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Safety Evaluation of *Ziziphus Mauritiana* Extract for its Utilization in Bread From Cholistan Desert, Pakistan

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Abstract: Considering health risk potential of various chemical additives in food, a study was conducted to evaluate the safety assessment potential of *Z. mauritiana* extract to be used in the bread manufacturing process. Mature and fresh leaves were subjected to drying at 60^{o}C for ethanol extraction. Various dosages of extract were provided to experimental rats for safety assessment. Blood samples were evaluated based on different parameters; liver working tests, kidney function tests, lipid profile, cardiac enzymes and serum proteins. Results explicated the non-remarkable influence of concentration on different characteristics of blood. ALT concentrations express the non-momentous and variable influence of concentrate. The plant is recommended for sustainable utilization and consumption in the food and pharmaceutical industry.
Keywords: *Ziziphus mauritiana*, Safety Evaluation, Liver, Kidney functions, Food

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1. Introduction

Ziziphus mauritiana is a small tree or shrub that grows in drought conditions (Awasthi and More, 2009). Its plants are dispersed in sub-tropical and warm areas of the world. It is an indigenous plant of the Indo-Pak subcontinent and China (Bhargava *et al*., 2005). It is also grown in Australia, Russia, Southern Europe, the Middle East, the USA and Asian countries like Pakistan, India, China, Thailand, Vietnam, and Korea (Reich, 1991; William, 2002). It is an extensively growing plant and is known by different local names (Upadhyay *et al*., 2012).

In Pakistan, *Z. mauritiana* producing areas are Bahawalpur, Multan, Faisalabad, Sargodha, Jhang (Punjab province), Hyderabad, Tandojam, Nawabshah, Mirpurkhas, Gadop and Malir Area of Karachi (Sindh). The average production of fruit per tree is 80-200 kg depending upon climatic conditions (Mukhtar *et al*., 2004). The desert of Cholistan is spread over an area of 26,000 km^2. Its location is Southern Punjab, Pakistan (Rao *et al*., 1989; Arshad *et al*., 2007). The climate of Cholistan is sub-tropical and is suitable for the growth of *Z. mauritiana*. The local people use *Z. mauritiana* to cure various diseases (Arshad *et al*., 2006;2007). The usage of medicinal herbs for treating ailments is as old as mankind. These plants have gotten more attention from research centers because of their importance for the safety of humanity (Najafi *et al*., 2010). The medicinal benefits of this vegetation are because of several bioactive compounds (Karthikeyan *et al*., 2003).

The major compounds present in *Z. mauritiana* are lipids, carbohydrates, alkaloids, saponins, triterpenic acid and glycosides. The leaves contain various compounds like triterpenic acid, volatile oils, glycosides, saponins and flavonoids The phenolic compounds have therapeutic effects such as antithrombotic, antibacterial, anti-allergic, antiviral, antimutagenic and antineoplastic. These effects are due to antioxidant substances and free radical scavenging potentials of various polyphenolic compounds present in it (Forster *et al*., 2003; Lee *et al*., 2004).

The concentrates of leaves, seeds and fruit of *Z. mauritiana* show properties to slow down the process of oxidation (Ndhala *et al*., 2006; Bhatia and Mishra, 2009). The bark and pulp extracts show cytotoxicity against cancer cells (Ramadoss *et al*., 2000; Vahedi, 2008). The fruits of *Z. mauritiana* are used to cure various ailments such as healing of wounds, pulmonary disorders and fever. These are used as active ingredients of Joshanda formulation, which are effective against chest problems. These also act as blood purifiers

and digestants. The dried fruits have laxative effects (Adzu *et al*., 2003; Goyal *et al*., 2012).

The alcoholic concentrate of *Z. mauritiana* roots inhibits diarrhea (Adzu *et al.,* 2003).. The powder of roots is applied to wounds for quick healing (Goyal *et al*., 2012). Pectin present in *Z. mauritiana* has bile acid binding, cholesterol-lowering and antidiarrheal properties (Xiao *et al*., 2013). Betulinic acid present in *Z. mauritiana* is a pentacyclic triterpene and lipophilic compound that has cytotoxic effects against cancer cell (Zhang *et al*., 2009).It also inhibits enzyme aminopeptidase-N, which is involved in the regulation of angiogenesis (Kwon *et al*., 2002).

Goyal *et al*. (2012) reported that seeds have calming and sedative effects. They have the potential to stop vomiting and nausea. They also provide relief against abdominal pain during pregnancy. According to Jarald *et al*. (2009), various extracts of *Z. mauritiana* fruits have anti-hyperglycemic potential. They also lower serum cholesterol, glucose, creatinine, triglyceride, urea, and glycosylated hemoglobin and improve HDL levels. The methanolic extract of *Z. mouritiana* has antioxidant and anti-diarrheal activities. The fruits have a hepato-protective effect (Dahiru *et al.,* 2006; Ndhlala *et al*., 2008). The seeds contain a volatile oil that promotes hair growth (Yoon *et al*., 2010).

Concentrate of *Z. mauritiana* inhibits the growth of liver tumor cells, malignant tumor cells and fibroblastic cells (Fu *et al*., 2005: Kumar *et al*., 2010). The methanolic extract shows antimicrobial effects against various bacterial and fungal strains. It also has antagonistic effects against Gram-negative and Gram-positive bacteria (Kayser and Arndt, 2000). Betulinc acid is an active compound isolated from *Z. mouritiana.* It inhibits the urease activity of *Helicobacter pylori* (Shin *et al*., 2009). Rutin minimizes resistance in airways (Jung *et al*., 2007). Rutin prevents osteopenia by increasing the osteoplastic activity of bones. It can also be known as an osteoblast stimulant (Molteni *et al*., 2000). People are nowadays more conscious of the health risks of chemical additives in foods. Therefore, Considering the health risk potential of various chemical additives in food, a study was conducted to evaluate the safety assessment potential of *Z. mauritiana* extract to be used in the bread manufacturing process.

2. Materials and Methods

Raw material (foliage of *Ziziphus mauritiana*) was collected from Cholistan. The necessary chemical substances were purchased from the supplier. After the

collection of leaves, washing and cleaning were carried out to remove dust particles. The moisture from the leaves was removed in the sun and then oven (hot air oven) dried at 60^{o}C for sixty minutes. Later, they were crushed finely and sieved to remove coarse particles. The ground sample was put in a soxhlet extraction apparatus in the presence of ethanol. After the evaporation of ethanol, the ethanolic extract was recovered and stored at 4^{o}C till further analysis.

2.1. Safety Assessment of concentrate

Before the application of antioxidant concentrate in food products, its safety was evaluated using Sprague Dawley rats. Various treatments like T_1=0.5, T_2=1.0, T_3=1.5 and T_4=2 g/kg body weight/day of plant extract together with T_0 (control group) were administered through oral cavity to different sets of study animals for two months. The blood specimens were collected and different parameters like bilirubin, AST, ALT (liver working tests), urea, creatinine (kidney function tests), cholesterol, triglycerides, HDL, LDL (lipid profile), LDH, CPK, CKMB (cardiac enzymes), total protein and albumin (serum proteins) were determined spectrophotometrically by means of kits (Hussain *et al*., 2012; Ahmad *et al*., 2013).

3. Results and discussion

3.1. *Ziziphus mauritiana* concentrate

Plant concentrate was gained using the soxhlet apparatus. Ethanol was used as a solvent. After the vaporization of ethanol, 1.30±0.10 % plant extract was obtained. Further assessment showed total polyphenols 25.92±1.51 mg(GAE)/g, antioxidants 6.30±0.32 g/ml and DPPH assay (free radicals scavenging activity)52.75±5.49 mg/ml.

3.2. Safety assessment of *Z.mauritiana* extract

The safety of concentrate was assessed, employing different treatments such as T_1=0.5, T_2=1.0, T_3=1.5 and T_4=2 g/kg.bw/day of plant concentrate to different sets of experimental animals (Sprague Dawley rats) for 2 months along with T_0 (control group). The rats were grouped in five sets with four rats in each set. Throughout the study period, no animal showed any symptoms of health complications. Later, the samples of blood were taken from experimental animals and studied concerning liver and kidney function tests, cardiac enzymes, lipid profile and serum proteins.

3.3. Liver function tests

Mean values (Table 01) explain the momentous influence of various treatments of concentrate on bilirubin, AST and ALT levels of blood in experimental rats. Data concerning bilirubin concentration shows

Fig. 01 (*Z. mauritiana*) A. Whole plant B.Fruit C. Flowers and leaves D. Seeds

non-momentous and arbitrary influence of different treatments of concentrate. The lot T_2 of rats exhibited maximum concentration (0.49±0.02[a] mg/dL), whereas the concentrations of 0.48±0.02[a], 0.47±0.02[a] and 0.46±0.02[a] mg/dL were shown by groups T_1, T_0 and T_4 correspondingly. While the lot T_3 of study animals revealed the lowest (0.44±0.02[a] mg/dL) value. In the case of AST, the group T_3 of rats showed the maximum (45.63±1.88[a] U/L) value, whereas the values of 44.50±1.34[a], 43.50±1.31[a] and 43.20±1.93[a] U/L were expressed by the sets T_0, T_4 and T_2 of study animals correspondingly. Nevertheless, the minimum concentration of 42.34±0.83[a] U/L was shown by group T_1.

The results concerning ALT concentrations express the non-momentous and variable influence of concentrate. The plot T_1 of rats exhibited the minimum concentration (50.14±2.83[a] U/L); whereas, the mean concentrations of 51.12±1.90[a], 52.79±2.76[a] and 53.01±2.22[a] were gained by the groups T_0, T_4 and T_2 accordingly. Whereas, the group T_3 of rats expressed the maximum concentration as 54.41±1.95[a] U/L. From the above results, it is obvious that concentrate had no detrimental influence on liver function parameters. These findings are in line with (Kumar *et al.,* 2022).

Table 01. Effect of concentrate on liver function tests in experimental rats

Treatments	**Bilirubin** (mg/dL)	**AST** (U/L)	**ALT** (U/L)
T_0	0.47±0.02[a]	44.50±1.34[a]	51.12±1.90[a]
T_1	0.48±0.02[a]	42.34±0.83[a]	50.14±2.83[a]
T_2	0.49±0.02[a]	43.20±1.93[a]	53.01±2.22[a]
T_3	0.44±0.02[a]	45.63±1.88[a]	54.41±1.95[a]
T_4	0.46±0.02[a]	43.50±1.31[a]	52.79±2.76[a]

*n=4, ± S.E., in columns means with the same letter are non-significant

3.4. Kidney function tests

The data (Table 02) exhibit that various dosages of concentrate exerted no remarkable impact on different parameters of the kidney. Means about urea concentration express that the category T_0 of study animals revealed the lowest concentration (29.32±1.25[a] mg/dL). Although the mean concentrations of

29.61±1.65[a], 30.46±1.90[a,] and 31.61±1.64[a] mg/dL were exhibited by sets, T_1 and T_2 accordingly. Nevertheless, the highest concentration of 32.06±0.77[a] mg/dL was expressed by the set T_4.

Table 02. Effect of concentrate on kidney function tests in experimental rats

Treatments	Urea (mg/dL)	Creatinine (mg/dL)
T_0	29.32±1.25[a]	0.62±0.01[a]
T_1	30.46±1.90[a]	0.63±0.01[a]
T_2	31.61±1.64[a]	0.61±0.02[a]
T_3	29.61±1.65[a]	0.64±0.02[a]
T_4	32.06±0.77[a]	0.65±0.03[a]

*n=4, ± S.E., in columns means with the same letter are non-significant

The results regarding creatinine explicitly that various levels of plant extract had a minor and haphazard impact on its concentration. The rats of group T_4 exhibited maximum (0.65±0.03[a] mg/dL) value, while the values of 0.64±0.02[a], 0.63±0.01[a] and 0.62±0.01[a] mg/dL values were shown by the sets T_3, T_1 and T_0 respectively. However, the class of experimental animals having the treatment T_2 expressed minimum concentration (0.61±0.02[a] mg/dL). The findings obtained from the above parameters clear that different levels of concentrate did not show dangerous effects on urea and creatinine concentrations of blood. These results are similar to the earlier study by Sharma et al. (2020).

3.5. Cardiac enzymes

The findings (Table 03) expound the minute influence of several dosages of concentrate on LDH, CPK, and CKMB levels of blood. The outcomes for LDH show that the set T_0 of rats expressed minimum mean concentration as161.75±4.07[a]U/L, whereas mean concentrations of 162.63±4.95[a], 163.25±4.56[a] and 163.75±2.64[a]U/L were revealed by the lotsT_2, T_1 and T_4 respectively. Nonetheless, the group of rats that received the treatment T_3depicted a value of 164.25±3.82[a] U/L. The results concerning CPK explicated that the highest value of 35.33±1.79[a] was obtained by set T_4 of rats; while, the values of 34.11±1.12[a],33.24±1.23[a] and 32.24±1.13[a] were expressed by the sets T_2, T_0 and T_3accordingly. Although the rats of class T_1 exhibited the lowest (31.88±1.15[a]U/L) value.

In the case of CKMB, the findings reveal that the set T_0 exhibited the least value of 82.10±4.00[a] followed by the mean values of 83.20±3.03[a], 84.73±2.84[a] and 85.86±1.76[a] for the groups T_2, T_1 and T_4 accordingly. Nevertheless, the set T_3 revealed the highest concentration(86.06±4.33[a]). The above outcomes show that various treatments of

Table 03. Effect of concentrate on cardiac enzymes in experimental rats

Treatments	LDH	CPK	CKMB
T_0	161.75±4.07[a]	33.24±1.23[a]	82.10±4.00[a]
T_1	163.25±4.56[a]	31.88±1.15[a]	84.73±2.84[a]
T_2	162.63±4.95[a]	34.11±1.12[a]	83.20±3.03[a]
T_3	164.25±3.82[a]	32.24±1.13[a]	86.06±4.33[a]
T_4	163.75±2.64[a]	35.33±1.79[a]	85.86±1.76[a]

*n=4, ± S.E., in columns means with the same letter are non-significant

Table 04. Effect of *Z. mauritiana* concentrate on lipid profile in experimental rats

Treatments	Cholesterol (mg/dL)	Triglycerides (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
T_0	95.38±3.13[a]	85.01±1.81[a]	36.00±1.22[a]	40.35±2.47[a]
T_1	97.23±3.69[a]	81.44±4.38[a]	33.91±0.87[a]	39.80±1.21[a]
T_2	94.88±2.46[a]	83.03±2.26[a]	34.27±1.73[a]	38.41±2.09[a]
T_3	92.14±1.87[a]	79.41±4.06[a]	33.00±1.91[a]	37.48±0.80[a]
T_4	90.64±3.76[a]	77.38±2.98[a]	35.11±1.20[a]	38.70±0.87[a]

*n=4, ± S.E., in columns means with the same letter are non-significant

concentrate had noxious effects on different cardiac enzymes of blood. These results coincide with an earlier study by Olaleye et al. (2019).

3.6. Lipid profile

Mean values (Table 04) explicate the minute influence of various levels of concentrate on cholesterol, triglycerides, LDL and HDL concentrations of rats. Results concerning cholesterol levels express that the group $T_4$of rats exhibited the minimum value (90.64±3.76[a] mg/dL), though the concentrations of 92.14±1.87[a], 94.88±2.46[a] and 95.38±3.13[a] mg/dL were explicated by the setsT_3, T_2 and T_0 correspondingly. However, the maximum concentration (97.23±3.69[a] mg/dL) was expressed by set T_1 of rats. The results regarding triglycerides exhibited the maximum concentration (85.01±1.81[a] mg/dL) for the control group (T_0), whereas the groupsT_1, T_2 and T_3 of rats exhibited the mean concentrations as 81.44±4.38[a], 83.03±2.26[a] and 79.41±4.06[a]mg/dL accordingly. However, the lowest mean concentration (77.38±2.98[a] mg/dL) was obtained by a set T_4 of rats.

The outcomes concerning LDL concentration expound that the set T_0 of rats expressed maximum concentration (36.00±1.22[a]mg/dL), while the values of 35.11±1.20[a] and 34.27±1.73[a] mg/dLwere showed by the sets T_4 and T_2 accordingly. Whereas, the lot T_3 of study animals exhibited the lowest mean concentrations

33.00±1.91[a] mg/dL. The outcomes regarding the effect of different levels of plant concentrate on LDL levels reveal that these did not cause any harmful effects in experimental animals.

Table 05. Effect of concentrate on serum proteins in experimental rats

Treatments	Total Protein (g/dL)	Albumin (g/dL)
T_0	5.93±0.26[a]	2.40±0.14[a]
T_1	6.16±0.42[a]	2.45±0.10[a]
T_2	5.95±0.09[a]	2.56±0.14[a]
T_3	6.14±0.06[a]	2.55±0.10[a]
T_4	5.99±0.23[a]	2.34±0.54[a]

Means about HDL concentrations show that the highest value of 40.35±2.47[a] mg/dL was showed by set T_0 of rats, though the sets T_1, T_2 and T_3 revealed mean values as 39.80±1.21[a], 38.41±2.09[a] and 37.48±0.80[a] mg/dL respectively. However, the value of 38.70±0.87[a] mg/dL was gained by set T_4 of rats. The above data explicates that various levels of concentrate had no detrimental influence on the lipid profile of experimental animals. These results are similar to earlier work done by Singh et al. (2018).

3.7 Serum proteins

The results (Table 05) expound that various treatments of concentrate had non-significant effects on serum proteins. The outcomes regarding total protein explicitly that the set T_0 expressed the minimum (5.93±0.26[a] g/dL) value. Whereas, the values of 5.95±0.09[a], 5.99±0.23[a] and 6.14±0.06[a] g/dL were shown by the sets of rats that received treatments T_2, T_4 and T_3 respectively. However, the maximum mean concentration of 6.16±0.42[a] g/dL was showed by the set T_1 of rats.

The results concerning albumin (Table 05) express that the set T_0 of rats exhibited the mean concentration as 2.40±0.14[a] g/dL, while the treatments T_1, T_2 and T_3 revealed concentrations as 2.45±0.10[a], 2.56±0.14[a] and 2.55±0.10[a] g/dL accordingly. Nevertheless, the class T_4 of experimental animals showed mean concentrations of 2.34±0.54[a] g/dL. The above findings show, that concentrate did not exhibit a detrimental impact on serum proteins of blood. According to the given results, it is clear that various levels of plant concentrate did not exert a harmful impact on different parameters (liver and kidney function tests, cardiac enzymes, lipid profile and serum proteins) of the blood of Sprague Dawley rats. Therefore, it may be utilized in various foods. These results are earlier stated by Kumar et al. (2017).

4. Conclusion

Z. mauritiana concentrate has various medicinal benefits due to the presence of flavonoids, triterpinic acids, alkaloids, saponins and glycosides. The extract showed no toxic effect on bilirubin concentrations in

blood. Regarding the outcomes of AST and ALT levels, the values were also statistically non-significant. The outcomes concerning the kidney function test exhibited a non-remarkable impact of the ethanolic extract on urea and creatinine concentrations of blood. As far as the results of cardiac enzymes, different treatments of plant extract did not show harmful effects. Different parameters of lipid profile and serum proteins also expressed the non-momentous impact of extract. It is safe for humans to use this plant extract in various products. Therefore, our study recommends its utilization in various products of food items and pharmaceutical drugs. Huge potential exists for plant extract utilization in food items discouraging chemical additives that pose health risks. Further studies are needed for the exploration of the flora of Cholistan for various useful additives replacing chemical additives in food.

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