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Assessment of renal and hepato-protective potential of guava leaves in male Sprague dawley rats

Makia Nasir[1], Muhammad Tahir-Nadeem[1*], Farhan Saeed[1], Tanvir Ahmad[2], Muhammad Imran[3]

[1] Department of Food Science, Government College University, Faisalabad, Pakistan
[2] Department of Statistics, Government College University, Faisalabad, Pakistan
[3] University Institute of Diet and Nutritional Sciences, Faculty of Allied Health Sciences, The University of Lahore, Lahore, Pakistan

*Correspondence to: mt.nadeem@gcuf.edu.pk; drtahirnadeem@ymail.com

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Abstract: Present research project was an attempt to explore the functional/nutraceutical worth of guava leaves from two locally grown varieties (Ruby & Safeda). For the purpose, guava leaves extract was fed to experimental male Sprague Dawley rats to explore the nutraceutical potential of guava leaves against hepatotoxicity. Two studies were performed on two types of rats i.e. study I (normal rats), study II (hepatotoxic rats). In both studies, 250 mg/kg each of pink guava leaves extracts (T_1) and white guava leaves extracts (T_2) was added in the feed. Feed intake and body weights of the rats were recorded. At the end of the first and eighth week of study, the blood samples of the rats were analyzed to check the effect of guava leaves extracts on renal functioning (Alkaline Phosphatase, Alanine Transaminase and Aspartate Transaminase) as well as liver functioning parameters including urea and creatinine. In both studies, comparatively higher feed consumption was observed in the control group than the rest of the treatments. At the end of study I, the highest weight (207±9.21 g) was observed in T_0 whereas, during study II, the maximum value (202±5.58 g) was found in T_2 (rats consuming white guava leaves extract) that indicates its effectiveness against hepatotoxicity. Regarding renal functioning tests, pink guava leaves were more effective in decreasing urea and creatinine levels in rats as compared to the white guava leaves in both study plans. Likewise, in each of study trial, pink guava leaves were more effective in reducing AST, ALT and ALP than white guava leaves and control. From the present investigation, it is deduced that guava leaves were effective against hepatotoxicity.

Key words: Guava; Renal functioning; Hepatotoxicity; Sprague dawley rats; Guava leaves.

Introduction

Liver protects the human body from the detrimental effects of microorganisms and various foreign toxic compounds through its enzymes (1). The liver plays important role in the body and produces several chemical compounds including cholesterol, bile acid, fibrinogen and albumin, which are of immense importance for normal body functioning (2, 3). Under certain conditions of disease or infection, liver may experience failure in normal functioning, commonly known as liver disorder. Globally, the prevalence of liver disorder has reached alarming levels especially in the industrialized countries where the people are directly or indirectly consuming versatile types of toxic compounds through food, soil and air. These compounds promote and lead to free radical-mediated lipid peroxidation that results in the breakdown of biomembrane and dysfunction of tissues, cell and liver toxicity (4, 5).

Various plants, including guava, have been reported to possess antioxidant activity due to high phenolic compounds which play important role in reducing chemically tempted liver damage. Guava is a member of the *Myrtaceae* family, about 133 genera and more than 38,000 species. Guava leaves and bark have an extensive history of remedial uses that are still working today (6). Around the world, various cultivar types of guava are being cultivated which differ extensively in flesh/pulp color, aroma & flavor and their seed composition. In Pakistan, 'Ruby' and 'Safeda' are the most widely grown species. The former cultivar is available in medium to large-sized fruit that possesses, strong odor, oval shape and popular for its red or pink flesh color. The latter cultivar, Safeda, grows medium-sized fruit with thin skin and white flesh (7). The fruits and various parts of the plant, especially leaves, are known to contain serval bioactive chemicals which add to its therapeutic importance. Among such compounds, the most important include certain polyphenolic compounds, saponins, terpenoids, tannins, coumarins, flavonoids and eugenol (8). Several researchers have reported the hepato-protective potential of guava leaves and recommended it as a liver tonic (9, 10).

Considering the above-mentioned benefits of guava especially its leaves, the occurrence of hepatotoxicity at a global level and expensive medication of the malady, the present study was planned to explore the therapeutic ability of guava leaves against renal dysfunctions and liver disorder in rats.

Materials and Methods

Procurement of raw materials

Commercially available white and pink guava

Table 1. Feeding plan for efficacy trial.

Groups	Feed plan
T_0	Control
T_1	Diet containing pink guava leaves extract (250 mg/kg)
T_2	Diet containing white guava leaves extract (250 mg/kg)

leaves were procured from of Institute of Horticultural Sciences, University of Agriculture, Faisalabad. The experimental rats were purchased from (NIH) National Institute of Health Islamabad and placed in the animal room of Department of Pharmacy, G.C. University, Faisalabad, Pakistan.

Preparation of guava leaves aqueous extract

The guava leaves were dried and crushed into powder. The guava leaves powders (250 mg) were added to hot water (300mL), the mixture was stirred, covered and allowed to steep for 5 minutes. The mixture was strained to get aqueous extract before offering to the rats.

Efficacy studies

For the evaluation of white and pink guava leaves extract against renal dysfunctions and hepatotoxicity, an efficacy trial was done on rats and the results are given below:

Feeding plan

The trial consisted of two studies; study I was conducted on normal rats whereas, study II comprised rats with liver damage achieved through injecting 90mg carbon tetrachloride /kg body weight. In each study, thirty rats were divided into three groups (T_0, T_1 and T_2), 10 rats in each group, and acclimatized by feeding a basal diet for one week. The control groups were fed on feed prepared by using corn oil (10%), corn starch (66%), protein (10%), cellulose (10%), mineral (3%) and vitamin mixture (1%) whereas, for T_1 and T_2 pink guava leaves extracts and white guava leaves extract were added at levels mentioned in Table 1. The diets prepared from the selected extracts were fed for 8 weeks. The temperature (23±2ºC) and relative humidity (55±5%) were maintained throughout the experimental period with a 12-hr light-dark cycle.

Study of physical parameters

Feed intake was monitored on daily basis, while body weights were recorded weekly throughout the experimental period. Half of the overnight fasted rats were sacrificed after one week of feeding trial to get baseline values while the rest of the animals were slaughtered at the end of the study. Blood samples were collected through cardiac puncture and EDTA coated tubes were used for serum collection (11).

Renal functioning tests

The serum samples were analyzed to check the effect of guava leaves on renal functioning parameters (urea, creatinine) in different groups of rats. The parameters were assessed using commercial assay kits through Microlab-300 Merck, Germany adopting the methods described by earlier researchers (12, 13).

Liver function tests

Hepatoprotective perspectives were assessed using respective commercial kits. The histological assay was conducted to assess the functional integrity of the liver through detection of Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) levels in blood samples.

Determination of alkaline Phosphatase (ALP) activity

It was determined with the help of Randox kits according to the method reported by Bassey et al. (14) which was slightly changed by Wright et al. (15).

Determination of alanine transaminase (ALT) and aspartate transaminase (AST) activities

ALT and aspartate transaminase (AST) activities were determined by using Randox kits according to the procedure reported by IFCC (16).

Statistical analysis

Completely Randomized Design (CRD) was used for experimentation. All the experiments were conducted in triplicates and the data obtained was analyzed using Minitab 16 computer-based statistical package (17).

Results

The prepared feeds were offered to two groups of rats i.e. normal and hepatotoxic rats for 8 weeks. At initiation, some of the rats were sacrificed to assess the baseline values. The results of the study are discussed as follows:

Feed intake

The data regarding feed intake shows a non-significant difference among the treatments for this parameter. However, increased feed intake can be observed over time. For the study I (normal diet), it is obvious from the mean values that maximum intake (21.96±0.13 g/rat/day) was recorded in T_0 group whilst minimum (15.41±0.11 g/rat/day) was calculated in T_1 group (rats fed on feed containing pink guava leaves extract). An increased feed intake was observed with time. Similarly, in Study II, the means in Table 2 show increased feed intake in hepatotoxic rats from 1st week of trial till 8th week of the study trial in all treatments although the difference the treatment was non-significant. Comparatively higher feed consumption was observed in the control group than the rest of the treatments.

Bodyweight gain

The data in Table 2 shows the mean values for body weight. It is explicated that body weight was affected substantially with treatments and study intervals. The results show that during Study I, the bodyweight increased in all treatments from 1st week to 8th week of the

Table 2. Effect of treatments on physical parameters.

Parameter	Study	WEEK I			WEEK VIII		
		T_0	T_1	T_2	T_0	T_1	T_2
Feed intake (g/rat/day)	I	15.98±0.18	15.41±0.11	15.79±0.13	21.96±0.13	20.05±0.18	20.86±0.15
	II	18.79±0.19	18.10±0.10	19.97±0.11	21.08±0.12	19.97±0.17	20.98±0.13
Bodyweight (g/rat)	I	132±4.23	127±5.18	126±3.25	207±9.21	205±9.74	198±5.25
	II	128±3.95	125±4.88	125±4.25	199±9.25	198±8.74	202±5.58

Study I: Normal rats, **Study II:** Hepatotoxic rats, **T_0:** Control, **T_1:** Diet containing pink guava leaves extract (250 mg/kg), **T_2:** Diet containing white guava leaves extract (250 mg/kg).

study period. The highest weight was observed in T_0 at 1st week (132±4.23 g) as well as at the termination of the study (207±9.21 g) than the rest of the treatments. Interestingly, during Study II, although the highest weight (128±3.95 g) was observed in T_0 at 1st week than T_1 and T_2 meanwhile after 8th week the highest value (202±5.58 g) for the body weight was noted in T_2 (Diet containing white guava leaves extract).

Renal functioning parameters

The results of the study for renal functioning parameters are given below:

Urea

The results of the study reflect the significant effect of guava leaves extracts on the urea level in rats. The mean values in Table 3 reveal that increased levels of urea were detected in blood samples of rats after consuming feed with guava leaves extract. The highest level was noticed in the T_0 (31.100±3.07 mg/dl) whereas, the lowest concentration (29.414±2.34 mg/dl) was recorded in T_1 during the study I. On the similar pattern, during study II the consumption of feed added with pink guava leaves extract lead to minimum urea levels (18.124±4.47 mg/dl) in blood samples as compared to intake of feed with white guava leaves extract (19.024±7.77 mg/dl) or control (49.346±7.83 mg/dl).

Creatinine

The data in Table 3 shows a significant effect of guava leaves extracts on creatinine level of rats. The mean values exhibit that creatinine levels in rats' blood samples were decreased by consumption of feeds with guava leaves extracts. It is obvious from the Table 3 that the highest creatinine level was noticed in the T_0 (0.7700±2.42 mg/dl) whereas, the lowest was recorded in T_1 (0.7380±3.07 mg/dl) during the study I. Likewise, minimum creatinine level (1.8720±4.47 mg/dl) was observed in T_1 while the maximum value (4.0960±7.83 mg/dl) was found in T_0 in study II. Therefore, it is clear that pink guava leaves were more effective in decreasing the creatinine level in rats as compared to the white guava leaves which are supported by the findings of Norazmir and Ayub (18).

Liver functioning tests

The results for liver functioning tests are described as follows:

Aspartate aminotransferase (AST) activity

It is obvious from the results (Table 4) that various treatments significantly affected the serum AST level. The mean values reveal that the highest value for AST (75.44±1.24 IU/L) was found in T_0 while the minimum (72.32±2.52 IU/L) was noted in T_1 during the study I. However, in study II higher AST value (80.34±3.72 IU/L) was noted in the T_2 group while the minimum value was noted in T_1 (75.24±2.44 IU/L). The results suggest therefore that pink guava leaves extract was more effective in reducing AST level as compared to the white guava leaves extract.

Alanine aminotransferase (ALT)

Alanine aminotransferase (ALT) is also an important indicator of liver damage. The data in Table 4 shows significant differences among treatments for serum ALT level. The highest value (56.358±3.07 IU/L) was determined in T_0 while the lowest level (55.08±2.52 IU/L) was noted in T_1 during the study I. Similarly, in study II, the highest AST value (86.89±3.72 IU/L) was detected in T_0 group whereas, the least value (55.42±2.44 IU/L) was found in T_1. Again, the pink guava leaves showed more effectiveness in decreasing serum ALT level in rats as compared to white guava leaves extracts or control.

Table 3. Effect of treatments on liver functioning parameters.

Parameter	Study	Treatments		
		T_0	T_1	T_2
Aspartate transferases (IU/L)	I	75.442±3.07a	72.322±2.34c	74.124±2.42b
	II	77.400±7.83a	75.246±4.47a	80.340±7.77a
Alanine aminotransferase (IU/L)	I	56.358±3.07a	55.0824±2.34c	55.904±2.42b
	II	86.898±7.83a	55.426±4.47c	58.744±7.77b
Alkaline phosphatase (IU/L)	I	62.128±3.07a	60.382±2.34c	61.104±2.42b
	II	114.73±7.83a	67.220±4.47c	74.748±7.77b

Study I: Normal rats, **Study II:** Hepatotoxic rats,**T_0:** Control,**T_1:** Diet containing pink guava leaves extract (250 mg/kg),**T_2:** Diet containing white guava leaves extract (250 mg/kg).

Table 4. Effect of treatments on renal functioning parameters.

Parameter	Study	Treatments		
		T_0	T_1	T_2
Urea (mg/dl)	I	31.100±3.07a	29.414±2.34c	30.772±2.42b
	II	49.346±7.83a	18.124±4.47c	19.024±7.77b
Creatinine (mg/dl)	I	0.7700±2.42a	0.7380±3.07a	0.7460±2.34a
	II	4.0960±7.83a	1.8720±4.47b	1.4500±7.77c

Study I: Normal rats,**Study II:** Hepatotoxic rats,**T_0:** Control,**T_1:** Diet containing pink guava leaves extract (250 mg/kg),**T_2:** Diet containing white guava leaves extract (250 mg/kg).

Alkaline phosphatase (ALP)

The data in Table 4 shows a significant effect of guava leaves extracts added feeds on alkaline phosphatase in blood samples. In the study, the maximum value (62.12±1.24) for the parameter was computed in T_0 while the least (60.38±2.52) was observed in T_1. A similar fashion was noted in study II, with the highest ALP level (114.73±3.72 IU/L) found in T_0 and the lowest value (67.22±2.44 IU/L) was detected in T_1. Pink guava leaves showed higher hepato-protective potential than other treatments.

Discussion

The positive effects of the treatments were only possible after more consumption of the feed. As the animals may show reluctance towards new taste or flavor of feed therefore, comparatively higher feed consumption was observed in the control group than the rest of the treatments. The reduction in the hepatotoxic group could be possibly explained by the toxic effects of CCl_4. Some researchers (19) reported reduced feed intake in hepatotoxic rats while increased intake by non-hepatotoxic when both fed on guava drink. Cokamoto et al*.,* (20) also observed reduced feed intake in rats after oral administration of carbon tetrachloride.

The kidney is the second organ most frequently affected by chemical/biochemical compounds ingested in the body. Therefore, renal functions were assessed by measuring the concentrations of creatinine and urea in plasma. Plasma urea and creatinine concentrations are increased in renal injuries therefore these can be used as an index of renal glomerular function (21). In the instant study, the consumption of feed added with guava leaves extract, especially pink guava, resulted in lower levels of urea and creatinine and similar results have been earlier reported by Norazmir and Ayub (18). This shows the protective role of guava leaves against renal dysfunctions.

Liver function test is crucial because the liver is the central organ in detoxification of compounds. A healthy liver is so crucial for protein metabolism since liver disease is frequently associated with alterations in proteins and disturbances of protein metabolism. Inadequate synthesis of total protein and albumin occurs due to liver disease. Liver damage also causes the release of increased amounts of many enzymes into the bloodstream that provides an excellent marker of tissue damage (21). In the case of liver functioning tests, the consumption of feed with added guava leaves extracts resulted in lower concentrations of various serum marker enzymes (ALT, AST, and ALP). The reduction in liver toxicity may be attributed to antioxidant effects of various bioactive compounds present in the extract like ascorbic acid, lycopene, rutin and many other compounds that might show positive effects by attacking free radicals, oxidized low-density lipoprotein cholesterols and inhibiting diacylglycerol acyltransferase and acetyl-CoA carboxylase activity responsible for the synthesis of triglyceride as well. As the consumption of guava leaves extract help in the healing of hepatic parenchyma and the regeneration of hepatocytes, the serum AST, ALT and ALP concentrations return to their normal levels in hypertensive rats (10, 19, 22, 23). However, it is worth mentioning that the level of consumption is very important because positive or hepatoprotective effects can only be achieved at certain lower levels nonetheless, the consumption of higher doses (>300 mg/kg body weight) may be deleterious and exhibit hepato-toxic effects as reported earlier in the study conducted on erythromycin-induced liver damaged rats (24). The positive effects of guava leaves on liver functioning by lowering serum marker enzymes (AST, ALT, ALP) and bilirubin levels are also evident from findings of Parmar et al*.,* (25).

The consumption of guava leaves extracts lead to better weight gain in the experimental rats. Likewise, the consumption of feed containing guava leaves extract, especially pink guava, resulted in lower levels of urea and creatinine that shows the protective role of guava leaves against renal dysfunctions. In the case of liver functioning tests, the intake of feed with added guava leaves extracts resulted in lower concentrations of various serum marker enzymes (ALT, AST, and ALP). Therefore, guava leaves based foods can be used effectively for protection against renal and liver dysfunctions.

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