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In vivo and *in silico* investigations of the toxicological and analgesic properties of unprocessed *Aloe vera* gel in experimental rat models

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Abstract: *Aloe vera* is a commonly used plant in both food and medicine industry. The potential toxicological side-effects of prolonged intake of *Aloe* extract have not been evaluated in detail. This work presents an in-depth toxicological study of the crude unprocessed *A. vera* gel in experimental rats. Acute and sub-chronic toxicity was evaluated in a 1 to 28-day long feeding schedule of the aqueous homogenized gel material. Hemoglobin, total protein, high density lipoprotein (HDL), low density lipoprotein (LDL), cholesterol, triglyceride, serum creatinine, serum alanine transaminase (SGPT), aspartate transaminase (SGOT) and alkaline phosphatase were examined and kidney and liver histology was performed. In the acute toxicity test, the behavioral aspects were also considered. A molecular docking assay was performed to investigate the binding affinities of pure *A. vera* compounds with liver and kidney toxicological marker enzymes, in order to assess the probable mode of action of selected *Aloe* constituents. Solubility factors for the active constituents were also studied to determine their possible miscibility with body fluids. The results from *in vivo* tests provided no major toxicological indications. Crude *Aloe* gel consumption up to 4 g/kg body weight (b.w.) showed no toxicological side effects. From the structural standpoint, *Aloe*-based bioactive molecules, such as *Aloe*-emodin, acetophenone, β-sitosterol, cholestenol and squalene showed promising binding affinity to qualify as alternative and complementary medicines. The synergistic roles of all *A. vera* constituents remain to be validated in human disease models.

Keywords: *Aloe vera*; nociception; molecular docking; *Aloe*-emodin; hepatitis B viral protein

INTRODUCTION

The genus *Aloe* has a long history of providing an array of health benefits as a traditional medicine. With about 400 *Aloe* species, one of the most frequently used is *Aloe vera* (L.) Burm.f. (Xanthorrhoeaceae). *A. vera* has served as a remedy for different ailments, such as wounds and burns, constipations, external and internal ulcers, hyperlipidemia, diabetes and many more [1-6]. It is also documented as a wound healing and anti-inflammatory agent in the Indian ayurvedic system, a complementary medicinal system of ancient India used as protective or preventive medication against many ailments. Only 8% of the online databases on *Aloe*, including research articles, deal with the toxicological effects of this plant, and are with or without any scientific references [7]. Less than 3% of the herbal product-related websites cite scientific literature regarding the usage, adverse effects, drug interactions and safety precautions regarding *A. vera* consumption or application. These findings indicate that the data regarding the toxicological effects of *A. vera* following short- or long-term consumption is incomplete. Moreover, when toxicological parameters are considered, only a few reports present toxicological studies on *Aloe* gel use. Orally supplemented *A. vera* extract was shown to protect from oxidative stress and restore blood reduced glutathione (GSH) concentration in experimental rat models of exposure to Arsenic (As), however, it did not reduce the concentration of As in tissues [8]. Crude *A. vera* ethanolic extract was reported to be a potent anti-oxidant against azoxymethane-induced stress in rats [9]. The *A. vera*-derived phytocompound *Aloe*-emodin exhibited

hepatoprotective activity in the experimental model of carbon tetrachloride-induced hepatic injury [10]. Anilakumar *et al.* [9] pointed to a probable synergy between *A. vera* phytocomponents that showed antioxidant effects in experimental rats. *Aloe* latex and whole leaf extract have been implicated in some cases of carcinogenesis, genotoxicity and *in vivo* toxicity in animal models. But the *Aloe* gel, which is free from latex and the epidermal layer, has not been evaluated systematically with regard to its potential toxicological effects [7,11].

We have considered the human-consumable aqueous preparation of homogenized, latex-free *A. vera* gel for feeding to Wistar albino rats of both sexes, in order to determine its acute and sub-chronic toxicological effects *in vivo*. The doses used were based on daily consumption of 50 g of *Aloe* gel by a healthy person weighing 60 kg. Available information indicates that ethnic people consume *Aloe* gel in a crude, unprocessed and fresh state [4]. Hence, we simply homogenized *A. vera* gel in water and used the fresh homogenate daily in each feeding schedule. Acute and sub-chronic toxicities were measured following 1 to 28-day feeding schedules, respectively. Animal death, behavioral parameters resulting from skin irritation, dizziness, as well as liver and kidney enzyme profiles, were evaluated along with other toxic and physiological biomarkers, including serum creatinine, triglyceride and cholesterol. We had previously confirmed insignificant changes in hematological parameters in rat models following extended *Aloe* gel consumption [4].

Molecular docking provides a prediction of the affinity of one molecule to another in a preferred orientation. The stability of such an association on a potential 3-dimensional surface of two moieties can further indicate their pharmaceutical utilities. We can even predict the probable solubility of compounds by mimicking their physiological conditions. Therefore, we screened and studied all the available phytochemical constituents of *Aloe* and we have selected 8 potentially bioactive molecules for molecular docking assays [12]. This can provide us with an understanding of the drug-like properties of the phytochemicals under study. These are the basic steps of *in silico* drug design.

MATERIALS AND METHODS

Ethics statement

The experiments were approved for the period 2013-16 by the Institutional Animal Ethical Committee (IAEC) of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), with registration number 840/ac/04/CPCSEA, dated 01/01/2004, of the University of North Bengal, West Bengal, India prior to commencement of the experiments.

Experimental animals

Wistar albino rats of both sexes, weighing 140-160 g, served as experimental animals for acute and sub-chronic toxicity and pain sensation. The animals were purchased from Ghosh Enterprise, Kolkata, India and were kept in the departmental animal house facility at 24±2°C, a 12h day-night cycle, and were fed with standard pellet obtained from Ghosh Enterprise and water *ad libitum*. All animals were acclimatized for a period of 10 days before initiation of the experiments. Four or fewer rats were kept per cage during the entire period.

Collection and processing of plant material

Naturally grown plants were collected in the post-monsoon seasons (September-November) from the Medicinal Plant Garden of the University of North Bengal, located at Siliguri (Darjeeling, West Bengal, India). The plant was identified and deposited in the Department of Botany, University of North Bengal. The voucher specimen was provided an accession number in the NBU herbarium (Accession No. NBU09884). The *Aloe* gel was collected after aseptically peeling off the outer green layer and latex part of the plant leaf. The gel was weighed and chopped into pieces before homogenization. Five g of *Aloe* gel was mixed with 3 mL of water during homogenization, to produce a final 7 mL mixture. Considering that 7 mL of the mixture contained 5 g of *Aloe* gel, the feeding doses contained the gel at concentrations of 1, 2, 4 and 5 g/kg body weight (b.w.). The oral administration of the plant material was done using an oral gavage. The process was repeated daily to ensure fresh consumption of unprocessed raw *Aloe* gel.

Chemicals

All the serum biochemical test kits for toxicological parameters were purchased from Coral Clinical Systems, Goa, India. Other chemicals, such as formalin and chloroform, were procured from Merck (USA) and SD Fine Chemicals (India). All chemicals were molecular biology grade.

Experimental design

Acute toxicity test

In the acute toxicity test, three groups of mature rats were selected, each contained 6 rats (3 males and 3 females). The first group (N) was not fed with *A. vera* gel; the other two were the experimental groups, TA1 and TA2 and were fed with 2 and 5 g of *Aloe* crude gel/kg b.w., respectively. The animals fasted overnight prior to feeding with a single dose of the gel homogenate. The mortality rate, salivation, fur irritation, sleep/dizziness, lethargy and diarrhea were observed for the next 24 h. This one-day long observation was further followed by a 7-day long screening for any toxicological effects. All the experiments were performed according to the OECD guideline 423 (Adopted on 17th December 2001) for acute oral toxicity study in rodents.

Sub-chronic toxicity

For the determination of sub-chronic toxicity, the animals were divided into four groups. All the groups contained 6 rats each (3 male and 3 females). The first group was considered as Normal (N), the second, third and fourth groups, T1, T2, and T3 respectively, were the treatment groups with a 28 day-long daily feeding schedule of *Aloe* gel at concentrations of 1, 2, and 4 g/kg b. w., respectively. After completion of the 28 day-long schedule, the animals were killed with anesthetic. Blood from each animal was collected separately by heart puncture. Kidney and liver tissues were collected and fixed in 4% formalin for histological processing. The clear serum was collected by allowing the blood samples to clot. Hemoglobin was measured immediately after blood collection. All the experiments were performed according to the standard guidelines with slight modifications [13,14].

In a previous study, we showed that the red and white blood cell (RBC and WBC, respectively) counts, total protein, total albumin remained normal in the experimental Wistar albino rats while evaluating the anti-arthritic properties of the same plant [4]. Next, the hemoglobin and the serum enzymatic parameters, including alkaline phosphatase (ALP), aspartate transaminase (SGOT), alanine transaminase (SGPT), and serum high density lipoprotein (HDL-D), low density lipoprotein (LDL-D), triglycerides, cholesterol, creatinine were measured using the appropriate assay kits, following the manufacturers' instructions provided with the Coral Biosystem's (Goa, India) product-specific user manual. All spectrophotometric assays were performed using in a Systronics VIS spectrophotometer (Model 105).

Formalin-induced paw licking test

Experimental animals were fed with the indicated doses of *Aloe* gel homogenate for 7 days (designated as T1, T2, and T3 respectively; doses as used in the sub-chronic toxicity tests i.e. 1, 2 and 4 g/kg body b.w.). Normal (N) group rats were fed with an equal amount of distilled water. On the 7th day, 50 μL of 2.5% formalin solution was injected into the right hind paw. The animals were kept under observation in a glass cage for the next 30 min. The duration(s) of paw licking by the animals during the first 5 min and then for the following 15-30 min after the formalin injection was recorded. The first five min was considered as neurogenic pain and the succeeding 15-30 min were considered as inflammatory pain [15-17]. The total paw licking time used by the animals during 30 min was an indicator of nociceptive pain. Morphine, used as the standard drug to compare the pain-ameliorating activity of the plant extract, was fed orally 30 min prior to formalin injection at a dose of 5 mg/kg b.w.

Molecular docking

Molecular docking analyses of selected toxicologic and analgesic marker proteins against the selected chemical constituents of *A. vera* were carried out as described [12,18]. Depending on the variations that may occur during sample preparation and variations of the phyto-constituents vis-a-vis seasonal and geographical changes, 10 major potent bioactive mol-

ecules were examined. All protein structures were retrieved from a protein data bank (http://www.rcsb.org/pdb/home). Docking was performed using Auto Dock Vina [18]. The X, Y and Z dimensions were chosen to be 100 for aspartate transaminase, glutamate dehydrogenase, hepatitis B viral protein (hepatitis Bx), and 80 for alanine transaminase, alkaline phosphatase. Center Grid Box was chosen to fit the protein. The exhaustiveness was set to 8. Each concerned protein was rendered ready by removing the water molecules and by adding polar. Gasteiger charges were added to the proteins on the basis of electronegativity equilibration and the non-polar hydrogens were merged. Gasteiger charges were also calculated for the respective ligands and all the torsions were allowed to rotate.

Solubility analysis

The basic solubility analysis of Log P and Log S values were determined using ALOGPS 2.1 [19] maintained by Virtual Computational Chemistry Laboratory (http://www.vcclab.org/lab/alogps/). Log P, the logarithm of the partial coefficient, is defined as the ratio of the concentration of a solute between two solvents specifically for unionized solutes. Log S value is a unit stripped logarithm (base 10) of the solubility measured in mol/L. Chemcalize.org beta (http://www.chemicalize.org/) by Chem Axon was used to determine the log D values at physiological blood plasma pH of 7.4. Log D is the distribution coefficient, log D, is the ratio of the sum of the concentrations of all forms of the compound (ionized plus un-ionized) in each of the two immiscible phases.

Statistical Analysis

All statistical analyses were performed using Kyplot ver. 5.0. In the Kyplot analysis, the data represented the mean±S.D., which was analyzed by one-way ANOVA. The results were considered significant when $p \leq 0.05$.

RESULTS

Acute toxicity test

No death of the animal was observed during the 1st day of the trial in the acute toxicity test. The animals were observed at regular 2 h intervals. All the animals were alive for the next 7 days and no death or change in the physiology and behavior was observed (Table 1). Normal weight gain was observed in all groups and no gross abnormal findings were documented in any of the groups. The *Aloe* gel was therefore considered to be non-toxic up to the dose of 5 g/kg b. w. and the lethality value was considered "unclassified" up to the feeding range.

Table 1. Behavioral changes in rat groups following a one-day single dose trial of *Aloe* gel for acute toxicity. The behavior was followed in each animal for 24 h after feeding with the plant extract.

Groups	N (Normal)	TA1 (2 g/kg b.w.)	TA2 (5 g/kg b.w.)
Number of animals	6	6	6
Number of deaths	None	None	None
Abnormal motor activity	-	-	-
Salivation	-	-	-
Fur irritation	-	-	-
Sleep/dizziness	-	-	-
Lethargy	-	-	-
Diarrhea	-	-	-
'-' denotes absent.			

Sub-chronic toxicity test

We observed that out of 6 animals per group, only one animal died in the T2 group (i.e. the medium dose group (2 g/kg b.w.)). This death was unrelated to the toxicological properties of the *A. vera* as no other toxicological signs were seen in other rats in the same nor in other dose groups. Following anatomical investigation, the dead animal was diagnosed with multiple cysts in the liver. Body weight changes were normal in all the groups (Fig. 1.). The changes observed in the histology of the livers and kidneys were also non-significant (Fig. 2.). The enzymatic parameters of the serum, ALP, SGPT and SGOT (Fig. 3A, B and C, respectively), showed no differences between the experimental and control animal groups. However, some non-significant variations were present in the serum profiles of creatinine, triglycerides, cholesterol, LDL-D and HDL-D (Fig. 3.D, E, F, G, and H, respectively). In addition, no change in the blood sugar level in the experimental groups was observed. The triglyceride level was normal in T1 and T3 but slightly decreased in T2. The serum cholesterol concentration was decreased in

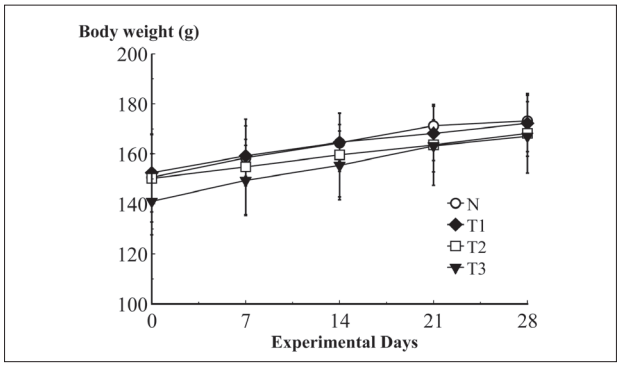

Fig.1. Diagram showing the body weight increment in experimental rat groups in the 28-day long sub-chronic toxicity assay. N refers to normal, T1, T2, and T3 refer to the second, third and fourth treatment groups, respectively, in a daily feeding schedule with *Aloe* gel (1, 2 and 4 g/kg b. w., respectively).

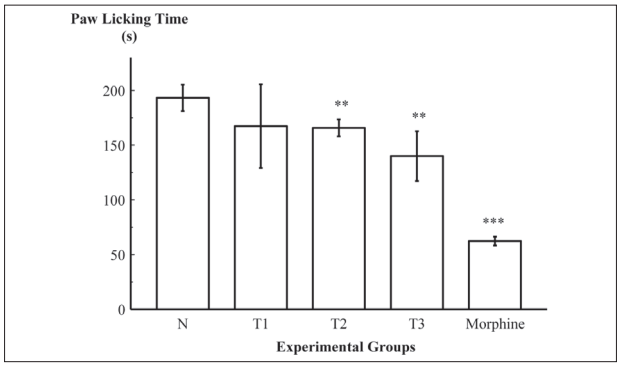

Fig.4. Bar diagram showing total paw licking time (s) in the formamide-induced paw licking test in different experimental groups. Dose groups T1, T2 and T3 showed decreased paw licking time, which revealed the analgesic property of the *Aloe* gel compared to the normal (N) group. Morphine was used as a standard analgesic drug. *** – significance at $P \leq 0.001$; * – significance at $P \leq 0.01$.

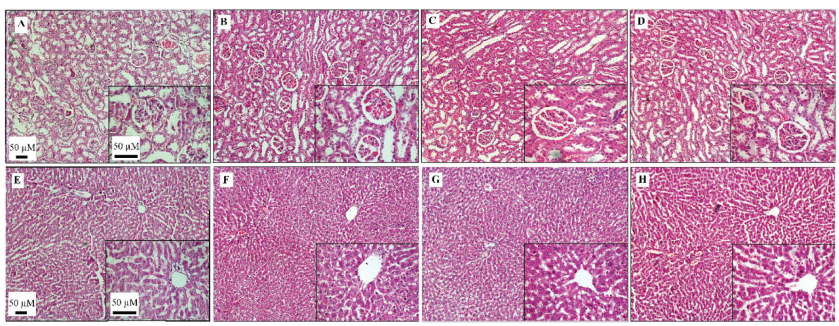

Fig.2. Histological study of kidney (upper panel) and liver (lower panel) of rats fed with unprocessed *Aloe* gel. **A** and **E** – normal, **B** and **F** – low dose (T1), **C** and **G** – medium dose group (T2); **D** and **H** – histological sections of the high dose group (T3). The larger photographs are 10X magnified, and the insets at the bottom right hand side of every photograph are 40X magnified. The size bar is in µM.

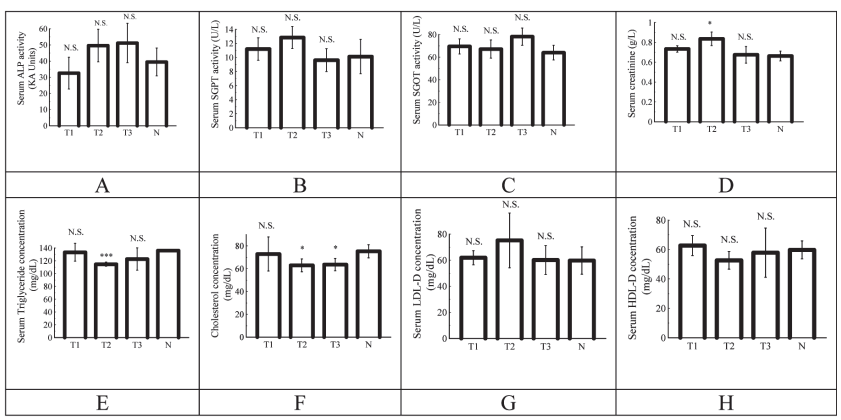

Fig.3. Different toxicological parameters in experimental rats fed with *Aloe* gel. N – normal animals; T1, T2 and T3 – low, medium and high dose *Aloe* gel-fed groups. *** – significance at $P \leq 0.001$; * – significance at $P \leq 0.05$.

T2 and T3, while the concentrations of HDL-D and LDL-D were at the physiological level in all experimental groups. SGPT and SGOT also exhibited insignificant differences in all the groups (Fig. 3).

Formalin-induced paw licking test

In the medium and high dose groups, the *Aloe* gel significantly reduced the nociception elicited by the injected formalin. The high dose group (T3) showed a better result than the low (T1) and medium (T2) groups (Fig. 4).

Molecular docking

The combination displaying the best binding affinity with the least root mean square deviation (RMSD) from zero was considered for our analysis. Glutamate dehydrogenase showed the best binding affinity on average with all the phytochemicals, followed by hepatitis Bx (Fig. 5A and B, Fig. 6). When we consider the interactions with respect to ligands, *Aloe*-emodin showed the best

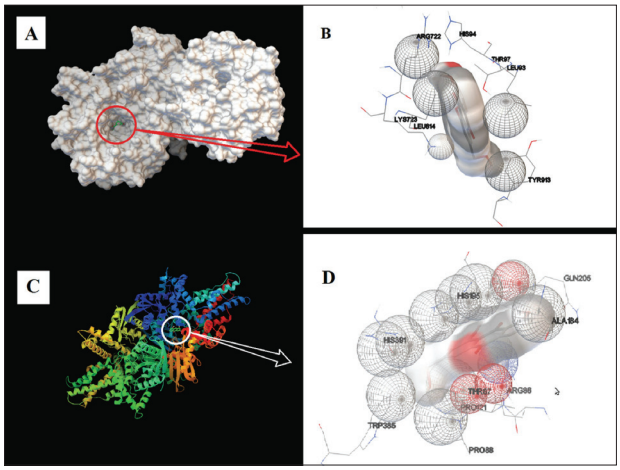

Fig. 5. A – Molecular docking of *Aloe*-emodin with hepatitis Bx protein. The encircled area shows the active binding site of *Aloe*-emodin. **B** – The binding site is enlarged in the right hand side indicating the participating amino acids. **C** – Docking image of glutamate dehydrogenase with *Aloe*-emodin. The encircled region indicates the best binding site, enlarged in **D**.

	Alanine transaminase	Alkaline phosphatase	Aspartate transaminase	Glutamate dehydrogenase	Hepatitis BX
Acetophenone	-5.2	-5	-5.3	-5.6	-6.1
Aloe-emodin	-6.9	-7.8	-7.5	-8.5	-8.6
Azelaic acid	-3.8	-5	-4.3	-4.8	-4.2
β-sitosterol	-7.3	-6.8	-6	-8.1	-8.3
Cholestanol	-6.8	-7.9	-5.8	-8.2	-7.1
Hydro-p-coumaric	-4.5	-5.7	-4.6	-5.9	-6
Hypoxanthine	-5	-5.5	-5.1	-5.9	-5.5
Oleic acid	-4.6	-4.8	-3.5	-4.9	-4.4
Salicylic	-5.5	-5.9	-6	-5.6	-5.8
Squalene	-5.6	-5.9	-4	-7.8	-7.1

High Low

Fig. 6. Heat map image of binding affinity strengths (Kcal/mol) of *Aloe* gel-derived pure compounds against toxicity marker enzymes. *Aloe*-emodin showed the best binding affinity with hepatitis Bx, followed by glutamate dehydrogenase.

result, followed by cholestanol. Parallel with the existing trend, *Aloe*-emodin had the best binding affinity of -8.6 kcal/mol with the standard control hepatitis Bx when individual interactions were considered. The 2nd best interaction was displayed by *Aloe*-emodin,

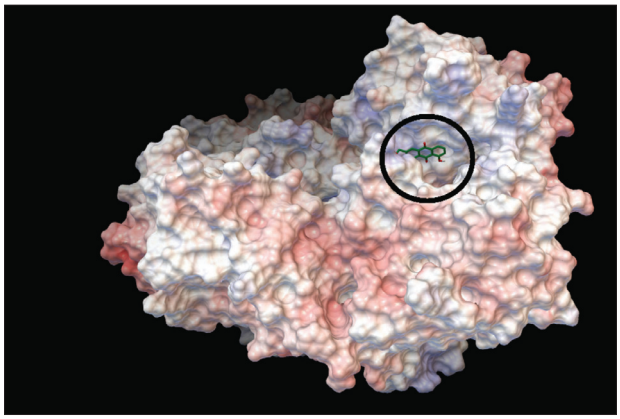

Fig. 7. Molecular docking of *Aloe*-emodin with Cox-2. The encircled region shows the best binding site of *Aloe*-emodin with Cox-2.

with a binding affinity of -8.5 kcal/mol with glutamate dehydrogenase (Fig. 5C and D, Fig. 6). When Cox-2 binding was examined, cholestanol had a binding affinity -6.5 kcal/mol, second to *Aloe*-emodin which had a binding affinity of -8.0 kcal/mol (Fig. 7).

Solubility analysis

According to the octanol-water partition coefficient, log P should be between -0.4 and +5.6. β-sitosterol, cholestanol, hypoxanthine, oleic acid, and squalene all lie outside the ideal range (marked red). The log S value is the solubility parameter of the phytochemicals. The ideal range of log S value lies around -5 mol/L. Compounds such as acetophenone, azelaic acid, hydro-p-coumaric, hypoxanthine and salicylic lie outside the range of good solubility. Another solubility parameter of importance is the log D value, with the ideal range between -3 and 3. Molecules with low log D values readily pass through cell membrane and have better solubility. Compounds that were out of range were β-sitosterol, cholestanol, oleic acid and squalene (Table 2).

DISCUSSION

The acute toxicity test revealed that unprocessed *A. vera* gel in Wistar albino rats was not toxic when the rats were fed a single dose of 5 g/kg b.w. (validated by a 7-day long observation), which also indicated that further testing for unprocessed *Aloe* gel is not nec-

Table 2. Solubility analysis of the *Aloe* gel-derived pure compounds. Log P is defined as the ratio of the concentration of a solute between two solvents specifically for un-ionized solutes. Log S value is a unit stripped logarithm (base 10) of the solubility measured in mol/L. Log D is the ratio of the sum of the concentrations of all forms of the compound (ionized plus un-ionized) in each of the two immiscible phases.

Chemicals	logP	logS	LogD
Acetophenone	1.65	-1.95	1.53
Aloe-emodin	1.27	-2.96	1.88
Azelaic acid	1.37	-1.92	-3.65
β-sitosterol	7.27	-7.35	7.84
Cholestenol	7.02	-7.41	7.52
Hydro-p-coumaric	1.15	-1.79	-1.28
Hypoxanthine	-0.74	-1.79	-0.42
Oleic acid	7.68	-6.37	4.40
Salicylic	1.96	-1.09	-1.52
Squalene	8.64	-5.91	10.42

essary [20]. Our findings can also serve as the basis for toxicological classification. Acute toxicity tests are not only performed to determine the precise lethality value but also to indicate the maximum dose for the survival of animals [21]. In traditional practice, the gel of this plant is consumed fresh after collection, without any processing. This was applied during preparation of the plant extract. In a previous study we showed that hematological parameters varied non-significantly following consumption of crude *Aloe* gel extract [4]. Now we determined that the highest dose of *Aloe* gel (5 g/kg b.w.) neither produced lethality nor elicited harmful effects in experimental rats after 7 days. No visually detectable behavioral abnormalities were observed (including body fur irritation, diarrhea, salivation).

A sub-chronic toxicity study was undertaken to assess the harmful effects associated with long-term repeated exposure of the animals to *Aloe* gel. It also provided information on organ-based toxicity [11]. The consumption of *Aloe* gel for 28 days did not initiate any deleterious changes or death, and all serum enzymatic and biochemical parameters remained unchanged. Generally, a loss in body weight relates to an immediate toxic effect of a plant, but no significant change was observed in any of the animal groups during the 28-day long feeding schedule [22]. Decreased food and water intake and loss of appetite are the early signs of physiological abnormalities. Our findings suggest that there were no interactions between the major metabolic pathways and *Aloe* gel constituents. This was further corroborated by the serum biochemical parameters in all animal groups. Blood glucose and hemoglobin were normal in all experimental groups. Serum creatinine was slightly elevated in the T2 group, which may indicate kidney malfunction; however, this data was not supported by the histological observation of the kidney. Creatinine is a by-product of muscle metabolism and is excreted by the kidneys. All other parameters, body weight and serum lipid, did not correlate with this finding. Moreover, the other experimental groups showed no such change in serum creatinine. Cholesterol decreased significantly in the T2 and T3 groups, indicating that *Aloe* gel consumption reduced the cholesterol content of serum, however, all groups had normal triglyceride content (100-140 mg/dL). Moreover, lowered levels of triglycerides in T2 and T3 groups indicated a potential positive effect of the *Aloe* gel in blood triglyceride regulation. LDL-D and HDL-D values showed no significant changes in the experimental groups. ALP, secreted from the bile duct and liver, is a bile duct malfunction marker when SGOT and SGPT levels are normal; on the other hand, SGPT, SGOT and ALP altogether are liver damage markers [23,24]. ALP, SGPT and SGOT remained at normal levels through the experimental feeding schedule. Histological analysis of kidney and liver tissues also supplemented the results of the biochemical experiments. There were no structural alterations in the hematoxylin-eosin stained kidney and liver sections. Kidneys possessed clear Bowman's capsule and vascularized glomerular region surrounded by proximal convoluted tubules. Both organs showed a normal arrangement of cells. Damage in parenchymal liver cells results in an elevation of SGOP and SGPT levels [23]. The obtained normal values of these two liver enzymes are supported by the normal appearance of the parenchymal region of the liver.

These parameters were supported by the *in silico* study of the active compounds of *A. vera* with the aforementioned enzymes through molecular docking analyses. GLDH, an important regulator of the urea cycle and an indicator of liver function was found to have the best interactions with the phyto-constituents, particularly with *Aloe*-emodin, cholestanol and β-sitosterol. The interaction profile of hepatitis Bx points to an overall good interaction with all exam-

ined ligands, with the best interaction observed between *Aloe*-emodin (binding affinity of -8.6 kcal/mol). Hepatitis Bx protein is a marker of liver dysfunction during hepatitis and upregulated expression of this protein increases the rate of hepatic carcinoma [24]. The strong interactions of *Aloe* gel components with hepatitis Bx protein indicated potential inactivation of this protein.

The other test proteins analyzed herein (SGPT, SGOT and ALP) are indicators of liver functioning, and all phytochemicals displayed moderate to average binding affinity with these proteins. Compounds with high binding affinities are supposed to alter the functioning of the protein(s). Thus, the strong binding observed between *Aloe*-emodin and Cox-2 could potentially downregulate the inflammatory pathway [25]. Our docking study revealed that there are numerous interaction sites for *Aloe*-emodin and other phyto-constituents with Cox-2. Compounds with high binding affinity should have a proper solubility index to reach the proper target protein. *Aloe*-emodin cleared all the logP, logS, LogD thresholds to qualify as a very good ligand. The logD value taken at a physiological pH of 7.4 ensures that *Aloe*-emodin can circulate through the plasma and reach the effector target to alter its functioning.

The formalin paw licking test is an established method to assess the anti-nociceptive activity of drugs in rodent models. We observed that the *Aloe* gel has potent pain-ameliorating activities. However, low doses decreased the paw licking time but variations between individual animals within the group resulted in high standard deviation, which is responsible for the non-significant change in T1 when compared to the control. Previous works reported by our group on the *Aloe* gel have established its efficacy as a potent anti-inflammatory and anti-arthritic agent [4,26,27]. The current work indicates that the consumption of unprocessed *Aloe* gel on a regular basis can ameliorate arthritic pain by its probable interaction with Cox-2.

To conclude, this work presents the first detailed examination of unprocessed crude *A. vera* gel-related toxicity after oral consumption, in an experimental rat model. *Aloe*-emodin, a unique constituent of the plant, has the best interaction, as per the molecular docking analyses, it has ideal solubility at physiological pH, and it can be considered for clinical trials. Our toxicological studies show no harmful toxicity in the rat when *A. vera* gel was consumed at doses up to 4 g/kg b.w for a month.

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Author contributions: S. Paul and D. Modak performed the *in vivo* experiments, analyzed the data and prepared the tables and figures; A. K. Chakraborty performed the *in silico* experiments, analyzed the data and prepared the related figures and tables. S. Paul and A. K. Chakraborty prepared and revised the manuscript. A. Sen and S. Bhattacharjee contributed to the concept and design of the experiments and data analysis, critical revision of the manuscript, and gave the final approval of the manuscript for publication.

Conflict of interest disclosure: Authors declare no conflict of interest.

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