Biochemical analysis of the crude extract of *Momordica charantia* (L.)

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Abstract: *Momordica charantia* (L.) commonly referred as bitter gourd, karela and balsam pear. Its fruit is used for the treatment of diabetes and related conditions amongst the indigenous populations of Asia, South America, India and East Africa. The study was conducted to find out the biochemical aspects of crude extract of whole fruit of *M. charantia* including seeds which includes blood test (Hemoglobin, RBC, Total leukocyte count, platelets count, HbA1C (Glycocylated heamoglobin Type A1C)), Lipid profile test and electrolyte balance. Hemoglobin (7.1±0.14), platelets count (827 $\times 10^9 \pm 1.95$), Cholesterol level (111±2), HDL (high density lipoproteins) (20±1.22) at 10mg shows marked increase in values as compared to control. While 25 mg dose shows insignificant result. Electrolyte balance are found significant at 10mg and 25mg except bicarbonates (Na^+=143±1.87, K^-=3.45±0.35, Cl^- =108±1.48). Another important property of *M. charantia* is the elevation of platelet counts, heamoglobin and specifically high-density lipoproteins (HDL). It also controls cholesterol, triglycerides, HDL, LDL and VLDL at low dosage (10mg). Further studies can be conducted to find out which phytochemical components acts on specific biochemical activity.

Keywords: *Momordica charantia*, Platelet, Blood biochemistry, HDL, Electrolytes.

INTRODUCTION

Biochemical analysis of blood samples are mostly used in healthcare to determine the pathological and physiological conditions of subject such as disease, mineral content, It is essential for a researcher to perform pre-clinical trials and confirm the effects of drug on various body organs and their functions;(Awe and Banjoko, 2013, Saba *et al.*, 2009).

Many of the drugs currently available in market have previous history of use as herbal remedies, such as opium, aspirin, digitalis, and quinine. According to the World Health Organization, approximately 25% of modern drugs used in the United States are derived from plants. About 7,000 medicinal compounds in the modern pharmacopoeia are derived from plants (Zhang *et al*., 2012). Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80% show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived (Fabricant and Farnsworth, 2001). There are benefits as well as drawbacks of using plants as the starting material for development of any drug. If someone use information that suggest that specific plants may yield useful drugs based on long-term use by humans as ethno-medicine, they can rationalize that any isolated active part of that compounds from that plants are likely to be safer than active compounds from plants with no history of human use (Fabricant and Farnsworth, 2001).

*M. charanti*a (L.) is one of the most popular vegetables in South Asia. It belongs to the cucurbit family along with squash, cucumber, watermelon, and muskmelon. It is native to China, India and Pakistan. It is the fast-growing plant, which grows throughout Asia and is becoming popular worldwide. It has great economic importance. Depending on location, bitter gourd is also known as bitter melon, Karela, or balsam pear (Sathishsekar and Subramanian, 2005). *M. charantia* (L.) also has importance from medical point of view. It has many biological activities like: antibiotic, antioxidant, anthelmintic, anti-diabetic, anti-mutagen, anti-inflammatory, antibacterial, anti-leukemic, antimicrobial, astringent, antimycobacterial, anti-tumor, anti-ulcer, antiviral, aperitive, aphrodisiac, cytotoxic, cytostatic, carminative, depurative, hypertensive, hormonal, hypocholesterolemic, hypoglycemic, hypotriglyceridemic, immuno-stimulant, insecticidal, laxative, lactagogue, purgative, refrigerant, stomachic, tonic and vermifuge etc (Budrat and Shotipruk, 2008). In the present study, crude extract of whole fruit of *M. charantia* has been administered to animals and evaluated for its effects on various biochemical parameters of blood.

MATERIALS AND METHODS

Materials and methods

Fresh fruit of *Momordica charantia* (L.) was purchased from local market. Authentication of the plant was carried out by Botanist and specimen voucher was deposited in department's herbarium (UK-MC-02032013), all chemicals used are of analytical grade. Animals were purchased from animal house of Dow University of Health sciences, Karachi, Pakistan.

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Preparation of crude extract

2Kg fresh fruit of *M. charantia* was washed and cleaned prior to air-drying at room temperature for 24 hour. The whole fruit along with seeds of *M. charantia* was cut into small pieces and mixed with 3 Liters of absolute methanol for 15 days to ensure complete extraction. The extract was filtered through Whatman's paper No. 4. Methanol was evaporated on a rotary evaporator at 50mm Hg at 40ºC. The evaporated plant extract was thicviscous material and was kept in air-tight bottle and stored at 5ºC until it was analyzed.

Healthy rabbits of either sex were used for this study. Animals were kept in a cage at room temperature and were feed with commercial feed and water. Approximate weight of the animals was 1 Kg. Animals were kept under observation for the period of 90 days.

Biochemical studies

Animals were divided into three groups (n=5), Group 1: Control, Group 2: Low dose treated (10mg), Group 3: High dose treated (25mg). Drug was administrated orally at interval of 24 hours for the period of 90 days. Physical behavior and signs of any sort of illness were observed daily. For biochemical analysis, blood was collected directly from heart by cardiac puncture. 10ml blood was collected in sterilized syringe using 1mg/ml EDTA as anticoagulant. Blood was estimated for haematological parameters viz, packed cell volume (PCV), platelets, haemoglobin (Hb), white blood cell counts (WBC), mean corpuscular haemoglobin concentration (MCHC), total leukocyte counts (WBC) using an automated dialyser machine (Cell-Dyn, Abbott, USA). Another portion of blood was dispensed into plain bottles, allowed to clot and centrifuged at 3500rpm for 10min and the clear sera aspirated off for biochemical evaluation viz; total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density cholesterol (VLDL-C) and triglycerides (TG) using commercial kits obtained from Randox Laboratories, UK. Electrolytes; Na^+, Cl^-, K^+ and HCO^{-3} levels were also estimated by standard methods (Sathishsekar & Subramanian, 2005; Rawi *et al*., 2011)

STATISTICAL ANALYSIS

ANOVA is applied on the data obtained, subjected to one way analysis of Variance for comparison among different groups. *P* value <0.05 was considered significant value and *P* <0.001 was considered as highly significant values (Hutcheson *et al*., 1998). Results are expressed as Mean ± SD.

RESULTS

After successful administration of drug to animals no abnormal signs of toxicity were observed after the ninety days of treatment. Following are the few biochemical parameters that are studied under this work.

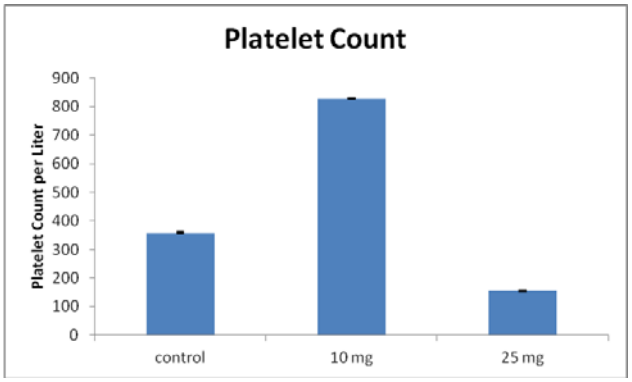

Fig. 1: Platelet Count of control and *M. charantia* extract treated rabbits. All values are expressed in Mean ± SD

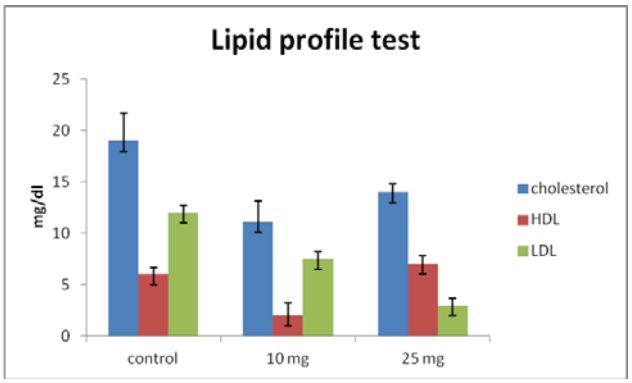

Fig 2: Lipid profile test of control and *M. charantia* extract treated rabbits. All values are expressed in Mean ± SD.

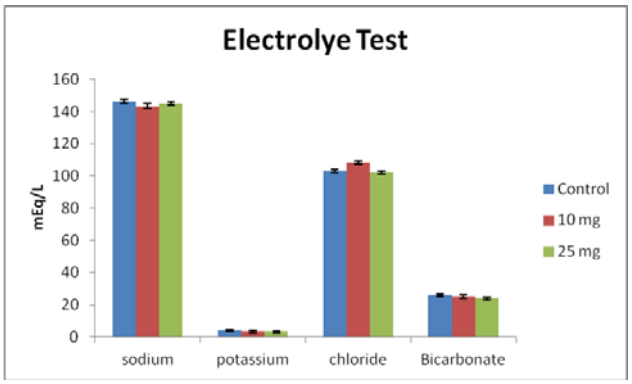

Fig. 3: Electrolyte Count of control and *M. charantia* extract treated rabbits. All values are expressed in Mean ± SD

Oral dosing of crude extract of *M. charantia* at two different concentrations for 90 days did not cause any abnormalities or illness in rabbits. Various biochemical parameters like complete blood count, lipid profile and electrolytes were studied under this work. Such biochemical parameters plays significant role in investigation and diagnosis of various diseases (Awe and Banjoko, 2013; Larrey, 2001, Malomo, 2000).

Table 1: Complete blood count of control vs treated rabbits with *M. charantia* extract after 90 days

Biochemical Analysis	Control (SD)	10mg *M. charantia* extract ± SD	25mg *M. charantia* extract ± SD	*P* value Control Vs 10 mg *M. charantia* extract	*P* value Control Vs 25mg *M. charantia* extract	*P* value 10mg Vs 25mg *M. charantia* extract
Hemoglobin	10.42±0.40	7.1±0.14	11.5±0.35	<0.0001	<0.0019	<0.0001
RBC	4.9 ±0.79	3.8±0.15	5.3±0.21	<0.0170	<0.3063	<0.0001
Hematocrit	33.2±2.25	25.2±0.25	36.5±0.35	<0.0001	<0.0120	<0.0001
MCV	67.3±1.3	64.3±0.16	68.9±0.75	<0.0001	<0.0001	<0.0001
MCH	21.2±1.9	18.3±0.52	21.75±0.52	<0.0124	<0.6048	<0.0001
MCHC	31.3±1.8	28.4±0.52	31.5±0.41	<0.0108	<0.8747	<0.0001
Total leukocyte count (WBC)	7.2×10^9±0.2	7.3×10^9±0.19	9.4×10^9±0.36	<0.4379	<0.0001	<0.0001
Platelet Count	357×10^9±6.50	827×10^9±1.95	155.2×10^9±1.10	<0.0001	<0.0001	<0.0001
HbA1C	4.14±0.1	5.07±0.33	4.08±0.07	<0.0008	<0.3485	<0.0002
PT (control)	14	14	14	-	-	-
PT Test	6±0.7	6.40±0.55	8±0.07	<0.3466	<0.0021	<0.0039
INR- International Normalize ratio	0.44±0.0	0.46±0.04	0.6±0.07	<0.5926	<0.0013	<0.0040
APTT Control	35	35	35	-	-	-
APTT Test	59±0.7	58±0.89	72±1.5	<0.2731	<0.0001	<0.0001
Blood Glucose Random	90±2.2	99±1.9	76±1.5	<0.0001	<0.0001	<0.0001
Calcium Serum	14.23±0.03	13.6±0.28	14.73±0.15	<0.0012	<0.0001	<0.0001

Value expressed as Mean ± SD, $p<0.05$ is significant, $p<0.0001$ is highly significant. Results show that at both doses of drug gives $p<0.0001$ high significant value for platelet count. With high significant results for hemoglobin as well whereas at lower dose HbA1c gives significant value and at higher dose it is insignificant.

Table 2: Lipid profile of control vs *M. charantia* extract treated rabbits after 90 days.

Biochemical Analysis	Control (SD)	10mg *M. charantia* extract ± SD	25mg *M. charantia* extract ± SD	(p value) Control Vs 10mg *M. charantia* extract	P value Control Vs 25mg *M. charantia* extract	P value 10mg Vs 25mg *M. charantia* extract
Cholesterol HDL Ratio	3.18±0.08	5.5±0.43	1.9±0.09	<0.0001	<0.0001	<0.0001
Cholesterol	19±2.7	11.1±2	14±0.84	<0.0001	<0.0056	<0.0001
Triglycerides	110±3.7	234±2.17	76±1.5	<0.0001	<0.0001	<0.0001
HDL	6±0.70	2±1.22	7±0.84	<0.0001	<0.0400	<0.0001
LDL	12±0.70	7.5±0.71	3±0.71	<0.0001	<0.0001	<0.0001
VLDL	22±1.5	47±1.4	15±0.71	<0.0001	<0.0001	<0.0001

Table 3: Electrolyte test of control vs *M. charantia* extract treated rabbits after 90 days

Biochemical Analysis	Control (SD)	10mg *M. charantia* extract ± SD	25mg *M. charantia* extract ± SD	(p value) Control Vs 10mg *M. charantia* extract	P value Control Vs 25mg *M. charantia* extract	P value10mg Vs 25mg *M. charantia* extract
Sodium	146±1.22	143±1.87	145±0.71	<0.0171	<0.1525	<0.0558
Potassium	4.28±0.13	3.54±0.35	3.70±0.07	<0.0022	<0.0001	<0.3466
Chloride	103±1	108±1.48	102±1.14	<0.0002	<0.4021	<0.0001
Bicarbonate	26±0.70	25±1.10	24±0.71	<0.2073	<0.0021	<0.0736

Value expressed as Mean ± SD, $p<0.05$ is significant, $p<0.0001$ is highly significant.

In table 1, study showed that the crude extract of plant at 10mg shows marked increase in platelet count (827×10^9± 1.95), increased Hb levels (11.5±0.35) at 25 mg, control HbA1c at 10mg (5.07±0.33). There is marked increased in total leukocyte count at 25mg (9.4×10^9±0.36) and insignificant at 10mg of drug.

Lipid profile of test drug (table 2) shows high significant values at 10mg of drug with increase in triglycerides values (234±2.17), decrease in HDL (2±1.22) and LDL (7.5±0.71) (when compared with control animal but at high dose 25mg it gives significant value with decrease in triglycerides (76±1.5), increase in HDL (7±0.84) and decrease in LDL (3±0.71) values.

In electrolytes study (table 3) significant control on sodium, potassium and chloride was observed at low dose (10mg), while potassium and bicarbonate show significant control at high dose (25mg) while insignificant values of sodium and chloride.

Fig. 2 of table 2 shows that *M. charantia* with seeds decreases total cholesterol at lower dose i.e. 10mg and also at higher dose 25 mg as compared with control readings. And it gives significant $p<0.0001$ values for increase in HDL and decrease in LDL at higher dose (25mg)

DISCUSSION

The main constituents of *M. charantia,* which are responsible for these effects are such as triterpene, proteins, steroid, alkaloid, inorganic, lipid, and phenolic compounds. The protein in bitter melon including protein MAP-30, α-momorcharin, and β-momorcharin were shown to have the ability for fighting against HIV. A steroid, charantin, contained mainly in the aerial parts, has been proven for its anti-diabetic activity. The phenolic compounds from bitter melon extracted by solvent extraction were reported to exhibit antioxidant activity (Saeed *et al*., 2010).

The significant change in the hematological values (table 1) after dosage indicates that the drug has significant impact on haemopoetic system. There is also a significant increase in count of thrombocytes, increase number of platelets is due to the stimulation of thrombocyte, the results call for extending the studies to isolation and identification of a particular phytochemical present in *M. charantia* which is responsible for increase in platelets count. It contains polypeptide-P that is natural insulin, which might be responsible for lowering the level of blood sugar (Saba *et al*., 2009).

In reference to (table 2) the lipid profile of test drug, difference of result in LDL and HDL is due to the oxidation of glucose that lowers the level of glutathione. These effects are due to high level of circulating glucose that resulted in high levels of LDL and triglycerides and low level of HDL (Chaturvedi and George, 2010). In our study *M. charantia* with seed shows this effect at lower dose (10mg) and at higher dose (25mg) it shows high level of HDL and lowered level LDL as compared with control.

Increased glucose oxidation has been shown to produce membrane damage by membrane lipid per oxidation and protein glycation (Gayathri and Kannabiran, 2008). This could be the reason for the altered flux in electrolyte balance. *M. charantia* restores the electrolyte balance by reducing lipid per oxidation index and retaining anti oxidants, this could be the reason for restoration of electrolytes when compared with control.

CONCLUSION

From this study, it is concluded that *M. charantia* with seed has efficacy to increase platelet counts at low dose and Hb level in high dose as well as in low dose controls cholesterol, triglycerides, HDL, LDL and VLDL levels signifying in blood purifier activity.

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