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Functional potential of *Aloe vera* juice against CCl_4 induced hepatotoxicity in animal model

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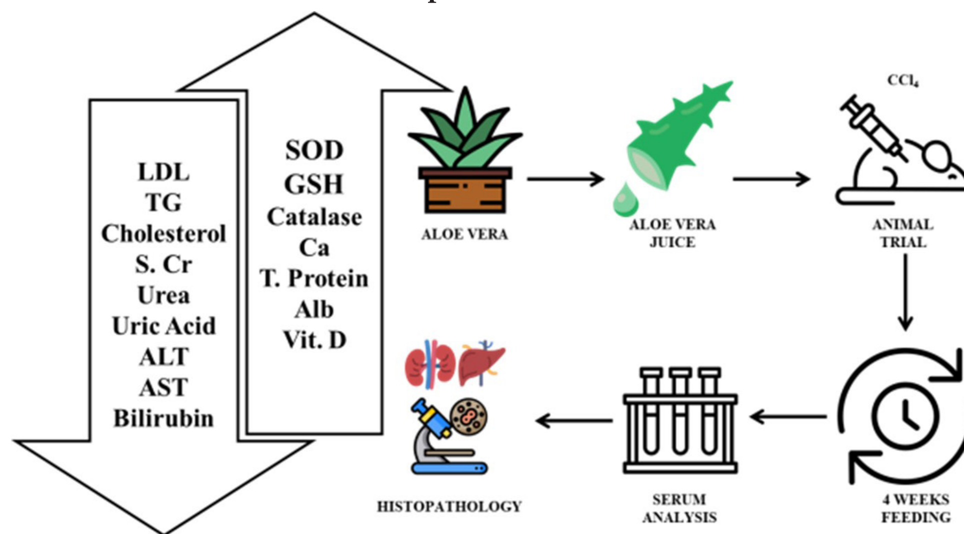
Abstract

The present study was designed to evaluate functional potential of *Aloe vera* juice against (CCl_4 induced) hepatotoxicity using animal model. Biochemical analyses including liver, renal enzymes, bone biomarkers and lipid profile were conducted after 4 weeks of oral *Aloe vera* juice administration. Liver enzymes, AST, ALP and total bilirubin were reduced up to 15-7%, 60-32.52% and 29-48%, respectively in *Aloe vera* juice treated rats i.e., 10 mL/kg and 20 mL/kg doses. Reduction in serum cholesterol (8%), LDL (12%) and triglycerides (15%) were observed. Urea was significantly reduced up to 11% which further reduced up to 30% at higher dose. Increased content of serum proteins (23-50%) and albumin (40-116%) were found at the different doses. Vitamin D was found maximum in G_4 i.e., 38.63 mg/dL as compared to lowest i.e., 5.12 mg/dL in G_2, similarly improvement in calcium i.e., 11-22% was observed in G_3 and G_4. The serum oxidative stress markers indicated raised superoxide dismutase (9%), glutathione (15%), catalase (9%) and reduced lipid peroxidation 78% at higher concentration of *Aloe vera* juice consumption. In addition, histological evaluation also depicted positive impact on hepatic and renal portfolio in all treated rats. All of the findings enlighten the potential of *Aloe vera* juice as a functional beverage.

Keywords: *Aloe* juice; functional beverage; hepatotoxicity; oxidative stress.

Practical Application: This study will help to develop Aloe vera juice based functional drinks for human consumption against hepatotoxicity

Graphical Abstract



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1 Introduction

In living system, liver is a vital organ for homeostasis and multiple physiological functions (Ranju et al., 2010). Liver is the prime detoxifier of exogenous toxins. Toxic secondary xenobiotic metabolites and chronic disorders are the leading cause of liver cirrhosis, fatty liver, portal hypertension, liver encephalopathy and hepatic ascites (Purohit & Cappell, 2015). Moreover, the liver damage may be attributed to excessive alcohol consumption, malnutrition, infections, anemia and drugs (Kim et al., 2016). According to Global Health data exchange, 2.60% mortality occurred globally in both male and female was due to liver diseases. The incidence of liver disease was 22.73% in World and 17.17% in Pakistan for both sexes. Contrary to world, ratio of mortality in Pakistan was 2.94% higher in both genders. In developing nations, liver remedies are uneconomical and hold numerous side effects. So, there is dire need to prevent and cure liver disorders alongside curtailing any sort of adverse impacts of the medicines which interact with hepatocytes (Suleria et al., 2015). Various plant derived compounds i.e., bioactives have been utilized frequently in clinical trials to cure diseases such as cancer, hyperglycemia, atherogenesis and liver disorders. Presently, nutraceuticals and functional foods are getting popularity owing to their unprecedented que of bioactive components. Henceforth, the consumption of above-mentioned food moieties provides prophylactic measures and enhance health status via disease prevention (Ramadan et al., 2021; Karacabey et al., 2021). Conclusively, the bioactives supplementation in dietary regime is an imperative method to deal with such diseases. Modern dietary approaches have diverted the attention of investigators toward certain plant-based products for possible liver remedies (Suleria et al., 2015). To explore the effects of functional foods or nutraceuticals on liver damage, hepatic steatosis carbon tetrachloride (CCl_4) is renowned chemical for induction of hepatic injury in animal models (Vulić et al., 2014).

Consequently, plants are attractive natural substitute and pose little or no adverse effect. *Aloe vera* plant is believed to be very effective in cosmetics and pharmaceutical applications (Gamboa-Gómez et al., 2015). An imperative pharmacological property which was revealed in *Aloe vera* includes antifungal activity, promotion of wound healing, anti-inflammatory, hypoglycemic effects, anticancer, gastro-protective and immunomodulatory property (Zodape & Bhise, 2016). In past, *Aloe vera* plant was known member of family *Liliaceae* but now it is recognized as own family named *Aloaceae.* It was originated from South and East Africa as well as in Mediterranean regions. Now a days, more than 400 species of *Aloe vera* has been grown throughout the World. Its remedial and medical applications extend back to 4th century BC, (Maan et al., 2018; Manvitha & Bidya, 2014) *Aloe vera* is much practiced and utilized medicinal herb in China, India, West Indies and Japan. As they are xerophytes, they are resilient to low water availability (Boudreau & Beland, 2006). There are approximately 75 nutrients and 200 biological active compounds together with sugars (monosaccharides such as mannose-6-phosphate and polysaccharides such as glucomannans), amino acids, enzymes, vitamins (vitamin A, C, E, and B12), minerals (i.e., zinc, copper, selenium, and calcium), saponins, anthraquinones (aloin and emodin), fatty acids (i.e., lupeol and campesterol), hormones (auxins and gibberellins), and even lignin has been derived from various parts of plants (Maan et al., 2018; Sholehvar et al., 2016). Flowers contain volatile compounds and ascorbic acid while leaves are source of organic acids, phenolics, enzymes, vitamins and minerals and rind contains pectin, lignin, cellulose, hemicellulose and polysaccharides (Boudreau & Beland, 2006).

Beverages particularly form the botanical origin are considered more beneficial by the consumers owing to their highlighted health benefits. Now a days, beverage culture is part of every society and is even occasion specific in various instances. Numerous factors are involved in drinking beverages but drinking on belief or for health benefits is pre-historic which is now customized in various forms (Adebo et al., 2021). People have changed their preferences from synthetic to natural products. Currently, nutraceutical industries are demanding to develop innovative products with certain functional properties (Cervantes-Martínez et al., 2014; Minjares-Fuentes et al., 2016).

Due to urbanization and increased costs of living in Pakistan, the common masses need cheap, safe and healthy food alternatives. There is a need of time to develop natural remedies against various life-style related disorders. To the best of our knowledge, this is the first study from Pakistan, in which 100% natural juice was processed from locally grown *Aloe vera barbadensis* and its functional potential was evaluated. Hence, the present study was conducted to assess the functional potential of *Aloe vera* juice against CCl_4 induced hepatotoxicity using animal model. The outcome of study will help to attract the Pakistani consumers for the consumption of locally processed *Aloe vera* juice as potential tool against life-style related disorders.

2 Materials and methods

2.1 Preparation of Aloe vera juice

Aloe vera plants were harvested from the field of Faculty of Agricultural Sciences, University of the Punjab. Leaves were rinsed and then green rind portion from all sides were also removed. After that inner fillet was crushed and homogenized at room temperature (25 °C) to make a slurry. This was filtered by passing through a filtration tank outfitted with vertical column having pore size of 0.1 mm to 0.8 mm. The sieve of the filtration column was enclosed with glass wool bed containing activated charcoal to remove unnecessary matter and pulp. The obtained juice was pasteurized at 60 °C for 30 minutes, cooled at 4 °C and stored in pre-sterilized plastic bottles.

2.2 Experimental design

For bio efficacy trail, 24 male Albino rats (Sprague Dawley) were acquired from the University of Veterinary and animal sciences (UVAS), Lahore. All the rats were reared in well-aerated cages and maintained at 22 ± 3 °C, relative humidity 50 ± 10 with 12 hours day and 12 hours night cycle. Animals were fed on standard laboratory diet which contains flour 82%, casein 4%, vitamins 1% and corn oil 10% during the entire period of trials. After one week of adoption, the rats were categorized into 4 different groups with 6 rats in each group as mentioned below.

2.3 Treatment plan

G_1 **Normal control:** Fed with basal diet only without any treatment

G_2 **Toxic control:** Administered with CCl_4 + Olive oil; 1:1 (2.5 mL/kg body weight) along with basal diet

G_3 **Treatment II:** Fed with *Aloe vera* juice (10 mL/kg/day) orally along with basal diet after administration of CCl_4 + Olive oil; 1:1 (2.5 mL/kg body weight)

G_4 **Treatment II:** Fed with *Aloe vera* juice (20 mL/kg/day) orally along with basal diet after administration of CCl_4 + Olive oil; 1:1 (2.5 mL/kg body weight)

The toxicant was injected intraperitoneally using syringe. After that, rats were euthanized and slaughtered for biochemical assessment of blood. In addition, liver, kidneys and heart were removed for the evaluation of *in vivo* oxidative stress markers such as lipid peroxidation, superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and histopathological examination.

2.4 Liver and kidney indices

Alkaline phosphatase (ALP), aspartate aminotransferase (AST), total bilirubin (TB), direct bilirubin (DB) and indirect bilirubin (IB) of the collected blood samples was scrutinized by the Human kits (Germany) as per directed instructions of the supplier. Fawcett & Scott (1960) methods were applied to determine the serum urea, creatinine and uric acid by using kit (Human Germany).

2.5 Serum lipid and protein level

Serum total cholesterol (TC) and total glycerides (TG) was estimated by the method of Rabey et al. (2019) using Human kit (Germany) while the prescribed method of Gordon et al. (1977) was followed to determine the high-density lipoprotein (HDL) using Human Kit (Germany). Friedewald et al. (1972) equation was applied for low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL). Serum total protein and albumin was also analyzed by the Human kit (Germany) Weissman et al. (1950) method, whereas Klatzkin et al. (2006) procedure was applied in estimating serum albumin using Sigma-Aldrich kit (USA). All the tests were performed according to the supplier instructions.

2.6 Vitamin D, calcium and phosphorous

Serum vitamin D was assessed by ELISA using Holick (2009) method while calcium and phosphorous was measured by the randox kit (Sarkar & Chauhan, 1967; Tietz, 1990).

2.7 Preparation of tissue homogenate

Tissue homogenate 10% (w/v) was prepared from heart, liver and kidneys in 10 mM phosphate buffer (pH 7.4) using a Potter Elvenjem glass homogenizer (Belco Glass Inc., Vineland, NJ, USA). The homogenate was centrifuged (13000 rpm) for 10 minutes at 4 °C. The homogenate was stored in refrigerator until further analysis.

2.8 Lipid peroxidation

Lipid peroxidation of tissue samples and serum in the form of malondialdehyde (MDA) was assessed by the Ohkawa et al. (1979) method in which 200 µL of sample (Serum, Tissue homogenate) and 200 µL of sodium dodecyl sulfate (8.1%) was added in a tube. After that 1.5 mL acetic acid 20% (pH 3.5) and 1.5 mL of 0.8% TBA were mixed. Now the mixture was made up to 4 mL with distilled water and heated for 60 minutes in water bath at 90 °C. The mixture was cooled with running tap water and then 1 mL distilled water and 5 mL n-butanol were added. The mixture was shaken vigorously, centrifuged for 10 minutes at 4000 rpm. The upper butanol layer was removed and absorbance of this layer was measured at 532 nm spectrophotometrically.

2.9 Superoxide dismutase

SOD enzyme was measured by the Kakkar et al. (1984) method. SOD activity was determined by adding 100 µL sample (Serum, Tissue homogenate), sodium phosphate (1.2 mL), phenazine methosulphate (100 µL), nitro blue tetrazolium (300 µL) and NADH (750 µL) in test tube after which mixture was centrifuged. Reaction was initiated after the addition of NADH, after 90 seconds incubation at temperature 30 ^{0}C, the reaction was hindered by the addition of 100 µL glacial acetic acid. Afterwards, the reaction mixture was agitated violently using n-butanol (4 mL) and whole mixture was allowed to stand for 10 minutes where butanol layer was separated using centrifugation. Chromogen intensity was measured in butanol layer at 560 nm using spectrophotometer.

2.10 Catalase activity

CAT was observed by Aebi (1974) method. Briefly, serum or tissue homogenate 100 µL was taken in cuvette and then added 1.9 mL 50mM phosphate buffer (pH 7.0). The reaction was initiated by 1.0 mL addition of 30 mM H_2O_2 and the decomposition of H_2O_2 was observed spectrophotometrically at 340 nm. CAT activity was expressed as U/mg of protein.

2.11 Glutathione

The method of Moron et al. (1979) was used to determine the GSH activity by adding 0.1 mL sample (Serum, Tissue) and 2.4 mL EDTA (0.02M) in glass tube. Mixture was placed in ice cold water bath for 10 minutes. Distilled water 2.0 mL and 0.5 mL TCA (50%) was added and placed in ice cold water bath for 10-15 minutes. Afterwards, the mixture was centrifuged for 10 minutes at 3000-3500 rpm and supernatant was separated in empty tube where 2 mL Tris HCl (0.15 M), 0.05 mL DTNB was added and the mixture was vortexed. After 2-3 minutes absorbance was noted at 412 nm.

2.12 Histopathological examination

Rats were dissected and their organs such as liver and kidneys were preserved in 10% neutral buffered formalin.

For histopathological examination, tissues were processed by sequential unit operation including cleaning, fixation, sectioning and staining. After preparation of tissue blocks, 0.4 μm thickness tissues were made using microtome. Hematoxylin and eosin staining was performed following the instructions described by the Bancroft & Gamble (2008).

2.13 Statistical analysis

The collected data were analyzed statistically using factorial under CRD using Statistix 8.1 software to find out the level of significance. Moreover, mean comparisons were done via Duncan multiple range test.

3 Results

3.1 Liver functioning enzyme

Serum hepatic biomarkers including AST, ALP, TB, DB and IB were determined to explore the effect of juice on rats. The substantial increment in serum hepatic biomarkers i.e., AST, ALP, TB, and IB were found in hepatotoxic groups, as shown in Table 1, which indicated the liver damage. The results exhibited the decrease of liver enzyme in serum of juice feeding groups G_3 and G_4. The liver enzymes including AST (reduced to 15.7-60%) by ingestion of 10-20 mL/kg *Aloe vera* juice compared to the CCl_4 toxic group. ALP was reduced up to 32-52% via supplementing basal diet with 10-20 mL/kg *Aloe vera* juice per day. Same trend was observed in the total bilirubin and indirect bilirubin concentration after the administration of *Aloe vera* juice via oral route. On the other hand, inverse relation was observed for direct bilirubin as its concentration was increased up to 50-70% by the rats with 10 to 20 mL/kg *Aloe vera* juice, respectively. Statistically, significant variation was observed among different groups including normal and hepatotoxicity induced group. Surprisingly, inhibitory relation was revealed in juice feeding groups as increasing the juice dose ameliorated the hepatotoxicity in rats which endorsed the nutraceutical potential of juice.

3.2 Serum lipid profile

The serum lipid profile affected by the liver induced toxicity is depicted in the Table 2. Parameters of lipid profile including LDL, HDL, cholesterol and triglycerides were significantly improved via administration of multiple doses of *Aloe vera* juice. The decreased concentration of total cholesterol, triglycerides, LDL and VLDL was observed in *Aloe vera* treated groups (G_3, G_4) and control group (G_1) as compared to toxic group (G_2) rats. The cholesterol level in the study was reported as 169.2 ± 6.72, 182.66 ± 6.28, 168.17 ± 9.41 and 168.67 ± 6.15 (mg/dL) in normal control, toxic control, *Aloe vera* juice group (10 mL/kg/day) and *Aloe vera* juice group (20 mL/kg/day), respectively. Average reduction of 8% was reported in cholesterol among the groups supplemented with *Aloe vera* juice compared to the toxic group. Similar detrimental trend was observed for triglycerides with 15% reduction and VLDL with 16-18% reduction levels in *Aloe vera* treated groups at various concentrations. HDL was significantly improved up to 15% in *Aloe vera* juice groups compared to CCl_4 toxic (G_2) group. Moreover, LDL was reduced from 6-12% via administration of 20 mL/kg and 10 mL/kg *Aloe vera* juice, respectively. It was found that lower dose of *Aloe vera* (10 mL/kg) was more efficient in reducing the LDL compared to all other groups except normal control in comparison with the toxic control. The administration of *Aloe vera* juice significantly attenuated hepatotoxicity induced serum lipid alteration. Statistical analysis revealed notable variation ($p > 0.05$) among groups.

3.3 Kidney functioning tests

Serum urea, creatinine and uric acid were also scrutinized as renal functioning biomarkers markedly increased in toxic group

Table 1. Liver function tests of *Aloe vera* juice treated rats induced with CCl_4 based liver toxicity.

Treatments	Aspartate Aminotransferase IU/L	Alkaline Phosphatase IU/	Total Bilirubin mg/dL	Direct Bilirubin mg/dL	Indirect Bilirubin mg/dL
G_1	20.4 ± 4.04 ^{d}	277 ± 6.12 ^{d}	0.84 ± 0.11 ^{c}	0.23 ± 0.02 ^{a}	0.61 ± 0.1 ^{c}
G_2	732.5 ± 86.05 ^{a}	774.5 ± 114.61 ^{a}	1.98 ± 0.3 ^{a}	0.14 ± 0.05 ^{b}	1.84 ± 0.31 ^{a}
G_3	616.83 ± 58.13 ^{b}	529.33 ± 24.06 ^{b}	1.4 ± 0.21 ^{b}	0.21 ± 0.01 ^{a}	1.19 ± 0.21 ^{b}
G_4	291.5 ± 51.37 ^{c}	371 ± 37.48 ^{c}	1.02 ± 0.12 ^{c}	0.24 ± 0.02 ^{a}	0.77 ± 0.1 ^{c}
***p*-Value**	0.000	0.000	0.000	0.000	0.000

G_1: Normal control; G_2: Toxic control; G_3: *Aloe vera* juice 10 mL/kg/day; G_4: *Aloe vera* juice 20 mL/kg/day. Data are mean ± SD, one-way ANOVA (level of significance; 5%). Heterogeneous superscripts in column are representing significantly different groups.

Table 2. Lipid profile of *Aloe vera* juice treated rats induced with CCl_4 based liver toxicity.

Treatments	Cholesterol mg/dL	HDL mg/dL	LDL mg/dL	Triglycerides mg/dL	VLDL mg/dL
G_1	169.2 ± 6.72 b	42.3 ± 1.68 a	76.84 ± 4.29 b	237 ± 10.65 a	47.4 ± 2.13 a
G_2	182.66 ± 6.28 a	36.71 ± 3.21 b	84.83 ± 4.44 a	245.5 ± 10.89 a	50.97 ± 3.73 a
G_3	168.17 ± 9.41 b	42.04 ± 2.35 a	74.29 ± 7.17 b	209.17 ± 20.07 b	41.83 ± 4.01 b
G_4	168.67 ± 6.15 b	42.17 ± 1.54 a	79.2 ± 5.27 ab	210.33 ± 6.65 b	42.75 ± 2.48 b
***p*-Value**	0.007	0.001	0.023	0.000	0.000

G_1: Normal control; G_2: Toxic control; G_3: *Aloe vera* juice 10 mL/kg/day; G_4: *Aloe vera* juice 20 mL/kg/day. Data are mean ± SD, one-way ANOVA (level of significance; 5%). Heterogeneous superscripts in column are representing significantly different groups.

Table 3. Kidney functioning tests of *Aloe vera* juice treated rats induced with CCl_4 based liver toxicity.

Treatments	Urea (mg/dL)	Creatinine (mg/dL)	Uric Acid (mg/dL)	Total Protein (g/dL)	Albumin (g/dL)
G_1	25.6 ± 2.97 [d]	0.72 ± 0.19 [d]	4.66 ± 1.54 [b]	8.02 ± 0.26 [a]	5.26 ± 0.23 [a]
G_2	73 ± 5.48 [a]	2.58 ± 0.29 [a]	10.32 ± 0.64 [a]	4.3 ± 0.47 [d]	1.87 ± 0.33 [d]
G_3	64.67 ± 7.17 [b]	2.03 ± 0.27 [b]	5.92 ± 1.91 [b]	5.27 ± 0.4 [c]	2.63 ± 0.25 [c]
G_4	51.5 ± 3.62 [c]	1.18 ± 0.28 [c]	5.95 ± 0.6 [b]	6.45 ± 0.54 [b]	4.03 ± 0.62 [b]
***p*-Value**	0.000	0.000	0.000	0.000	0.000

G_1: Normal control; G_2: Toxic control; G_3: *Aloe vera* juice 10 mL/kg/day; G_4: *Aloe vera* juice 20 mL/kg/day. Data are Mman ± SD, one-way ANOVA (level of significance; 5%). Heterogeneous superscripts in column are representing significantly different groups.

(G_2) (73, 2.58, 10.32 mg/dL) in comparison to normal group (G_1) rats (25.6, 0.72, 4.66 mg/dL). The most efficient group in reducing the urea and creatinine was *Aloe vera* juice 20 mL/kg group (G_4) compared to toxic control group (G_2). Serum urea was reduced up to 11% and 30% in *Aloe vera* group with doses of 10 mL/kg and 20 mL/kg, respectively while serum creatinine was reduced up to 21% and 54% in *Aloe vera* group with doses of 10 mL/kg and 20 mL/kg, respectively. Uric acid was declined to 42-43% in *Aloe vera* juice treated groups. Conclusively, treatment with juice for the period of 4 weeks distinctly decreased the elevated serum level of urea, creatinine and uric acid as represented in the Table 3. On the other hand, total protein and albumin levels were significantly improved in *Aloe vera* juice administered group. Total protein was increased up to 23-50% in *Aloe vera* juice treated groups and albumin was increased up to 40% and 116% in *Aloe vera* juice at 10 mL/kg and 20 mL/kg doses, respectively. Kidney biomarkers were found significantly varied ($p < 0.05$) among the different groups.

3.4 Bone health biomarker

Serum vitamin D and calcium were significantly improved after administration of *Aloe vera* juice for 4 weeks compared to toxic control group. It was found maximum in G_4 (38.63 IU/L) followed by G_1 (35.2 IU/L) and G_3 (24.02 IU/L). Serum vitamin D was increased up to 369% and 655% in groups fed with 10 mL/kg and 20 mL/kg *Aloe vera* juice, respectively along with basal diet in CCl_4 treated rats. Hence, the administration of *Aloe vera* juice normalized the serum vitamin D level. Vitamin D also plays a prime role in bone health and calcium homeostasis. Calcium was improved up to 11% and 22% in 10 and 20 mL/kg *Aloe vera* juice supplementation. Serum calcium level was minimum in G_2 (i.e., 7.48 mg/dL) and maximum in G_1 (i.e., 10.42 mg/dL). The G_3 and G_4 showed improvement in calcium level as mentioned in the Table 4. Contrary to serum vitamin D and calcium, serum phosphate levels were decreased up to 23% and 33% after ingestion of *Aloe vera* juice 10 mL/kg and 20 mL/kg day, respectively. The highest level of phosphorus was present in G_2 and lowest in G_1 and G_4 as the serum phosphorus was found antagonistic to calcium. The results showed improvement in biochemical markers due to *Aloe vera* juice which ameliorated the hepatotoxicity induced by CCl_4. Statistically, significant ($p < 0.05$) variation was found among the different groups.

Table 4. Bone health parameters of *Aloe vera* juice treated rats induced with CCl_4 based liver toxicity.

Treatments	Vitamin D IU/L	Calcium mg/Dl	Phosphorus mg/dL
G_1	35.2 ± 3.27 [a]	10.42 ± 0.66 [a]	4.2 ± 0.77 [c]
G_2	5.12 ± 1.88 [c]	7.48 ± 0.6 [d]	7.18 ± 0.48 [a]
G_3	24.02 ± 3.35 [b]	8.32 ± 0.31 [c]	5.55 ± 0.61 [b]
G_4	38.63 ± 4.09 [a]	9.12 ± 0.51 [b]	4.78 ± 0.21 [c]
***p*-Value**	0.000	0.000	0.000

G_1: Normal control; G_2: Toxic control; G_3: *Aloe vera* juice 10 mL/kg/day; G_4: *Aloe vera* juice 20 mL/kg/day. Data are mean ± SD, one-way ANOVA (level of significance; 5%). Heterogeneous superscripts in column are representing significantly different groups.

3.5 Lipid peroxidation

Lipid peroxidation (MDA, nmol/mL) of rat's blood, liver kidney and heart tissues are presented in the Table 5. Lipid peroxidation level in serum was observed as 11.16 ± 1.87, 28.58 ± 6.84, 6.61 ± 1.48 and 6.06 ± 0.44 (nmol/mL) in normal control, toxic control, *Aloe vera* juice group (10 mL/kg/day) and *Aloe vera* juice group (20 mL/kg/day), respectively with maximum reduction found in *Aloe vera* juice (10 mL/kg) compared to toxic control group (G_2). The mean value of organs also followed the same pattern of lipid peroxidation as the blood. The lipid peroxidation was found maximum in G_2 (liver: 27.7 nmol/mL, kidney: 13.7 nmol/mL) as compared to G_3 (liver: 23.48 nmol/mL, kidney: 8.42 nmol/mL) and G_4 (Liver: 9.7 nmol/mL, kidney: 11.7 nmol/mL). The provision of *Aloe vera* juice to the rats mitigates the oxidative stress in blood as well as organs. The results confirmed the prophylactic role of *Aloe vera* juice in decreasing lipid peroxidation. The blood, liver and heart tissues showed positive correlation with juice dose as the increase in *Aloe vera* juice dose decrease the stress in rats.

3.6 Superoxide dismutase

The mean value of SOD is also shown in the Table 5 below. The results indicated that intake of *Aloe vera* juice increased SOD of G_3 (1.99 µg/mL) and G_4 (1.94 µg/mL) in comparison to G_2 (1.77µg/mL) rat's blood. Tissue of Liver, heart and kidney also exhibited the same trend as the blood. Hence, the administration of CCl_4 affected the considerable physiological function of rat's tissue. It is noteworthy that the pivotal reduction in SOD level of blood and organ tissues in G_2. The treatment of rats with *Aloe vera* juice consequentially increased SOD of G_3 and G_4. Rats indicated the positive behavioral response of *Aloe vera* juice to support antioxidative system of their body. The results endorsed the potential nutraceutical function of *Aloe vera* juice against CCl_4 induced toxicity.

3.7 Glutathione estimation

Liver is the main site of GSH synthesis in the organism and transfers it to the other body organs through plasma. Serum

GSH level was reduced after the injection of CCl_4 in rats which showed the *in vivo* oxidative stress as the minimum level of GSH in G_2 group as compared to all other groups, as represented in Table 5. Serum GSH level improved 11% and 15% in 10 and 20 mL/kg in *Aloe vera* juice supplementation. The G_4 rat's serum contains 6.35 µg/mL GSH which clearly displayed the effect of *Aloe vera* juice. The liver tissue also exhibited 5% increase in G_3 and 6% increase in G_4 as compared to G_2. Similarly, tissues of liver and kidney showed the increased level of GSH in G_3 and G_4 as compared to G_2. So, the improvement in hepatic tissue causes to increase the level of GSH in juice feeding groups. Statistically, significant variation ($p > 0.05$) was found for blood, liver and heart but non-significant variation for kidney tissues among different groups.

3.8 Catalase estimation

The hepatotoxicity induced rats showed reduced CAT level in G_2 rats, described in Table 5, illustrating the damaging effect of CCl_4. The serum, liver, heart and kidney tissue exhibited 114.16, 39.92, 102.91 and 105.44 U/mL CAT, respectively in G_2. The minimum level was found in G_2 liver tissues as compared to all groups indicating the highest liver damage by the use of toxicant. The maximum level of CAT activity occurred in blood, liver, heart and kidney tissue of G_4 i.e., 124.76 106.73,125.84 and 113.75 U/mL, respectively. Highest CAT content was seen as 9% improvement in serum, 167% in liver of G_4 compared to toxic group (G_2) in this trial. However, the *Aloe vera* juice feeding mitigates the toxicant severity as indicated by the comparative higher CAT activity of G_4 rats. The enhanced enzymatic activity showed the remedial effect of juice against hepatic stress. Statistical analysis for data of CAT also revealed significant variations ($p < 0.05$) among differently treated groups for rat's serum, liver and heart tissues while non-significant variations for kidney tissues.

3.9 Histopathology

Histopathological examination of liver in hepatotoxic rat model performed in the present study revealed significant effects of *Aloe vera* juice ingestion, as shown in Figure 1. Liver histopathology of G_1 (normal control; without any disease, fed with basal diet) displayed normal and intact hepatocytes without any infiltration of fatty vacuoles and signs of inflammation. Contrary to G_1, the liver histopathology of G_2 revealed signs of moderate chronic inflammation, lobular necrosis of mild nature, hepatocellular apoptosis along with edema and hemorrhage. On the other hand, liver histopathology G_3 and G_4, reported mild chronic inflammation on portal area without any sign of hemorrhage and with lesser signs of hepatocellular apoptosis as well as edema. In addition, histopathology of G_1 kidney, illustrated in Figure 2, revealed the glomeruli normal in size, intact renal tube with no signs of hemorrhage or inflammation while in G_2 group kidney glomeruli decrease in size and number having moderate inflammation. Signs of Renal tubular necrosis and hemorrhage were also observed with mild edema. Inversely, treated groups (G_3, G_4) kidney showed mild inflammation

Table 5. *In vivo* oxidative stress indices of *Aloe vera* juice treated rats induced with CCl_4 based liver toxicity.

		Serum		
Treatments	Malondialdehyde nmol/mL	Superoxide Dismutase µg/mL	Glutathione µg/mL	Catalase U/mL
G_1	11.16 ± 1.87^{b}	2.91 ± 0.81^{a}	6.19 ± 0.32^{a}	119.26 ± 2.32^{b}
G_2	28.58 ± 6.84^{a}	1.77 ± 0.25^{b}	5.50 ± 0.20^{b}	114.16 ± 6.11^{b}
G_3	6.61 ± 1.48^{bc}	1.99 ± 0.25^{b}	6.11 ± 0.15^{a}	117.73 ± 1.71^{b}
G_4	6.06 ± 0.44^{c}	1.94 ± 0.21^{b}	6.35 ± 0.2^{a}	124.76 ± 5.19^{a}
***p*-Value**	0.00	0.002	0.00	0.004
		Liver		
G_1	12.09 ± 0.39^{c}	1.85 ± 0.26^{ab}	6.22 ± 0.68^{a}	101.41 ± 16.49^{a}
G_2	$27.7^{a} \pm 0.53^{a}$	1.57 ± 0.29^{b}	4.54 ± 0.06^{b}	39.92 ± 0.04^{b}
G_3	23.48 ± 1.41^{b}	2.08 ± 0.19^{a}	4.76 ± 0.06^{b}	48.08 ± 0.07^{b}
G_4	9.7 ± 0.38^{d}	2.11 ± 0.12^{a}	4.83 ± 0.01^{b}	106.73 ± 8.14^{a}
***p*-Value**	0.00	0.064	0.001	0.00
		Heart		
G_1	12.03 ± 0.77^{ab}	1.68 ± 0.12^{a}	5.49 ± 0.68^{a}	108.38 ± 4.78^{b}
G_2	11.15 ± 0.46^{b}	1.72 ± 0.22^{a}	4.44 ± 0.35^{b}	102.91 ± 2.47^{b}
G_3	12.24 ± 0.45^{a}	1.97 ± 0.61^{a}	4.45 ± 0.14^{b}	109.15 ± 1.31^{b}
G_4	6.55 ± 0.19^{c}	2.04 ± 0.14^{a}	4.41 ± 0.08^{b}	125.84 ± 4.58^{a}
***p*-Value**	0.00	0.511	0.024	0.00
		Kidney		
G_1	12.55 ± 0.79^{b}	1.74 ± 0.1^{c}	5.67 ± 0.44^{a}	107.87 ± 6.27^{a}
G_2	13.7 ± 0.1^{a}	2.14 ± 0.29^{b}	4.69 ± 0.37^{a}	105.44 ± 14.12^{a}
G_3	8.42 ± 0.89^{c}	2.69 ± 0.21^{a}	4.99 ± 0.8^{a}	113.69 ± 0.87^{a}
G_4	11.7 ± 0.19^{b}	2.13 ± 0.1^{b}	5.06 ± 0.06^{a}	113.75 ± 1.75^{a}
***p*-Value**	0.00	0.002	0.178	0.526

G_1: Normal control; G_2: Toxic control; G_3: *Aloe vera* juice 10 mL/kg/day; G_4: *Aloe vera* juice 20 mL/kg/day. Data are mean ± SD, one-way ANOVA (level of significance; 5%). Heterogeneous superscripts in column are representing significantly different groups.

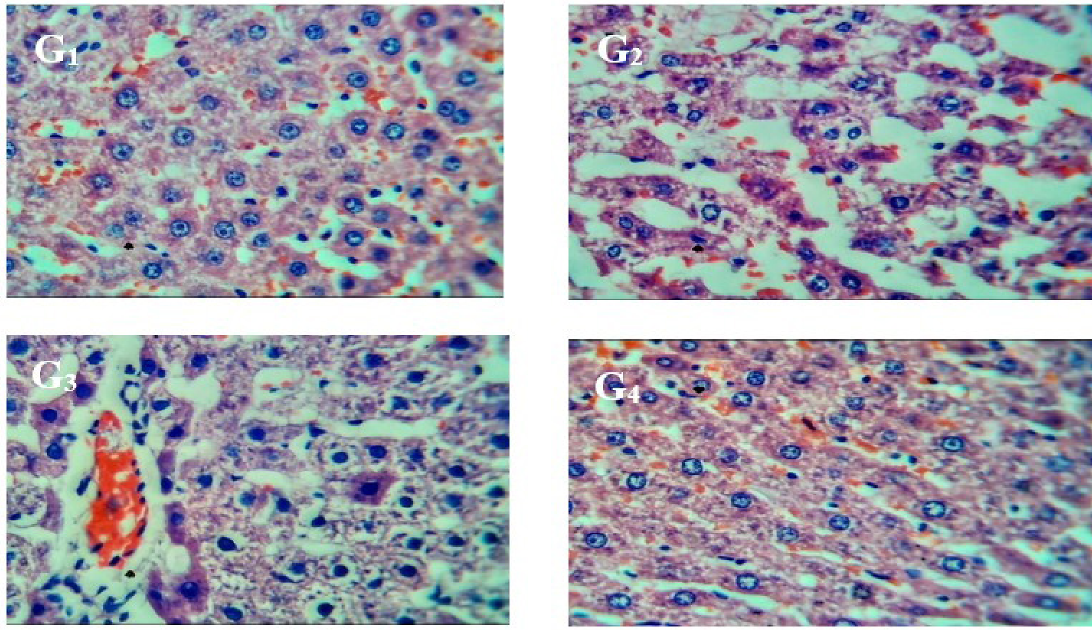

Figure 1. Liver histopathology of rats fed with *Aloe vera* juice at 100 X after staining with Hematoxylin and eosin. G_1: Normal control; G_2: Hepatotoxic control; G_3: *Aloe vera* treated hepatotoxic groups (10 mL/kg); G_4: *Aloe vera* treated hepatotoxic groups (20 mL/kg).

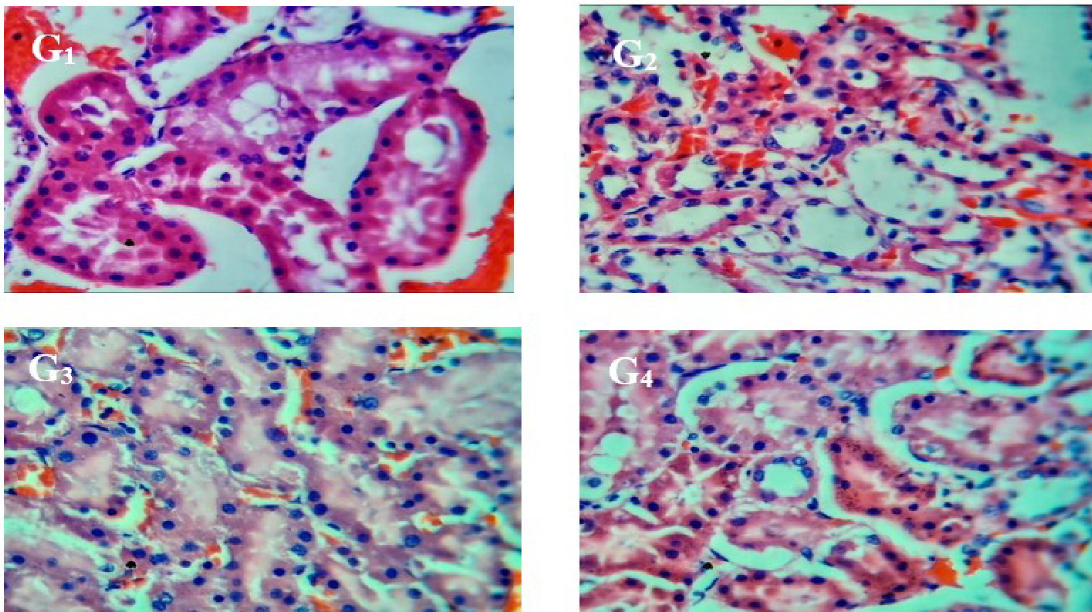

Figure 2. Renal cells histopathology of rats fed with *Aloe vera* juice at 100 X after staining with Hematoxylin and eosin. G_1: Normal control; G_2: Hepatotoxic control; G_3: *Aloe vera* treated hepatotoxic groups (10 mL/kg); G_4: *Aloe vera* treated hepatotoxic groups (20 mL/kg).

with decrease in size and number of glomeruli. Renal tubular necrosis and hemorrhage were disappeared. Histopathological examination also validates the biochemical examination.

4 Discussion

The present investigation reports the functional potential of *Aloe vera* juice against liver induced injury as the dietary bio-actives are the excellent tool to combat metabolic malfunctioning. The effectiveness of the juice clearly exhibited that our processing does not affect the health beneficial components of *Aloe vera*. Most of the previous studies was done on *Aloe vera* based extract and little data reported the effect of *Aloe vera* based juice. ALP, AST and bilirubin are responsive biomarker implicated directly in liver tissue damage and toxicity (Akinloye et al., 2021). Our findings are supported by the Al-Shinnawy et al. (2014) who revealed

hepatotoxicity induced rabbits showed significantly high liver indices e.g., ALT, AST, ALP and administration of *Aloe vera* juice found to be effective in reducing liver enzymes in toxicity induced groups. In another study *Aloe vera* polysaccharides administration in aflatoxins B1 induced hepatotoxicity (Albino Rats) the serum ALT and AST reduced significantly near to normal rats group value (Cui et al., 2017).

Moreover, the administration of extract (*Aloe vera*) for 30 days in anti-tuberculosis drugs induced hepatotoxicity cause to restoration of liver function enzyme. The results also support the *Aloe vera* juice could be used by the chronic disease patience with multiple drugs to secure their liver function as dietary bio actives are excellent tool to cop up the chronic metabolic malfunction (Zodape, 2011). The results of this investigation showed that *Aloe vera* juice administration significantly reduced the serum ALT, AST, TC and TG which is in line with the finding of Cui et al. (2014). In addition, the *Aloe vera* polysaccharides administration to the rats increases the GSH and SOD activity prominently and decreases MDA activity as compared to alcohol induce toxicity group which is also in line with our results. In another study, similar results were reported that the administration of *Aloe vera* in alcohol induced hepatotoxicity group decrease lipid peroxidation and increase GSH and SOD (Wu et al., 2006). Likewise, lipid peroxidase activity was raised to 39.50 ± 2.1 which was restored back to 20.43 ± 3.2 (nmoles/mg) after consuming *Aloe vera* juice in ethanol induced ulcerative rats. Another oxidative stress marker GSH was identified declining in ulcer induced animals i.e.,52.56 ± 3.4 U/mL. When *Aloe vera* juice was administered the activity of this enzyme was raised (83.15 ± 4.2 U/mL) while the activity of SOD was significantly decreased which was possibly restored back by the *Aloe vera* juice (62.70 ± 4.3 U/mL) (Gopinathan & Rameela, 2014). Badgujar et al. (2020) evaluated that unprocessed *Aloe vera* provide substantial effect on CAT activity by protecting the cell from oxidative damage.

Present findings of *Aloe vera* juice on lipid profile is reinforced by Atik et al. (2020) by exposing rats to cigarette smoke which stimulate the release of catecholamine, leads to elevated level of VLDL and LDL concentration. Smoking exerts its damaging effects on HDL functioning, biosynthesis and maturation. Moreover, in another study conducted by the Deora et al. (2021) in which *Aloe vera* treated group exhibit significant reduction ($p < 0.05$) in triglycerides and VLDL. These investigations endorsed the *Aloe vera* ability to lower blood lipid profile which could be excellent tool for chronic heart diseases.

Hepato/nephro-cellular dysfunction was recently reported by the Akinloye et al. (2021) in which 250 mg/kg body weight of *Aloe vera* gel significantly lowered the serum urea, uric acid and creatinine as compared to alcohol induced toxic group. Serum albumin results were also in line with our study. While in another study, serum urea and creatinine were significantly attenuated in *Aloe vera* 100 mg/kg body weight treated group. Moreover, 200 mg/kg dose of *Aloe vera* protect the renal tubular damage as revealed by the histological results (Chatterjee et al., 2012). In this regard, current investigation reported the functional role of *Aloe vera* juice on kidney enzymes in preserving higher tissue enzyme levels in blood of treated animals than the animals without *Aloe vera* juice ingestion which endorsed the above studies.

In addition, renal toxicity was determined in human by the administration of *Aloe vera* juice to forty diabetic patients. Their renal biochemical markers revealed no adverse effects on blood urea, serum creatinine and uric acid (Yongchaiyudha et al., 1996). In combining all previous investigations, it could be concluded that the attenuation of liver oxidative stress by *Aloe vera* juice due to its ability to scavenge the free radicals. Moreover, the restorative activity of *Aloe vera* could be due to bioactive substances. These bioactives are responsible for therapeutic effects of *Aloe vera* juice and could account for its modulatory ability to ameliorate hepatotoxicity (Akinloye et al., 2021).

5 Conclusion

The current research implies the functional potential of locally grown *Aloe vera* in hepatotoxicity induced rats. The provision of *Aloe vera* juice significantly improved the oxidative stress and reversed liver damage secondary to hepatotoxicity via lipid peroxidase reduction and increase production of SOD and GSH only after 4 weeks of administration. The devised functional beverage could be useful for reducing multiple clinical ailments related to liver disease or hepatotoxicity. However, further investigations should be conducted including biomarkers of gene expressions and metabolomics in order to further validate the effects of *Aloe vera* juice in prophylaxis and prevention of liver damage.

Ethical approval

The experimental procedures were approved by the ethical committee.

Conflict of interest

The authors declare no conflict of interest regarding this manuscript.

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