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Research Article

Antioxidant potential of guava leaves extracts and their effects on hyperlipidemia

Eman Manzoor, Ashfa Ghani*, Moazzam Rafiq Khan, Muzna Sultana, Aniza Ishaque, Ehtisham Nasir, Muhammad Fahad Latif, Talha Riaz & Asad Sohail
National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan.

Abstract: The present study was carried out to check out the effect of guava leaves extract on hyperlipidemia. Guava leaves were taken from different orchards of Faisalabad after this mineral and proximate analysis were performed. Three varieties of guava were taken and extract of these three varieties were prepared with different solvents. Among these extracts one of the best variety extract was given to the rats for a period of 30 days. Blood samples were taken at the start, middle and at the end of the study to analysis the level of cholesterol. The maximum effect on the value of glutathione in hypercholestrolemic rats was seen with pink guava leaves extract in S5 the value of glutathione at 0 day was 41.59 ± 1.73 and it increased up to 44.55 ± 2.94 at 30th day. The maximum effect on the value of TBARS in hypercholestrolemic rats was seen with pink guava leaves extract in S5 the value of TBARS at 0 day was 5.58 ± 0.52 and it increased to 6.31 ± 0.87 at 30th day. The maximum effect on the value of urea in hypercholestrolemic rats was seen with pink guava leaves extract in S5 the value of urea at 0 day was 26.45 ± 1.74 and it decreased to 25.54 ± 0.79 at 30th day. Maximum effective results were seen with the ethanolic extract of pink guava leaves the values at 0 day was 0.06 ± 0.02 while it decreased to 0.14 ± 0.03 at 30th day. Least effect on the creatinine was seen with the surahi guava leaves extract in S4 group the value at 0-day was0.96 ± 0.03 while at 30th day it decreases to 0.90 ± 0.03. Mean value of ALT was high in those rats were given pink guava leaves extract as compared to gola extract followed by extract of surahi leaves extract. Mean value of AST was high in those rats were given pink guava leaves extract as compared to gola extract followed by extract of surahi leaves extract. The mean value of DPPH was highest with ethanolic extract of pink guava leaves and its mean value was 66.87 ± 2.67 it was followed by ethanolic extract of gola guava leaves and its value was60.29 ± 3.11 and third best extraction was with ethanolic extract of Surahi leaves and its mean value was 56.75 ± 3.44 on the base of varieties. The mean value of FRAPP was highest with ethanolic extract of pink guava leaves and its mean value was 91.55 ± 2.85 it was followed by ethanolic extract of gola guava leaves and its value was 85.78 ± 1.72and third best extraction was with ethanolic extract of Surahi leaves and its mean value was 80.54 ± 3.05 on the base of varieties. Mean value of TG was minimum in normal rats that was 120.33, value of TG in hyperlipidemic rats was highest that was 164.44, value of TG in rats those were given gola leaves extract its value was 178.15 mg/dl, mean value of TG was 194.85 in rats those were given surahi leaves extract and mean value of TG in rats was 174.85mg/dl in rats those were given extract of pink guava leaves so value of TG was minimum in those rats that were given extract of pink guava leaves so extract of pink guava leaves was best among the gola and surahi. Mean value of cholesterol was minimum in normal rats that was 100.33, value of cholesterol in hyperlipidemic rats was highest that was 218.47mg/dl, value of cholesterol in rats those were given gola leaves extract its value was 202.88 mg/dl, mean value of cholesterol was 232.94 mg/dl in rats those were given surahi leaves extract and mean value of cholesterol in rats was 200.22mg/dl in rats those were given extract of pink guava leaves so value of cholesterol was minimum in those rats that were given extract of pink guava leaves so extract of pink guava leaves was best among the gola and surahi leaves extract. Mean value of LDL was minimum in normal rats that was 47.33, value of LDL in hyperlipidemic rats was highest that was 148.47, value of LDL in rats those were given gola leaves extract its value was 89.88 mg/dl, mean value of LDL was 128.44 mg/dl in rats those were given surahi leaves extract and mean value of LDL in rats was 86.22mg/dl in rats those were given extract of pink guava leaves so value of LDL was minimum in those rats that were given extract of pink guava leaves so extract of pink guava leaves was best among the gola and surahi. The mean value of HDL in rats was 35.52mg/dl in rats those were given extract of pink guava leaves so highest value of HDL was highest in those rats that were given extract of pink guava leaves so extract of pink guava leaves was best among the gola and surahi.

Key words: Guava, antioxidant, hyperlipidemia, HDL, LDL

***Corresponding Author:**
Mr. Ashfa Ghani,
National Institute of Food Science and Technology,
University of Agriculture, Faisalabad, Pakistan.
E-mail: ashfaghani@yahoo.com

Introduction

Fruits and vegetables have different antioxidant potential (Prior and Cao, 2000). It is wise to make use of a variety of fresh fruits and vegetables rather than consuming the few commodities with the highest potential of antioxidant capacity. The dietary guidelines of USDA, 2000 says that an individual should take at least five servings of fruits and vegetables that is two fruits' serving and three vegetables' servings a day. As vegetable and fruits are enriched in carotenoid so it is easier to utilize fresh food rather than using the supplements of carotenoid because they are useful in lowering the DNA damage and LDL oxidation (Southon, 2000). Leaves of guava extract have been utilized commercially in Taiwan, China, Japan and Korea and these extracts are commonly used as dietary supplements having many pharmacological effects. The leaves extract contains main part of a variety of polyphenols and flavonoids. In the tropic's guava is a small tree that is thirty-five feet in height. It relates to the family of Myrtaceae. Leaves and bark possessed a long history of medical use and that practice is still in now days (Nwinyi *et al.,* 2008). Various portions of guava plants can be used for medical purpose to fix the diseases e.g. gastrointestinal diseases, malaria, infection in intestine, antiperistalsis, cough, ulcers, toothache, swelling in gums and many other medical conditions. This fruit weighing 160-170g contain more than 4 times ascorbic acid than an orange (220-230mg/100g), and possess more content of micronutrients like Mg, K and few more important micronutrients (Mahendran, 2010). Some guavas have organic acids' with very good distribution like tartaric acid, oxalic acid, vitamin C, citric acid and malic acid etc. That have capacity to perfectly used in other fruit's juices for preservation purpose or to add up antioxidant activities of any fruit product (Zheng *et al*., 2007).

Guava fruit is good example of palatable organic product, called as apple for poor because it has less effort, easily accessible and healthful profile. It is taken to be super natural fruit product, since they are rich in vitamin A and ascorbic acid in peel additionally that is rich in dietary fibers. It has a suitable measure of dietary minerals Mg, K and other supplements (Mahendran, 2010). Hyperlipidemia can be explained as hoisted level of VLDL and LDL in blood. Great amount of LDL and VLDL add to the high blood cholesterol level and collection of biomolecules prompting coronary heart ailment, harm that is not reversible to heart muscle tissues and blocking in supply of blood to brain (Dhandanya *et al.,* 2012). The agents for cancer prevention are usually phytochemicals and have good results (Sumino *et al.,* 2002). Guava considered as a sub-acidic natural fruit since dissolvable solids of it are related to broad range of sugar and natural acids. Mash of organic product of guava gives sharpness of 0.3 to 0.8 percent (weight/volume) and large with contrast in interspecies. In many other varieties of this fruit causticity have been seen to be fall in the scope of 0.37 to 0.96 percent (Sharma *et al.,* 2009). This is exceptionally rich wellspring of cell's reinforcement that have great measure of lycopene, zeaxanthin and lutein.

Guava fruit is enriched in phenols, tannins, flavonoids, fundamental oils, carotenoids, lectins and saponins as well. Leaves of this fruit separate make hypolipidemic and hypoglycemic affect in alloxan treated on rats that are diabetic. This concentrate limits the risk of coronary diseases by exceeding the HDL cholesterol level in blood and by lowering the level of glucose in blood, triglycerides, sum cholesterol VLDL and in addition LDL. Deguchi *et al.,* (2013) explained that the fluid leave remove limit the working of alpha-amylase, maltase and sucrose in vitro conditions. Treatment with recently arranged concentrates of leaves of guava fruit tremendously lowers the blood glucose level and the lipid profile levels in diabetic yellow skinned person rats (WV *et al.,* 2009 and Rafiq *et al.,* 2009 and PK. Rai *et al.,* 2010). Leaves extract make hypolipidemic and hypoglycemic affect in diabetic rats treated with alloxan. This concentrate limits the possibility of coronary illnesses by increasing the blood level of HDL cholesterol and it lowers the blood glucose level, TC, TG, VLDL and LDL. Treatment with latest arranged guava leaves concentrates of this fruit immensely lowers the blood glucose level and level of lipid in diabetic yellow skinned rats (JW.WV *et al.,* 2009 & Rafiq *et al.,* 2009 & Rai *et al.,* 2010).

This fruit is full in carotenoids and polyphenols that are most important classes of cancer prevention agent shades that provide them potential for cell reinforcement. These shades provide shading to organic product skin, guavas of red orange color have polyphenol, carotenoid, retinoid and professional sources of vitamin A than that guavas of yellow green (Joseph, 2011).

Materials and Methods

Procurement of Raw Material

Leaves of guava were collected from various orchards of Faisalabad city. The leaves were collected after collection leaves were washed and then for the purpose of drying leaves were air dried for few days. The research was done in the processing lab of fruits and vegetables of NIFSAT, UAF.

Extraction Analysis:

Different 3 varieties of guava leaves will be taken and by using different concentrations of solvents of ethanol and water 3 extracts will be prepared by Mazumdar *et al.,* (2015).

Ethanolic extract of pink leaves

For the preparation of 50 % ethanol extract 262.5ml of ethanol was added to the distilled water solution and 25g of sample was taken and keep on adding distilled water until 112.5ml of volume of extract is attained.

Ethanolic extract of surahi leaves

For the preparation of ethanol extract 187.5ml of ethanol was added to the distilled water solution.25g of sample was taken this solution was made up to the volume of 187.5 ml by the addition of distilled water

Ethanolic extract of gola leaves

For the preparation of 90% ethanol extract 337.5ml of ethanol and water solution was made. We kept on adding distilled water until its volume was made up to the 37.5ml.

Treatment plan for solvent extraction

Solvent	Treatments	Guava leaves
Ethanol	T_0	Gola
Ethanol	T_1	
Ethanol	T_2	
Ethanol	T_3	
Ethanol	T_4	Surahi
Ethanol	T_5	
Ethanol	T_6	
Ethanol	T_7	Pink
Ethanol	T_8	

In vitro studies

Free radical scavenging ability

Free radical scavenging DPPH ability of guava leaves extract was determined by using the method of Ghafoor. (2014).

Ferric reducing antioxidant power (FRAP) assay

The reducing power was evaluated by the measurement of capability of those extracts that can easily reduce the femetripyridyltriazine into blue colored ferrous and it can be detected at 593 nm as narrated by Omidreza *et al.,* (2005).

Selection of Best Treatment

Best treatment was selected on the basis of antioxidant assay and efficacy of extraction one of the best treatments should be selected from each kind of guava leaves.

In Vivo Studies

For efficacy trial rats were purchased from National Institute of Health (NIH), Islamabad. Pakistan. At the 1st week of study some rats would be sacrificed to obtain the baseline values for each study. Five types of studies will be carried out individually and each group will be consisting of 3 rats. In study I rats were fed on normal diet in study II rats were fed on high cholesterol fed diet whereas study III, IV AND V will be given high cholesterol plus guava leaves extract diet. During this trial of 1-month selective diets should be provided to the related group of rats to check out the therapeutic effect of guava leaves. Drink and feed intake will be measured throughout the experimental duration.

Study I: Normal diet

For an initial period of week 1, special diet was provided to the rats so that they can be adjusted to the environment. Later on, the diets consist up of guava leaves extract should provide for the trial of 1 month Feed and drink intakes was checked out and noted on daily basis throughout this experimental trial .At the end of the trial fasted rats overnight were slaughtered and their blood samples were collected to measure the cholesterol level, LFTs and RFT values.

Feed and drink intakes

Feed and drink intake are the basic necessity of living things. Average amount of drink and feed intake was measured in daily basis. The average amount of feed falls in the range of 20-30g per rat per day and water intake falls in the range of 20-30ml per rat per day.

Treatments used in the treatment of rats

Study I	Study II	Study III	Study IV	Study V
*G1 Negative control	*G2 Positive control	G3 Hypercholesrolemia rats+gola leaves extract	G4 Hypercholestrolemic rats +surahi leaves extract	G5 Hypercholestrolemic rats +pink leaves extract

*G1= Normal rats
*G2 =High cholesterol induced rats

Serum Lipid Profile
Serum lipid profile of cholesterol was measured by the method of Asano *et al.,* (2008). serum lipid profile will consist of HDL, LDL and triglycerides

Antioxidative status test
Serum catalase and superoxide dismutase will be ascertained following method of Reddy *et al.,* (2011).

Glutathione assay
Glutathione peroxidase assay was finding out by using the method of Arun*et al.,* (2002). Whereas, reduced glutathione (GSH) was determined by the method of Arun *et al.,* (2002).

Thiobarbituric acid reactive substances (TBARS)
The deliberation of thiobarbituric acid reactive substances (TBARS) was found out in the tissue by using the method of Donnan (1950).

RFT and LFTs
To determine the RFT it will include the blood urea nitrogen and serum creatinine. In LFT WE WILL determine the serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) by Fen-Yu *et al.,* (2006).

Antioxidants status test
Determination of glutathione peroxidase was determined by using the method of Baluchnejadmojarad *et al.,* (2017) and TBARs assay will be finding out by using the method of Gok *et al.,* (2016)**.**

Statistical Analysis
Statics will be subjected to each parameter to determine the level of significance (Montgomery, 2008).

Results and discussion

Glutathione
Table 1 showed that mean value of glutathione at o day was lower than mean value of glutathione at 30th day and mean value of glutathione that was 37.15 ± 1.8 and 40.04 ± 2.44 µmol/L respectively. The mean value of glutathione for normal rats at 0 day was 37.78 ± 2.40 and it increase to 40.37 ± 1.85 at 30th day. It has been observed that level of glutathione first decreased in hyper cholestrolemic rats the value of glutathione at 0 day was 32.40 ± 2.02 and at 30th day value of glutathione was 37.27 ± 2.58. The maximum effect on the value of glutathione in hyper cholestrolemic rats was seen with pink guava leaves extract in S5 the value of glutathione at 0 day was 41.59 ± 1.73 and it increased up to 44.55 ± 2.94 at 30th day. The second best effect was seen with gola leaves extract in S3 the level of glutathione at o day was 40.29 ± 0.91 and it increased to 42.31 ± 1.89 the minimum effect on the value of glutathione was seen with surahi leaves extract the value of glutathione in rats that were treated with surahi leaves extract at 0 day was 33.70 ± 2.29 and it increased upto 36.55 ± 2.95 at 30th day so this shows that pink guava leaves extract showed significant effect on the level of glutathione.

Table 1. Mean values of GPX

Days	Studies					Mean
	S1	S2	S3	S4	S5	
0	0.49 ± 0.016	0.12 ± 0.017	0.14 ± 0.013	0.16 ± 0.01	0.19 ± 0.006	0.2159^{b}
30	0.51 ± 1.85	0.11 ± 0.04	0.17 ± 0.02	0.18 ± 0.02	0.10 ± 0.013	0.2323^{a}
Mean	0.4977^{b}	0.1144^{c}	0.1453^{ab}	0.1705^{c}	0.1924^{a}	

TBRAS
Statistical data showed significant trend in values of TBRAS in serum of rats those were under efficacy trial. Table for analysis of variance showed significant effect of groups of rats. Table (2) showed the means values of TBRAS into 5 groups. 15 rats were taken for the efficacy trial of 30 days and these rats were divided into five groups each group consist of 3 rats. While table 4.5.2 showed that mean value of glutathione at 0 day was lower than mean value of glutathione at 30th day and mean value of TBRAS that was 6.81 ±

0.40 and 7.54 ± 0.79 respectively. The mean value of glutathione for normal rats at 0 day was 5.34 ± 0.4 and it increase to5.61 ± 0.50µmol/L at 30th day. It has been observed that level of TBARS first increased in hyper cholestrolemic rats the value of TBARS at 0 day was 8.51 ± 0.47 and at 30th day value of TBARS was 9.51 ± 0.51. The maximum effect on the value of TBARS in hyper cholestrolemic rats was seen with pink guava leaves extract in S5 the value of TBARS at 0 day was 5.58 ± 0.52 and it increased to 6.31 ± 0.87 at 30th day. The second best effect was seen with gola leaves extract in S3 the level of TBARS at o day was 7.09 ± 0.10 and it increased to 7.62 ± 0.97 at 30th day, the minimum effect on the value of TBARS was seen with surahi leaves extract the value of TBARS in rats that were treated with surahi leaves extract at 0 day was 7.57 ± 0.46 and it increased up to 8.11 ± 1.12 at 30th day so this shows that pink guava leaves extract showed significant value of the level of TBARS.

Table 2. Mean values of TBRAS

Days	Studies					Mean
	S1	S2	S3	S4	S5	
0	5.34 ± 0.44	8.51 ± 0.47	7.09 ± 0.10	7.57 ± 0.46	5.58 ± 0.52	6.8180^{b}
30	5.61 ± 0.50	9.51 ± 0.51	7.62 ± 0.97	8.11 ± 1.12	6.31 ± 0.87	7.5410^{a}
Mean	5.4733^{c}	9.0083^{a}	7.3583^{b}	8.1142^{ab}	5.9433^{c}	

Kidney function test

Statistical data showed significant trend in values of urea in serum of rats those were under efficacy trial. Table (3) showed the means values of urea into 5 groups. 15 rats were taken for the efficacy trial of 30 days and these rats were divided into five groups each group consist of 3 rats. While table 4.5.2 showed that mean value of urea at 0 day was lower than mean value of urea at 30th day and means value of urea that was 27.51 ± 1.93 and 29.00 ± 1.94 respectively. The mean value of urea for normal rats at 0 day was 22.27 ± 2.20 and it increase to26.26 ± 2.30µmol/L at 30th day. It has been observed that level of urea first increased in hyper cholestrolemic rats the value of urea at 0 day was 25.73 ± 1.78 and at 30th day value of urea was 29.07 ± 2.24. The maximum effect on the value of urea in hyper cholestrolemic rats was seen with pink guava leaves extract in S5 the value of urea at 0 day was 26.45 ± 1.74 and it decreased to 25.54 ± 0.79 at 30th day. The second best effect was seen with gola leaves extract in S3 the level of urea at 0 day was 25.58 ± 1.63 and it increased to 28.04 ± .85 at 30th day, the minimum effect on the value of urea was seen with surahi leaves extract the value of urea in rats that were treated with surahi leaves extract at 0 day was 37.55 ± 2.29 and it decreased to 36.11 ± 2.11 at 30th day so this shows that pink guava leaves extract showed significant decrease in value of the urea.

Table 3. Mean value for urea

Days	Studies					Mean
	S1	S2	S3	S4	S5	
0	38.74 ± 0.45	55.33 ± 7.50	45.66 ± 6.65	47.22 ± 5.02	46.22 ± 9.53	46.551^{a}
30	21.16 ± 1.11	57.88 ± 7.73	32.01 ± 11.55	39.51 ± 9.93	23.22 ± 9.53	34.757^{b}
Mean	29.952^{b}	56.61^{a}	38.84^{a}	43.26^{a}	34.61^{a}	

Table (4) showed the means values of creatinine into 5 groups. 15 rats were taken for the efficacy trial of 30 days and these rats were divided into five groups each group consist of 3 rats. Normal rats were denoted by S1 group and mean value of creatinine in normal rats at 0 day was 0.51mg/dl while at 30th day the value of creatinine for normal rats was 0.29.S2 was denoted to the rats in which hypercholesterolemia was induced by high cholesterol fed rats in these rats mean value of creatinine at 0 day was 2.64mg/dl while at 30th day the value of creatinine was decreased to 2.75mg/dl. S3 was denoted to those rats who were receiving the ethanolic extract of gola guava leaves and in these rats mean value of creatinine was 1.77mg/dl while at 30th day there was not a significant change in creatinine level as compare to 0 day. Maximum effective results were seen with the ethanolic extract of pink guava leaves the values at 0 day was 0.06 ± 0.02 while it decreased to 0.14 ± 0.03 at 30th day. Least effect on the creatinine was seen with the surahi guava leaves extract in S4 group the value at 0-day was0.96 ± 0.03 while at 30th day it decreases to 0.90 ± 0.03.

Table 4: Mean values of creatinine

Days	Studies					Mean
	S1	S2	S3	S4	S5	
0	0.51 ± 0.12	2.64 ± 0.065	1.77 ± 0.013	1.84 ± 0.10	0.06 ± 0.24	1.67[a]
30	0.29 ± 0.095	2.75 ± 0.12	1.61 ± 0.30	1.67 ± 0.14	0.14 ± 0.21	1.56[b]
Mean	0.3983[b]	2.69[a]	1.69[a]	1.76[a]	1.51[a]	

Liver function test

Table (5) showed the means values of ALT into 5 groups. 15 rats were taken for the efficacy trial of 30 days and these rats were divided into five groups each group consist of 3 rats. Normal rats were denoted by S1 group and mean value of ALT in normal rats was 72 IU/L. S2 was denoted to the rats in which hypercholesterolemia was induced by high cholesterol fed rats in these rats mean value of ALT was 224.25 IU/L. S3 was denoted to those rats who were given of extract of surahi guava leaves and in these rats mean value of ALT was 103.08IU/L. S4 groups was denoted the rats those were given ethanolic extract of surahi guava leaves and mean value of ALT in rats 119.91IU/L. S5 group was denoted to the rats those were given pink guava leaves and its mean value of ALT was 91.91IU/L. Mean value of ALT was high in those rats were given pink guava leaves extract as compared to gola extract followed by extract of surahi leaves extract. Table (6) showed the means values of AST into 5 groups. 15 rats were taken for the efficacy trial of 30 days and these rats were divided into five groups each group consist of 3 rats. Normal rats were denoted by S1 group and mean value of AST in normal rats was 4083 IU/L. S2 was denoted to the rats in which hypercholesterolemia was induced by high cholesterol fed rats in these rats mean value of AST was 181.33 IU/L. S3 was denoted S3 was denoted to those rats who were given of extract of surahi guava leaves and in these rats mean value of AST was 116.15IU/L. S4 groups was denoted the rats those were given ethanolic extract of surahi guava leaves and mean value of AST in rats 136.91IU/L. S5 group was denoted to the rats those were given pink guava leaves and its mean value of AST was 86.22IU/L. Mean value of AST was high in those rats were given pink guava leaves extract as compared to gola extract followed by extract of surahi leaves extract.

DPPH

Table 7 showed that mean value of DPPH was highest with ethanolic extract of pink guava leaves and its mean value was 66.87 ± 2.67 it was followed by ethanolic extract of gola guava leaves and its value was 60.29 ± 3.11 and third best extraction was with ethanolic extract of Surahi leaves and its mean value was 56.75 ± 3.44 on the base of varieties. On the base of solvents, methanol was second best solvent and the mean value for TFC with methanol was 35.27 ± 3.5and minimum value of TFC was recorded with water and its mean value was 29.44 ± 2.56.

Table 5. Mean values of AST

Days	Studies					Mean
	S1	S2	S3	S4	S5	
0	49.00 ± 5.31	176.46 ± 6.07	122.67 ± 8.99	143.96 ± 9.55	90.86 ± 7.65	116.60[a]
30	32.15 ± 2.33	186.00 ± 7.34	109.96 ± 6.44	129.73 ± 4.35	81.57 ± 5.88	107.52[b]
Mean	40.83[b]	181.24[a]	116.15[a]	136.85[a]	86.22[a]	

Table 6: Mean values of ALT

Days	Studies					Mean
	S1	S2	S3	S4	S5	
0	72 ± 9.77	219 ± 2.52	110.86 ± 6.55	126.66 ± 8.33	95.66 ± 8.06	124.74[a]
30	54 ± 7.81	229.71 ± 7.66	97.43 ± 7.55	112.25 ± 7.55	86.66 ± 3.89	115.57[b]
Mean	63[c]	224.25[a]	103.08[ab]	119.46[ab]	91.21[b]	

Table 7: Mean value of DPPH

Solvents	Varieties			Mean
	Pink	Gola	Surahi	
Methanol	39.12 ± 4.63	36.07 ± 4.08	30.62 ± 3.61	35.27[b]
Ethanol	66.87 ± 2.67	60.29 ± 3.11	56.75 ± 3.44	61.30[a]
Water	33.62 ± 2.55	29.25 ± 2.97	25.44 ± 2.16	29.44[c]
Mean	46.54[a]	41.87[b]	37.61[c]	

Table no.8 Mean values of FRAPP

Solvents	Varieties			Mean
	Pink	Gola	Surahi	
Methanol	78.37 ± 3.66	75.44 ± 2.44	70.07 ± 2.78	74.63[b]
Ethanol	91.55 ± 2.85	85.78 ± 1.72	80.54 ± 3.05	85.96[a]
Water	68.77 ± 2.78	65.88 ± 2.26	60.44 ± 3.71	65.03[c]
Mean	79.57[a]	75.70[b]	70.35[c]	

FRAPP

Table 8 showed that mean value of FRAPP was highest with ethanolic extract of pink guava leaves and its mean value was 91.55 ± 2.85 it was followed by ethanolic extract of gola guava leaves and its value was 85.78 ± 1.72 and third best extraction was with ethanolic extract of Surahi leaves and its mean value was 80.54 ± 3.05 on the base of varieties. On the base of solvents, methanol was second best solvent and the mean value for FRAPP with methanol was 74.63 ± 4.33minimum value of TFC was recorded with water and its mean value was 65.03 ± 2.91.

Water intake

ANOVA table for source of variance showed that effect of studies on water intake was significant and effect of weeks on water intake was significant while interaction of weeks and study was found non- significant. Table 9 shows the trend of water intake of rats and normal rats will drink less water than remaining four groups, rats those are hyper cholestrolemic will drink plenty of water, rats those were given extract of surahi guava leaves will drink more water than gola leaves extract and least amount of water is drank by those rats that were given extract of pink guava leaves. Water intake was high during 1st week and it was decreased till the end of tenure. Mean value for S1, S2, S3, S4 and S5 are 21.29, 30.0, 27.95, 28.73, and 22.34 respectively. And trend for mean values during 1st, 2nd, 3rd and 4th week was 24.99, 27.43, 26.63 and 25.20 respectively.

Feed intake

I noted the amount of feed that rats were taking during tenure of research table 10 showed that effect of studies on feed was significant, effect of weeks on feed was highly significant while interaction of weeks and study on feed was found to be non-significant. It was seen that normal rats were taking a lot of food as compare to other groups hyper cholestrolemic rats were taking minimum least food. Feed intake was increasing till the end of the last week of research. Normal rats were taking a lot of food followed by S5, S3, S4 and S2 respectively. Means values of feed intake of S1, S2, S3, S4 and S5 were 29.18, 21.48, 27.62, 23.20 and 29.14 respectively. While mean values of feed intake during 1st,2nd, 3rd, 4th and 5th week were 27.37, 24.373, 25.73 and 27.36 respectively.

Table 9: Mean values of water intake

Weeks	S1	S2	S3	S4	S5	Means
1st week	22.85 ± 1.60	28.17 ± 0.15	27.43 ± 2.36	27.94 ± 3.41	18.55 ± 2.55	24.99[a]
2ndweek	20.18 ± 2.71	29.07 ± 1.00	25.83 ± 1.00	29.6 ± 3.56	21.5 ± 3.05	27.43[a]
3rdweek	22.69 ± 2.82	31.21 ± 0.28	24.96 ± 2.23	18.55 ± 2.87	26.01 ± 1.60	26.43[a]
4thweek	19.98 ± 3.22	31.56 ± 0.77	26.70 ± 2.45	21.5 ± 3.01	23.30 ± 0.40	25.20[a]
Means	21.29[b]	30.00[a]	27.95[a]	28.73[ab]	22.34[ab]	

Table 10: ANOVA table for mean values of feed

Weeks	S1	S2	S3	S4	S5	Means
1st week	22.85 ± 1.60	28.17 ± 0.15	27.43 ± 2.36	27.94 ± 3.41	18.55 ± 2.55	27.37[a]
2ndweek	20.18 ± 2.71	29.07 ± 1.00	25.83 ± 1.00	29.6 ± 3.56	21.5 ± 3.05	24.72[a]
3rdweek	22.69 ± 2.82	31.21 ± 0.28	24.96 ± 2.23	18.55 ± 2.87	26.01 ± 1.60	25.72[a]
4thweek	19.98 ± 3.22	31.56 ± 0.77	26.70 ± 2.45	21.5 ± 3.01	23.30 ± 0.40	27.02[a]
Means	29.183[b]	21.481[a]	27.616[a]	23.20[ab]	29.136[ab]	

Table for mean values of triglyceride

Table for mean values of TG at 0 day was 171.23 and mean value of TG at 30th day was 161.56mg/dl. Mean value of TG was minimum in normal rats that was 120.33, value of TG in hyperlipidemic rats was highest that was 164.44, value of TG in rats those were given gola leaves extract its value was 178.15 mg/dl, mean value of TG was 194.85 in rats those were given surahi leaves extract and mean value of TG in rats was

174.85mg/dl in rats those were given extract of pink guava leaves so value of TG was minimum in those rats that were given extract of pink guava leaves so extract of pink guava leaves was best among the gola and surahi.

Table 11: Mean values of cholesterol

Days	S1	S2	S3	S4	S5	Mean
0 day	148 ± 10.58	253.86 ± 6.63	259.97 ± 9.49	242.46 ± 2.52	236.86 ± 5.45	185.63[a]
30 day	128.67 ± 10.11	243.09 ± 9.75	259.96 ± 9.49	249.41 ± 9.62	227.57 ± 11.37	174.30[b]
Mean	100.33[c]	218.47[a]	202.88[a]	232.94[a]	200.22[b]	

ANOVA table for cholesterol shows that effect of days on cholesterol was significant, effect of study on cholesterol was found to be highly significant while their interaction was found to be non-significant. Table for mean values of cholesterol at 0 day was 185.63 and mean value of cholesterol at 30th day was 174.30mg/dl. Mean value of cholesterol was minimum in normal rats that was 100.33, value of cholesterol in hyperlipidemic rats was highest that was 218.47mg/dl, value of cholesterol in rats those were given gola leaves extract its value was 202.88 mg/dl, mean value of cholesterol was 232.94 mg/dl in rats those were given surahi leaves extract and mean value of cholesterol in rats was 200.22mg/dl in rats those were given extract of pink guava leaves so value of cholesterol was minimum in those rats that were given extract of pink guava leaves so extract of pink guava leaves was best among the gola and surahi leaves extract.

Table 12: Mean values of LDL

Days	S1	S2	S3	S4	S5	Mean
0 day	65 ± 10.58	170.86 ± 6.63	159.47 ± 2.52	176.97 ± 9.49	153.86 ± 5.45	105.63[a]
30 day	45.67 ± 10.12	160.09 ± 9.74	167.41 ± 11.28	152.79 ± 9.40	144.57 ± 11.37	94.50[b]
Mean	47.33[c]	148.47[a]	89.88[ab]	128.44[ab]	86.22[b]	

ANOVA table for LDL shows that effect of days on LDL was significant, effect of study on LDL was found to be highly significant while their interaction was found to be non-significant. Table for mean values of LDL at 0 day was 105.63 and mean value of LDL at 30th day was 94.50. Mean value of LDL was minimum in normal rats that was 47.33, value of LDL in hyperlipidemic rats was highest that was 148.47, value of LDL in rats those were given gola leaves extract its value was 89.88 mg/dl, mean value of LDL was 128.44 mg/dl in rats those were given surahi leaves extract and mean value of LDL in rats was 86.22mg/dl in rats those were given extract of pink guava leaves so value of LDL was minimum in those rats that were given extract of pink guava leaves so extract of pink guava leaves was best among the gola and surahi.

Table 13: Mean values of HDL

Days	S1	S2	S3	S4	S5	Mean
0 day	47.33 ± 4.72	22.73 ± 4.30	29.01 ± 3.51	18.74 ± 3.51	31.85 ± 5.99	29.934[b]
30 day	65 ± 9.92	27.02 ± 10.15	25.67 ± 4.95	34.05 ± 7.76	57.18 ± 10.16	35.85[a]
Mean	62.17[a]	15.88[b]	18.40[b]	17.40[b]	35.52[a]	

ANOVA table for HDL shows that effect of days on HDL was significant, effect of study on HDL was found to be highly significant while their interaction was found to be non-significant. Table for mean values of HDL at 0 day was 29.94 and mean value of HDL at 30th day was35.85mg/dl. Mean value of HDL was highest in normal rats that was 62.17, value of HDL in hyperlipidemic rats was minimum that was 15.88, value of HDL in rats those were given gola leaves extract its value was 18.40 mg/dl, mean value of HDL was 17.40 in rats those were given surahi leaves extract and mean value of HDL in rats was 35.52mg/dl in rats those were given extract of pink guava leaves so highest value of HDL was highest in those rats that were given extract of pink guava leaves so extract of pink guava leaves was best among the gola and surahi.

Conclusion

The extracts obtained from pink guava leaves were found to possess strong antioxidant potential. Maximum effective results were seen with the ethanolic extract of pink guava for kidney & liver functioning test as well as for cholesterol.

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