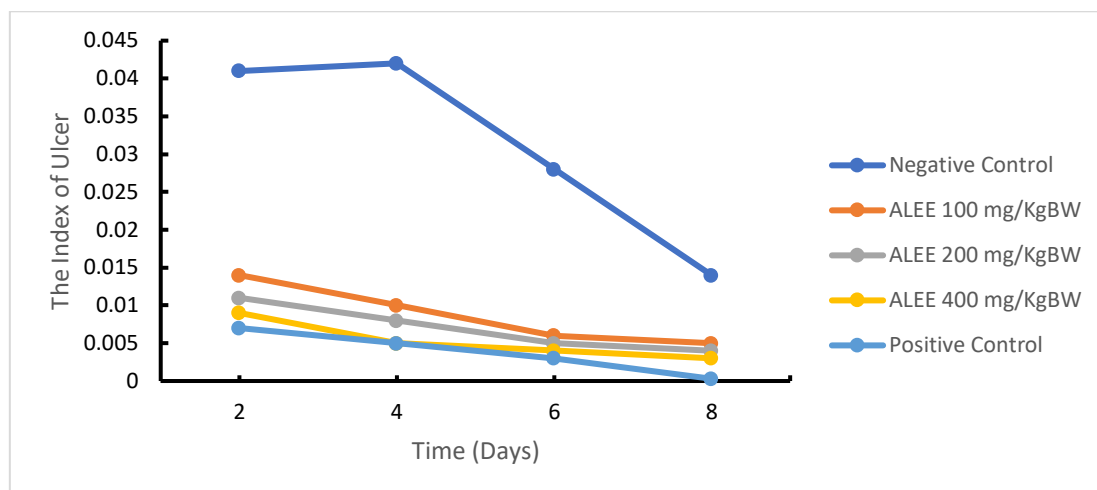


Figure 3. The index of ulcer

The ethanol extract of avocado leaf 400 mg/kgBW group's mean ulcer index, as indicated by Table 6 and Figure 3, is 0.003 ± 0.005 . The mean ulcer index of 0.0003 ± 0.0006 for the positive control group is not significantly different from the mean ulcer index of 0.014 ± 0.013 for the negative control group. The application of an ethanol extract from avocado leaves at a dose of 400 mg/kgBW reduced the ulcer index.

The Gastric Liquid's pH

Data from an 8-day study measuring the pH of rat stomach juice are shown in Table 7 and Figure 4 below:

Table 7. pH of gastric liquid

Group	Average pH \pm SD			
	2 days	4 days	6 Days	8 Days
Negative Control (CMC Na)	2.30 ± 0.17	2.40 ± 0.10	2.47 ± 0.06	2.57 ± 0.12
Positive Control (Cimetidine 200 mg)	4.50 ± 0.17	5.00 ± 0.26	5.63 ± 0.15	6.27 ± 0.42
Avocado Leaf Ethanol Extract 100 mg/kgBW	3.27 ± 0.55	3.73 ± 0.40	4.23 ± 0.15	4.63 ± 0.35
Avocado Leaf Ethanol Extract 200 mg/kgBW	3.83 ± 0.25	4.23 ± 0.21	4.50 ± 0.10	4.80 ± 0.10
Avocado Leaf Ethanol Extract 400 mg/kgBW	4.13 ± 0.15	4.53 ± 0.25	4.47 ± 0.25	5.53 ± 0.57

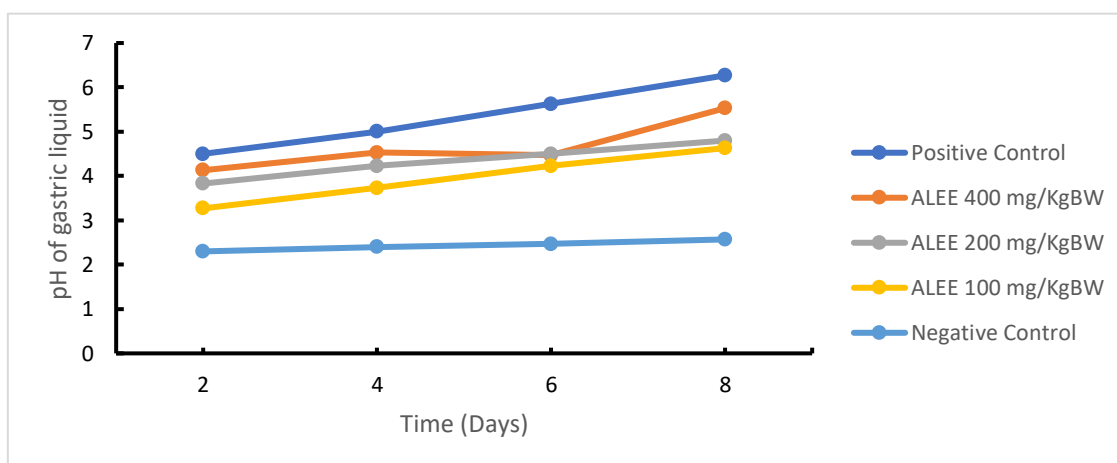


Figure 4. pH of gastric liquid

The average pH of the gastric liquid in the 400 mg/kgBW group of ethanol extract of avocado leaf is 5.53 ± 0.57 in Table 7 and Figure 4. This is significantly different from the negative control group's average pH of 2.57 ± 0.12 but not significantly different from the positive control group's average pH of 6.27 ± 0.42 . Raising pH is achieved by administering 400

mg/kgBW of avocado leaf ethanol extract; a stomach's normal pH is indicated by a gastric pH above 3.

Conclusion

The study's findings, which examined the impact of 400 mg/kgBW of avocado leaf ethanol extract on the reduction of stomach acid concentration in aspirin-induced rats, demonstrated that acid concentration, with the following results: (a) The ethanol extract from avocado leaves can lower the average stomach acid concentration from 22.67 ± 2.08 to 16.00 ± 2.65 . This is significantly different from the negative control group's mean of 33.33 ± 1.53 at an acid concentration of 27.67 ± 2.52 ; (b) Compared to the negative control group, which had a mean number of ulcers from means of 12.67 ± 3.21 to 5.33 ± 0.58 , the avocado leaf ethanol extract can lower the mean number of ulcers from means of 5.00 ± 1.73 to 1.67 ± 2.08 ; (c) The avocado leaf ethanol extract dramatically lowers the mean ulcer index from 0.009 ± 0.009 to 0.003 ± 0.005 , relative to the negative control group, whose mean ulcer index increased from 0.041 ± 0.016 to 0.014 ± 0.013 ; (d) The ethanol extract of avocado leaf can raise the mean pH from means of 4.13 ± 0.15 to 5.53 ± 0.57 , it differs noticeably from the negative control group, whose mean pH ranges from 2.30 ± 0.17 to 2.57 ± 0.12 .

Suggestion

The researchers propose that, in order to obtain more precise results regarding the ethanol extract of avocado leaves' ability to reduce gastritis, a lipid peroxidation test should be carried out. The study's findings indicate that the extract has a reducing effect on gastric acid.

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