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In vivo antioxidant potential of fruit mucilage from two varieties of *Cucumis melo subsp. agrestis* against oxidative stress induced toxicity

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Abstract: To evaluate *in*-vivo antioxidant potential of fruit mucilage from *Cucumis melo* variety *momordica* (PM) and variety *agrestis* (KM) using rats as experimental animals, the fruits were collected, identified, dried and pulverized. Mucilages were isolated from the fruit powders by microwave-assisted method. Aqueous extracts obtained were filtered to remove fruit pulp. Each filtrate was centrifuged at 4000xg rpm for 15 min. Each supernatant was precipitated with 3 volumes of 95% ethanol and maintained overnight at 4°C. These precipitates were filtered and lyophilized. In vivo antioxidant activity was determined using rats for 14 days. Paracetamol (75mg/Kg, i.p.) for inducing oxidative stress and Vitamin C & Vitamin E (200mg/Kg each, p.o.) as standard treatment were used. PM and KM were given in 500mg/Kg and 1000mg/Kg, p.o. doses in separate groups. SOD, MDA, GSH and CAT levels were estimated in organs (liver, kidney, heart, brain) of all groups using standard procedures. Toxic control showed prominent toxicity in the liver. The levels of GSH, CAT and SOD were raised and MDA levels were reduced in all organs of test and standard groups. The levels of antioxidant biomarkers varied in all remaining groups. The overall results are significant suggesting strong antioxidant potential of PM and KM.

Keywords: Mucilage, *Cucumis melo*, antioxidant, superoxide dismutase, malondialdehyde.

INTRODUCTION

Normal aerobic cellular metabolism produces reactive oxygen species (ROS) which play a vital role in intracellular signaling, physiological mechanisms and homeostasis. To regulate these physiological mechanisms human body is equipped with antioxidant systems which help to maintain the levels of these free radicals (Katerji *et al*., 2019). Any condition that causes imbalance in oxidation–reduction system or disproportionate level of oxidants, may lead to decline in the levels of antioxidant (Salim, 2017; Singh *et al*., 2019). Under oxidative stress (OS) conditions excessive free radicals are produced, that damage the macromolecules of cellular structure such as proteins, lipids, and nucleic acids. Disruption of cell organelles and structure leads to disease manifestation (Katerji *et al*., 2019). Natural defense mechanism of the body against oxidative stress could be improved by the use of herbal products (Peng *et al*., 2011). On the other hand, the use of the natural antioxidant resources like fruits and vegetables is a useful approach to control the manifestation of oxidative stress induced diseases without any side effects (Zhao, 2009). Therefore, it is an emerging trend to enrich the human diet with foods consisting of high content of antioxidant compounds (Aguiar *et al*., 2020).

Various phytochemicals and secondary metabolites such as bioactive polysaccharides obtained from various extracts of fruits and vegetables have the ability to control the generation of reactive oxygen species and induce endogenous antioxidant defense (Liu *et al*., 2018). Mucilage is a source of such polysaccharides. Among various plant families Curcurbitaceae family comprises mucilage rich plant species. Mucilage of several members of Cucurbitaceae family has been studied and was evaluated for its antioxidant efficacy (Motiwala *et al*., 2015). Wild musk melon or *Cucumis melo* subsp. *agrestis* (CMA) belongs to this family. Several species of *Cucumis melo* were also studied for their antioxidant potential (Gopalasatheeskumar and Kalaichelvan, 2021). In current study, the antioxidant activity of mucilage of two varieties of *Cucumis melo subsp. agrestis* from *Cucurbitaceae* family was assessed against oxidative stress induced toxicity.

MATERIALS AND METHODS

Collection and drying of fruit

The dried *Cucumis melo* sub specie *agrestis* variety *agrestis* fruit was purchased from local herb market of Lahore, Pakistan. The fresh fruit of *Cucumis melo* subspecie *agrestis* variety *momordica* was obtained from outskirts of Lahore, Pakistan. Fruits samples were authenticated by a taxonomer of GCU, Lahore, Punjab, Pakistan. Voucher specimens (3738 and 3739, respectively) were submitted to the herbarium. Fruits were dried under shade and were pulverized to coarse powder for extraction of mucilage.

Extraction of mucilage

Mucilage was extracted from fruits by method as described by Vittori (2002) with slight modifications. The fruit powders were soaked in distilled water for 24 h

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before extraction then microwave assisted extraction was done for 3 minutes (800W irradiation). The resulting aqueous extracts obtained were filtered through double layer cheesecloth to remove pulp and were centrifuged at 4000xg (4˚C) for 15 minutes. The supernatant of each sample obtained was treated with three of its volume of 95% ethanol to precipitate the mucilage and filtered. Precipitates were finally lyophilized and were ground to powder form. The powdered PM and KM were stored in dried, air tight bottles at room temperature.

Animals

35 wistar rats of both genders weighing 120-130g were purchased from animal house of Punjab University College of Pharmacy, Lahore and were kept in the controlled temperature (22±4°C) and humidity (50-60%). They had free access to water and diet. The study was carried out as per recommendation of ethical Committee of animal care and experimentation, under voucher number No. D/418 /DZ. All the animals were divided into 7 groups as normal control, toxic group, standard group and experimental groups.

Acute Toxicity study

Acute toxicity study of PM and KM was carried out according to OECD guidelines. OECD guidelines. Wistar rats were administered oral 5g/Kg/day dose of PM and KM separately. No behavioral changes and mortality were observed for 14 days. The LD_{50} would be greater than 5g/Kg of PM and KM (Shastri *et al*., 2017).

In-vivo antioxidant study

The study was conducted for 14 days. Group N (Normal control) was given normal saline orally. Group T (Toxic control) received paracetamol 75mg/kg once intraperitoneal. Group S (Standard control) was given standard treatments: Vitamin E and Vitamin C, 200mg/kg/day, p.o (Saleem *et al*., 2014; Ho *et al*., 2012). Group PM1 and PM2 were given PM 500 and 1000 mg/kg/day orally respectively. Group KM1 and KM2 were given KM 500 and 1000 mg/kg/day orally respectively (Shastri *et al*., 2017). In order to induct hepatorenal damage by oxidative stress, 3h before starting experiments (Day 0), all groups (except normal control) were administered paracetamol intraperitoneally (75mg/kg). Weight of rats (Group N- KM2) was recorded on Day 0, 7^{th} day and 14^{th} day (Saleem *et al*., 2014). At the end of the study, blood was collected from rats by inserting syringe into heart of anesthetized rats and the serum was obtained after centrifugation (Shastri *et al*., 2017).

Preparation of tissue homogenates

After dissecting rats, the organs like kidney, liver, heart and brain were separated. All the organs were immediately immersed in ice-cold 10mmol/l of phosphate saline buffer with 7.4 pH and 25% homogenized tissue solutions were prepared. These homogenates were centrifuged for ten minutes (4000 rpm) at 4˚C to remove cellular remains. The supernatant obtained was stored in chiller at -80˚C till estimation of biological markers for antioxidant activity.

Estimation of antioxidant biological markers in tissue homogenates

Quantification of Catalase (CAT) activity

In a cuvette, 100µL tissue homogenate, 1.9mL of 50mmol/L phosphate buffer (pH 7.0) and 1.0mL of 2mmol/L crisply arranged H_2O_2 were added. Blank and standard were prepared with same method. Absorbance of all the tests was measured against blank at 240nm. Utilizing different concentrations of CAT standard dilutions, a calibration curve was established. Condition of direct relapse was applied to degree the catalase action in µg/mg of tissue (Kumar *et al*., 2008).

Quantification of Glutathione (GSH)

In different test tubes 0.1mL of each tissue homogenate and 0.1mol/L Phosphate buffer (pH 7.4) were blended with 2.4mL 0f 0.02mol/L Ethylenediaminetetraacetic acid i.e., EDTA. To accelerate the tissue proteins, each test tube was put in ice for 10-15min and 2.0mL distilled water and 0.5mL of TCA (50%) were added. Centrifuged the reaction mixtures at 3000-3500 r/min. Another cleaned set of test tube was taken for supernatants of each reaction mixture and 2.0mL of 0.15 mol/L Tris-HCl and 50µL of 6mmol/L 5,5′-Dithio-bis (2-nitrobenzoic acid) were added and vortexed. Measured the absorbance at 412 nm against blank. Virtually blank was managed excluding homogenized tissues. GSH standard (concentration of 40-200µg/mg) was used for making calibration curve of GSH. Direct relapse condition was utilized to estimate glutathione in homogenized tissue solutions and the concentration was represented in µg/mg of tissue (Moron *et al*., 1979).

Quantification of Superoxide dismutase (SOD)

To study the SOD activity followed the method of Kakkar *et al.* described by (Saleem *et al*., 2014). 200µL of tissue homogenate, 1.2mL of 0.025mol/L Tetrasodium phosphate buffer (pH 8.3), 100µL of 186µmol/L phenazine methosulphate and 300µL of nitro blue tetrazolium (NBT) were put in test tubes. To start the reaction, 200µL of 780µmol/L reduced nicotinamide adenine dinucleotide (rNAD) was included to the reaction mixture and incubated at 30˚C for 1.5 min. 1.0mL pure acetic acid was added to halt further response. 4mL of *n*-butanol was mixed to the reaction mixture by vigorous shaking and permitted to stand for 10min and finally centrifuged. Butanolic layer was isolated and then the concentration of chromogen was measured at 560nm against blank prepared exclusive of tissue homogenates. calibration curve was developed with concentrations of standard ranging from 10-100µg/mg. A straight relapse condition was utilized to degree superoxide dismutase action.

Quantification of Malondialdehyde (MDA)

Added all the chemicals (8.1% sodium docecyl sulphate, 0.75 mL of 0.8% TBA and of 20% acetic acid each and 300 µL of double distilled water) into 100µL of tissue homogenate, mixed and homogenized by vortex mixer. The resulting mixture was heated in water bath at 95°C for 1 h. Added 2.5mL of 1:15 V/V pyridine-butanol mixture and 0.5mL of extra pure water to the cooled reation mixture, and centrifuged for 10 minutes at 3000rpm. The absorbance of the supernatant was measured relative to the blank prepared exclusive of tissue homogenates, at 532nm. The concentration of MDA standard used was 10-100mmol/g for calibration curve. Direct relapse conditions were used to access the level of malondialdehyde in homogenized tissue solutions in nmol/g (Ohkawa *et al*., 1979).

Biochemical analysis

Complete blood count of all the control and test group rats was studied using Randox kit (UK) with the help of Humalyser. LFTs (Liver function tests) including ALP (Alkaline Phosphatase), Total Bilirubin and ALT(Alanine Aminotransferase), RFTs (Renal Function Tests) including Creatinine and Urea along with quantification of lipids including Triglycerides (TGs), High density Lipoprotein (HDL), Cholesterol, Low Density Lipoprotein (LDL), Very Low-Density Lipoprotein (VLDL) were studied using Randox UK kits (Saleem *et al*., 2014).

STATISTICAL ANALYSIS

The results were scrutinized by utilization of SPSS version 17. One way ANOVA and post hoc Tukey's test were applied to find out statistical variances among groups/treatments. *P-value <0.05 was considered as statistically significant.

RESULTS

In order to evaluate the antioxidant potential of PM and KM, free radical scavenging effects of KM and PM were assessed against oxidative stress by estimation of levels of antioxidant bio-markers and LFTs, RFTs and lipid profile. CBC was quantified in normal, toxic and all the test groups to determine the effects on blood.

Estimation of antioxidant bio-markers in homogenized tissues

The mucilage of both fruits exhibited prominent *in*-vivo antioxidant effects in liver, brain, heart and kidney. In liver tissue homogenates, studies showed that at 1000mg/Kg dose of PM and KM, the level of GSH significantly (*P<0.05) increased with significant reduction (*P<0.05) in MDA levels. At 500mg/Kg dose of PM and KM the levels of CAT were increased while the level of SOD maximized with only PM treatment as compared to KM treated groups. The results are shown in table 1.

In brain there was an increase in GSH, CAT and SOD levels and the level of MDA was significantly reduced (*P<0.05) in all treated groups in comparison to the toxicity group. Activation of GSH, CAT and SOD and reduction of MDA was maximum (*P<0.05) with PM treatment at 1000 mg/kg dose, shown in the table2.

In the heart the level of GSH, CAT and SOD was highest by standard control as compared to other controls and treated groups. On the other hand, KM at 1000mg/kg dose

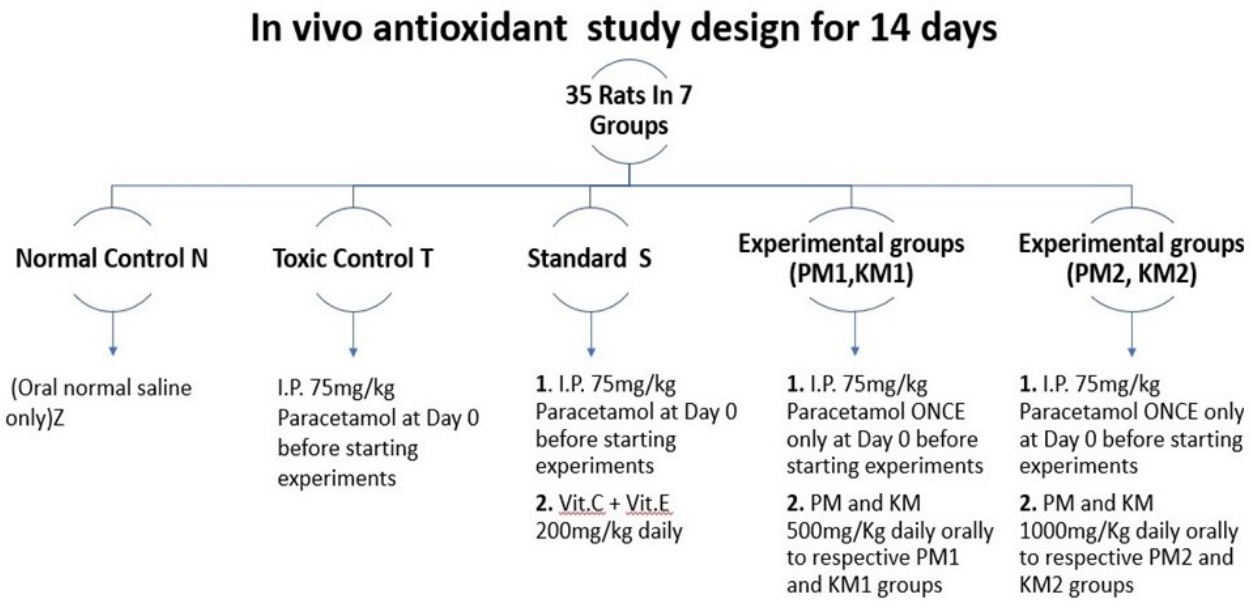

Table 1: Estimation of antioxidant biomarkers in liver homogenates

Groups	Dose (mg/Kg)	GSH (µg/mg)	CAT (µg/mg)	SOD (µg/mg)	MDA (nmol/g)
N	-	2.40 ± 0.12*	7.48 ± 0.05*	17.38 ± 0.41*	34.98 ± 0.08*
T	75, i.p.	1.61 ±0.15	5.10 ± 0.02	9.68 ±0.50	96.22 ±0.05
S	200 each, p.o.	2.65 ±0.04*	8.01 ± 0.01*	19.28 ± 0.05*	69.01 ± 0.53*
PM1	500, p.o.	2.43 ± 0.05*	7.63 ± 0.03*	16.76 ± 0.04*	61.01 ± 0.03*
PM2	1000, p.o.	2.49 ± 0.01*	8.68 ± 0.07*	19.59 ± 0.04*	59.05 ± 0.03*
KM1	500, p.o.	2.12 ± 0.03*	7.98 ± 0.04*	15.78± 0.05	63.12 ± 0.21*
KM2	1000, p.o.	2.48 ± 0.02*	8.23 ± 0.04*	18.76± 0.02*	60.51 ± 0.06*
F value:		Within groups: 3.122		Between groups: 63.49	

Table 2: Estimation of antioxidant biomarkers in brain homogenates

Groups	Dose (mg/Kg)	GSH (µg/mg)	CAT (µg/mg)	SOD (µg/mg)	MDA (nmol/g)
N		3.39 ± 0.04*	10.20 ±0.02*	18.61 ±0.02*	34.28 ±0.02*
T	75, i.p.	1.89 ± 0.01	5.05 ± 0.04	9.56 ± 0.01	92.24 ± 0.04
S	200 each, p.o.	3.02 ± 0.01*	8.56 ± 0.01*	17.87 ±0.01*	50.68 ±0.02*
PM1	500, p.o.	2.81 ± 0.05*	7.28 ± 0.02*	14.19 ±0.01*	58.19 ±0.01*
PM2	1000, p.o.	3.09 ± 0.03*	8.94 ± 0.02*	17.91 ±0.01*	50.51± 0.02*
KM1	500, p.o.	2.69 ± 0.02*	7.05 ± 0.02*	14.01 ±0.01*	59.11 ±0.02*
KM2	1000, p.o.	2.89 ± 0.01*	8.16 ± 0.01*	17.27 ±0.01*	54.22 ±0.02*
F-value:		Within groups: 3.161		Between groups: 64.53	

Table 3: Estimation of antioxidant biomarkers in heart homogenates

Groups	Dose (mg/Kg)	GSH (µg/mg)	CAT (µg/mg)	SOD (µg/mg)	MDA (nmol/g)
N		3.48 ± 0.04*	11.30 ± 0.02*	18.02 ± 0.02*	39.08 ± 0.02*
T	75, i.p.	2.75 ±0.02	6.48 ±0.02	9.04 ±0.02	71.01 ± 0.02
S	200 each, p.o.	4.02 ± 0.46*	8.82 ± 0.06*	13.71 ± 0.02*	61.56 ± 0.01*
PM1	500, p.o.	3.21 ± 0.02*	7.02 ± 0.10*	11.82 ± 0.02*	67.28± 0.01*
PM2	1000, p.o.	3.31 ± 0.02*	7.73 ± 0.01*	12.57 ± 0.01*	66.17± 0.01*
KM1	500, p.o.	3.01 ± 0.01*	7.25 ± 0.01*	12.51 ± 0.02*	66.03±0.02
KM2	1000, p.o.	3.37 ± 0.01*	8.04 ± 0.01*	13.52 ± 0.02*	60.01± 0.03*
F-value:		Within groups: 0.2794		Between groups: 137.6	

Table 4: Estimation of antioxidant biomarkers in kidney homogenates

Groups	Dose (mg/Kg)	GSH (µg/mg)	CAT (µg/mg)	SOD (µg/mg)	MDA (nmol/g)
N		3.58 ± 0.16*	9.49 ± 0.01*	19.61 ± 0.02*	36.59 ± 0.02*
T	75, i.p.	1.69 ± 0.02	5.58 ± 0.02	9.58 ± 0.01	94.01 ± 1.72
S	200 each, p.o.	3.09 ± 0.04*	8.29 ± 0.01*	18.56 ± 0.01*	68.17 ± 0.02*
PM1	500, p.o.	2.78 ± 0.02*	7.58 ± 0.02*	17.19 ± 0.01*	73.99 ± 0.04*
PM2	1000, p.o.	3.12 ± 0.01*	8.32 ± 0.01*	18.71 ± 0.01*	67.99 ± 0.02*
KM1	500, p.o.	2.74 ± 0.04*	7.39 ± 0.02*	17.02 ± 0.01*	75.12 ± 0.02*
KM2	1000, p.o.	2.89 ± 0.02*	8.10 ± 0.01*	18.12 ± 0.01*	69.79 ± 0.04*
F-value:		Within groups: 0.4868		Between groups: 75.63	

The results are given as mean ± S.D. *P<0.05 when compared with toxic control group

showed maximum increase (*P<0.05) in the levels of GSH, CAT and SOD while MDA level was significantly reduced (*P<0.05) by KM 1000 mg/kg dose as compared to toxic and PM treated group. As shown in the table 3.

In kidney homogenates, a remarkable upsurge in superoxide dismutase (SOD), catalase (CAT) and Glutathione (GSH) levels and a distinct decrease in MDA levels (*P<0.05) was recorded in all treatment and control groups as compared to toxicity group. The highest increase (*P<0.05) in levels of SOD, CAT, GSH and reduction in MDA levels (*P<0.05) was observed in PM treated group at 1000mg/kg dose as shown in the table 4.

Study of liver functions

Slight variations in total bilirubin levels in all test groups were observed with no significant differences <P>0.05) in comparison to toxic group. The levels of ALT and ALP in reduced the most in the standard treatment group, while the PM treatment group showed the highest reduction in

Table 5: Effect of various treatments on LFTs of rats

Groups	Dose (mg/Kg)	Total Bilirubin (mg/dL)	ALP (IU/L)	ALT (IU/L)
N	-	0.56 ±0.05	110.66 ± 2.05*	81.33 ± 1.52*
T	75, i.p.	0.58 ±0.14	242.02 ± 3.01	144.31 ± 2.50
S	200 each, p.o.	0.54 ±0.11	127.01 ± 3.56*	82.66 ± 3.50*
PM1	500, p.o.	0.57 ±0.10	150.65 ± 5.41*	88.65 ± 2.51*
PM2	1000, p.o.	0.55 ±0.11	131.02 ± 1.95*	85.51 ± 2.06*
KM1	500, p.o.	0.57 ±0.12	151.01 ± 3.51*	89.33 ± 2.95*
KM2	1000, p.o.	0.56 ±0.05	142.31 ± 2.48*	87.03 ± 2.01*
F-value	Within groups: 0.149		Between groups: 33.84	

Table 6: Effect of various treatments on RFTs of rats

Groups	Dose (mg/Kg)	Serum creatinine (mg/dL)	BUN (mg/dL)
N	-	0.41 ± 0.03*	20.18 ± 0.05*
T	75, i.p.	0.78 ± 0.03	31.35 ± 0.06
S	200 each, p.o.	0.49 ± 0.03*	25.69 ± 0.06*
PM1	500, p.o.	0.57 ± 0.02*	28.11 ± 0.02*
PM2	1000, p.o.	0.31 ± 0.03*	21.95 ± 0.02*
KM1	500, p.o.	0.59 ± 0.03*	28.12 ± 0.02*
KM2	1000, p.o.	0.31 ± 0.02*	21.99 ± 0.05*
F-value:	Within groups: 0.218	Between groups: 34.42	

Results are given in mean ± S.D. *P<0.05 when compared with toxic control group.

Table 7: Study of treatment effect on lipid profile

Groups	Dose (mg/Kg)	LDL (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)	Triglyceride (mg/dL)	Cholesterol (mg/dL)
N	-	45.66± 1.52*	48.01± 2.18*	36.98± 2.48*	251.98±2.62*	128.33±2.10*
T	75, i.p.	84.67±2.08	31.12± 2.08	60.08± 2.09	388.41±3.51	197.02±2.42
S	200 each, p.o.	65.01± 2.01*	51.32± 2.06*	33.99± 1.01*	269.89±2.01*	141.98±2.55*
PM1	500, p.o.	71.33± 2.51*	44.31± 2.08*	45.25± 2.51*	296.31±3.98*	138.00±2.63*
PM2	1000, p.o.	48.62± 2.08*	47.99± 2.00*	34.08 ± 1.02*	268.34±3.00*	129.61±2.09*
KM1	500, p.o.	65.33± 2.52*	42.86± 1.52*	44.31± 1.00*	300.01±4.04*	148.25±2.07*
KM2	1000, p.o.	49.33± 1.51*	43.66± 1.52*	39.02± 2.06*	271.12±2.61*	130.62±2.51*
F-value		Within groups: 0.279		Between groups: 133.2		

Results are given in mean ± S.D. *P<0.05 when compared with toxic control group.

ALT and ALP at a dose of 1000 mg/kg (*P<0.5) (table 5).

Study of renal function tests

Results indicated reno-protective effect with maximum reduction in the levels of BUN and serum creatinine in groups treated with 1000mg/kg dose of PM and KM as compared to toxicity group (*P<0.05).

Estimation of lipid profile

In the standard control group and the PM and KM treatment groups, triglycerides, cholesterol, LDL and VLDL levels were significantly reduced (*P<0.05). In PM 1000mg/Kg group, the levels of all parameters (except HDL) were reduced significantly (*P<0.05) compared to toxic group. The HDL value of PM (1000mg/Kg) group was increased remarkably, which was roughly equal to the estimated level of normal control group. Compared with toxicity control group, there are significant differences (*P<0.05) in the levels of all lipids in all experimental groups (table 7).

Study of treatment effect on CBC

All complete blood count parameters (except platelet count) in the trial groups sustained within the normal range, although the results of the treatment groups were significantly different (*P<0.05) compared with the toxicity control group. The highest drop in platelet count was observed in the group treated with KM 500mg/Kg (table 8).

Assessment of effect of treatment on the body weight of rats

The effect of treatment with PM, KM and other controls on body weight of rats was assessed in all study groups.

Table 8: Study of treatment effect on CBC

Groups	Dose mg/kg	RBCs (10^{12}/L)	Hb (g/dL)	RDW %	MCV (fL)	PCV %	PLT (10^9/L)	MPV (fL)	WBC (10^9/L)	MCHC (g/dL)	MCH (pg)
N	-	9.15± 0.01*	14.22± 0.05*	17.98± 0.10*	47.05± 0.05*	38.21± 0.10*	391.02± 1.41*	6.40± 0.15*	4.46± 0.15*	30.60± 0.14*	15.10± 0.15*
T	75, i.p.	8.98± 0.02	10.19± 0.02	15.78± 0.03	44.39± 0.02	26.98± 0.05	101.78± 2.52	5.29± 0.15	3.49± 0.05	22.28± 0.03	12.95± 0.05
S	200 each, p.o.	9.03± 0.01	14.26± 0.02*	17.82± 0.03*	46.86± 0.04*	38.28± 0.02*	372.32± 0.52*	6.43± 0.02*	4.43± 0.02*	30.25± 0.02*	15.02± 0.01*
PM1	500, p.o.	9.07± 0.02	14.10± 0.02*	17.79± 0.04*	46.83± 0.02*	38.26± 0.04*	316.00± 2.00*	6.35± 0.02*	4.45± 0.02*	29.98± 0.09*	15.07± 0.02*
PM2	1000 p.o.	9.11± 0.02	14.18± 0.05*	17.91± 0.02*	47.04± 0.02*	38.81± 0.02*	328.31± 2.08*	6.39± 0.04*	4.42± 0.05*	30.31± 0.03*	15.09± 0.03*
KM1	500, p.o.	9.05± 0.01	14.08± 0.02*	17.71± 0.02*	46.76± 0.04*	38.12± 0.02*	310.01± 3.06*	6.34± 0.04*	4.41± 0.02*	30.14± 0.02*	15.05± 0.02*
KM2	1000, p.o.	9.09± 0.02	14.11± 0.05*	17.82± 0.02*	47.01± 0.02*	38.24± 0.98*	329.17± 0.08*	6.38± 0.04*	4.47± 0.04*	30.28± 0.04*	15.08± 0.05*
F-value:			Within groups: 1.267				Between groups: 65.69				

RBCs: Red Blood Cells; Hb: Hemoglobin; RDW: Red cells distinction width; MCV: Mean corpuscular volume, measurement of mean volume of red blood cells; PCV: Packed cell volume; PLT: Platelet; MPV: Mean platelets volume; MCHC: Mean corpuscular hemoglobin concentration (mean Hb content per cell); MCH: Mean Corpuscular Hemoglobin (Hb average mass per cell). The results are given in mean ± S.D. *P<0.05 when compared with toxic control group.

Table 9: Estimation of treatment effect on body weight of rats

Groups	Dose (mg/kg)	Weight (g)		
		Day 0	Day 7	Day 14
N		125.87 ± 1.65	126.14 ±1.73	127.56 ± 1.69*
T	75 *i.p.*	126.03 ± 1.40	125.18 ± 4.29	124.01 ± 5.93
S	200 each *p.o.*	126.00 ± 3.91	128.05 ± 4.05*	129.35 ± 3.36*
PM1	500 *p.o.*	126.01 ± 2.60	128.06 ± 2.42*	128.95 ± 3.37*
PM2	1000 *p.o.*	126.09 ± 1.63	131.05 ± 3.09*	132.00 ± 2.64*
KM1	500 *p.o.*	125.89 ± 4.19	127.93 ± 3.89*	129.11 ± 4.53*
KM2	1000*p.o*	125.91 ± 1.89	128.31 ± 2.81*	129.75 ± 3.15*
F-value:	Within groups: 3.600		Between groups: 7.940	

The results are given as mean ± S.D. *P<0.05 when compared with toxic control group.

There was a rise in the average weight of rats in all the trial groups excluding the toxicity group in which the body weight was reduced in comparison to the pretreatment weight at Day 0. At the end of the study (Day 14) the rats treated with PM at 1000mg/Kg dose had maximum weight gain (*P<0.05) (table 9).

DISCUSSION

Earlier studies have demonstrated that members of the Cucurbitaceae family have good antioxidant potential (Salehi *et al*., 2021) and recent studies show that extracts from *Cucumis* have antioxidant effects (Arora *et al*., 2011). Most of the toxic chemicals cause damage in organs by direct or indirect induction of lipid peroxidation (Asha, 2001). Liver and kidney damage is caused by the overdose of paracetamol and it produces toxic effects in both human beings and rats. Hence, paracetamol is an appropriate investigational toxin to promote the hepatorenal injury (Placke *et al*., 1987). The increase in MDA levels in organs suggests enhanced lipid peroxidation leading to tissue damage (Khan *et al*., 2012). The effects of mucilage on the activation of defense enzymes are dose dependent. There was a variation in effectiveness at 500 and 1000mg/Kg dose among the organs (liver, heart, brain and kidney). The levels of GSH, CAT and SOD were remarkably increased at 1000mg/Kg dose. The results demonstrated that ALP, ALT and total bilirubin decreased in groups treated with PM and KM as compared to toxic control further suggesting antioxidant potential of PM and KM. Oxidative stress caused by paracetamol toxicity led to disturbance in the profile of lipid factors. Comparing with toxicity group, high VLDL, LDL, cholesterol and the triglycerides levels (by the paracetamol intoxication) were decreased with treatment with standard, PM and KM. Similarly, the levels of creatinine and Blood Urea Nitrogen (BUN) declined in treatment groups in comparison to the levels of toxicity group. The body weight of rats was increased in all treated groups in comparison to their pretreatment body weights except that of toxicity group. In general toxic agents or drugs usually reduce body weight as a result of intoxification (Shahare *et al*., 2013). KM and PM at 1000mg/Kg and 500mg/kg doses were found non-toxic rather they ameliorate the oxidative toxicity induced by paracetamol. All these results emphasized on *in*-vivo

antioxidant potency of KM and PM. Previously no data was available on *in*-vivo antioxidant activity of mucilage from fruits of *Cucumis melo subsp. agrestis* variety *momordica* (PM) and variety *agrestis* (KM). This is the first report according to our knowledge.

CONCLUSION

This work concluded that PM and KM both showed good antioxidant potential.

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