



## Assessment of Ascorbic Acid and Some Minerals from the White Table Grapes (*Vitis vinifera* L.) Collected from Various Markets of District Khairpur

ASLAM KHAN<sup>1</sup>, GHULAM QADIR SHAR<sup>1</sup>, ABDUL RAHEEM SHAR<sup>2\*</sup>, RABIA PARVEEN MEMON<sup>1</sup>, ATTA HUSSAIN RIND<sup>2</sup>, SAHIB GHANGHRO<sup>1</sup>

<sup>1</sup>Institute of Chemistry, Shah Abdul Latif University Khairpur

<sup>2</sup>Government Degree College, Thari Mirwah

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### Corresponding author

[araheem.shar@salu.edu.pk](mailto:araheem.shar@salu.edu.pk)

### ABSTRACT

The current study examines the physical and chemical composition of white grapes collected from various local markets of district Khairpur. The samples from 10 towns i.e., Hingorja, Khairpur, Thari Mirwah, Sobhodero, Kotdiji, Setharja, Kumb, Ranipur, Gambat, and Suigas. were collected and labeled as G1 to G10 respectively. The samples were analyzed for Physical parameters (pH & Moisture) and chemical parameters (Vitamin C, potassium, magnesium, sodium, calcium and iron) by standard methods. The pH was observed in the range of 3.80 to 4.40 while the moisture was observed as 76.70% to 81.30% in grapes collected from different markets of study area. The range of Vitamin – C collected from samples was observed as 5.23 - 6.82. UV-Visible spectrophotometer was used to measure the concentration of Vitamin C and Atomic absorption spectrophotometer was used to determine the concentration of metals. The range of selected minerals measured was found as, K<sup>+</sup> (33.47 mg/kg - 38.46 mg/kg), Mg<sup>2+</sup> (73.87 mg/kg - 78.26 mg/kg), Na<sup>+</sup> (24.23 mg/kg - 27.78 mg/kg), Ca<sup>2+</sup> (45.98 mg/kg - 50.23 mg/kg) and Fe<sup>3+</sup> (2.88 mg/kg - 4.24 mg/kg). Concentration order of chemical parameters was found as, Magnesium > Calcium > Potassium > Sodium > Vitamin C > Iron. The highest concentrations of Vitamin C, Mg and Fe measured in samples were collected from Kumb town. The higher concentration of K<sup>+</sup> and Ca<sup>2+</sup> were found from samples of Gambat, while higher Na content was found from samples of Ranipur. Therefore, current work may provide valuable information to diet conscious and nutritionists to remove vitamin C and mineral deficiency in their diet.

**Keywords:** Moisture, Vitamin C, minerals and Atomic absorption.

### INTRODUCTION

Citrus fruits are highly significant sources of medicines and foods which can be classified as climacteric and non-climacteric. Avocados, pears and bananas are included in climacteric category whereas strawberry, pineapple, melon, orange, lemon, and grapefruit are included in non-climacteric category (Mansour., 2019). Grapefruit, lemon, and orange are rich sources of ascorbic acid (Batista-Silva et al., 2018). Antiscorbutic vitamin, L – ascorbic acid or Vitamin – C are other names of ascorbic acid. Vegetables and fruits supply > 90% of vitamin C in human diet. Most of the organisms synthesize vitamin C from glucose but so many species including man and other animals must get it from their foodstuff (Phillips et al., 2018). A very important role is played by vitamin C in human body to protect from various infectious diseases. It is significant in synthesizing of collagen, carnitine, steroid hormones and neurotransmitters and bile acid synthesis from cholesterol, also increases bioavailability of iron (Motora, 2017).



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It also possesses anti – fungal, anti – tumor, antioxidant, anti-inflammatory and inhibition performance (Siddique, et al., 2021). The deficiency disease named scurvy may be caused due to lack of vitamin- C (Gandhi, et al., 2023). Its deficiency may also cause weakness, edema, meager wound healing and hemorrhages under the skin (Epigenetics). Grapes are found attached to the stem and belong to the berry family. Clusters or bunches of grapes are made various berries. The fruit of the grape possesses various medicinal and nutritional properties for customers and is one of the most pleasant eatable foods. Fruit is good sources of fat (0.3 to 0.4%), proteins (0.5 to 0.6%), carbohydrates (12 to 18%) and moisture (82%). Moreover, significant amounts of minerals and vitamins are also present in the fruit such as K (0.1 to 0.2%), Ca (0.01 to 0.02%), P (0.08 to 0.1%), vitamin A (0.001 to 0.0015%) and vitamin C (0.1 to 0.2%). Grape (*Vitis vinifera L.*) is a fruit which is utilized on large scale all over the world. In addition to being a well-liked table fruit, it is also used as raw material to generate wine all over the world. Literature reveals that better health conditions of heart are associated to phenolic compounds in grapes in the inhabitants of Mediterranean area further enhancing the consumption of grapes (Apostolou et al 2013). At present millions of tons of grapes are generated all over the world producing stems as by-products (Fuchs et al., 2020). Generally open areas are selected to discard these by-products or may be used as compost, creating atmospheric threats because of its polyphenolic contents (Teixeira, et al., 2014). However, serious problems may be caused due to the accumulation of these by-products, which require recycling plans to get ecological satisfying yields (Duan, Xet al., 2012; Aravindhnan, et al., 2009). Consequently, the scientific community wants to use the by-products from wine industry to obtain phenolic compounds to be utilized in different areas (Gouvinhas, et al., 2018; Piñeiro, et al 2013). Recent studies have shown that degenerative diseases can be prevented by using phytochemicals because of their antioxidant properties along with anti – cancer, anti-inflammatory and antimicrobial properties (Tsao, 2010; Salehi, et al., 2019).

Various factors may influence the mineral content in grapes and wine such as characteristics of soil mineral quantity, accumulation by the shoot and fruit, climatic influences on transpiration rate and uptake temperament of the rootstock (Jackson, 2008)). Such minerals are essential for human beings' food, and consequently are nominated as essential elements. Various elements form this group like Sodium, Potassium, Magnesium, Calcium, Zinc and Iron and others as well which are required in trace amounts for instance, Selenium, Cobalt, Nickel, Chromium, Manganese and Copper (Mir-Marques, et al., 2016;

Añón et al., 2014). Major role is played by essential elements in our body to work in appropriate way, suppose the health of teeth and bones and structural parts of enzymes is contributed by calcium, magnesium, potassium, manganese, and selenium (Alegría-Torán et al., 2015). For body immunity, enzyme reactions, cell growth, copper, zinc, iron and cobalt elements are highly important (Kim et al 2019). Essential elements play a vital role in a various biological, chemical, and physical processes such as building block of life in which essential elements like carbon hydrogen, oxygen and nitrogen form the basis of biomolecules like carbohydrates, proteins, and nucleic acids. Iron, Zinc and copper are crucial for enzyme function, which facilitate various metabolic function reactions (Jomova, K et al., 2022) In oxidation-reduction reactions iron, copper and manganese are used which are important for energy production and other cellular processes. Sodium, potassium, and chloride helps to maintain proper nerve function and transmission of nerve impulse. Calcium, phosphorous and manganese are essential for maintaining strong bones and teeth. Iron, zinc and selenium support immune system function and help protect against infections. Selenium, manganese and copper have antioxidant properties, protecting cells from damage caused by free radicals. Iodine, zinc and chromium are involved in hormone regulation and balance (Kostova, I., 2023) Calcium, potassium and sodium play roles in cellular signaling pathways. Furthermore sodium, potassium and chloride help in regulate fluid balance and blood pressure. These are just a few examples of impotence of essential elements (Ali, 2023).

The main purpose of this study is to analyze the vitamin C content and mineral content from grapes consumed in the study area. As well as to compare the contents with literature reported at national and international level.

## **MATERIALS AND METHODS**

### **STUDY AREA**

District Khairpur belongs to division Sukkur, province Sindh of country Pakistan. The population of district Khairpur was found at 5<sup>th</sup> number according to the 2017 census. Khairpur city is the headquarter of district Khairpur. There are eight Talukas or sub – districts such as, Nara, Faiz Ganj, Gambat, Sobho Dero, Kingri, Kot Diji, Mirwah and Khairpur. The longitude and latitude of district Khairpur is extended as 68.77°E and 27.53°N. The geographical area of 15,910 square kilometres is covered by study area and it is located at a height of 50 m above sea level. The average annual rain fall in the area is about 12 mm, while climate is generally hot as well as dry in

summer, and temperature range is found as 27 – 47 °C (Abbasi et al., 2017).

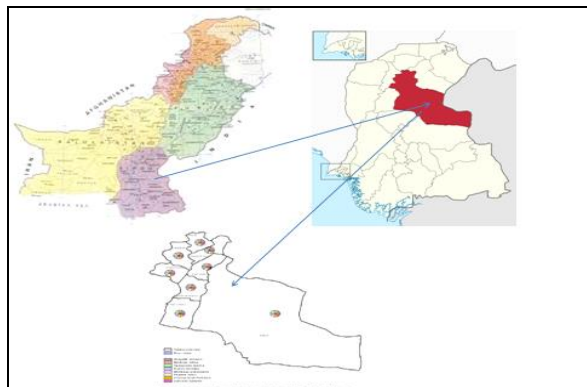


Figure 1 Map of area under study from where samples of grapes were purchased

### SAMPLE COLLECTION

Samples of grapes were collected from ten markets of district Khairpur. Markets included Higorja, Khairpur, Thari Mirwah, Sobhodero, Kotdiji, Setharja, Kumb, Ranipur, Gambat and Suigas (Table 1).

S. No	Sample Location	S. ID	S. No	Sample Location	S. ID
1	Hingorja Town	G1	6	Setharja	G6
2	Khairpur City	G2	7	Kumb	G7
3	Thari Mirwah	G3	8	Ranipur	G8
4	Sobhodero	G4	9	Gambat	G9
5	Kotdiji	G5	10	Suigas	G10

Three replicates of each sample were collected from each market and at least one kilogram was bought for the purpose of analysis. All grape samples were washed carefully with tap water and then de-ionized water to remove dust particles and adhering contaminants. Composite samples were prepared by mixing all samples collected from ten markets. Samples were prepared for analysis of vitamin – C and minerals like, Iron, Sodium, Calcium, Magnesium and Potassium.

### MATERIALS

Materials used in this study included glacial acetic acid, de-ionized water, meta-phosphoric acid, 2,4-

dinitrophenylhydrazine, thiourea, sulphuric acid, ascorbic acid and bromine water. All reagents and chemicals used in this study were A.R grade.

### STANDARD VITAMIN – C SOLUTION

Stock solution of 1000 mg/L was prepared from ascorbic acid. 1.000g of ascorbic acid was weighed accurately and dissolved in de-ionized water. The volume of the solution was made up to the mark in 1000 mL measuring flask using de-ionized water. The calibration curve was prepared by diluting the stock solution of known strength.

### SAMPLE PREPARATION FOR VITAMIN C

Grape samples were dried in an oven to remove water content completely. Mortar and pestle were used to grind the dried samples to make powder. In a test tube 10g of each sample was taken, and then reagent of meta-phosphoric acid – acetic acid solution was mixed. Contents of test tubes were shifted to 100 mL volumetric flasks and then shaken vigorously. The volume of the measuring flasks was made up to the mark by adding the reagent meta-phosphoric acid – acetic acid. Sample solutions were filtered, centrifuged at the rpm of 4000 for 15 minutes and vitamin C was analyzed using double beam UV-Visible spectrophotometer (CECIL CE 9500).

### SAMPLE PREPARATION FOR MINERALS

Collected samples from selected locations were washed with water to remove dirt particles and other contaminants. Complete moisture content was removed by oven dried. Mortar and pestle were used to grind the dried residues to make powder. From powdered samples 2.00 g were weighed accurately and transferred to conical flasks. Concentrated nitric acid (10 mL) was added to powdered samples kept in conical flasks. Samples were digested using electric hot plates, after total evaporation of acid; 5 mL of hydrogen per oxide was added and heated again on electric hotplates. Contents of conical flasks were dissolved, and volume was made up to the mark with 50 mL of de-ionized water and evaporated again, total volume of 40 mL was made by adding de-ionized water. The 2% nitric acid solution was used to prepare all metals under study.

### PROCEDURE VITAMIN C ANALYSIS

To filter solution bromine water was added. To remove excess of bromine and color of solution few drops of thio-urea were mixed. To all samples plus standard solutions 2,4-dinitrophenylhydrazine was added. In 100 mL volumetric flask volume was made up to the mark with acetic acid. All samples were then analyzed by UV-Visible double beam

spectrophotometer (*CECIL CE 9500*) and the absorbance was taken at  $\lambda_{\max}$  of 521 nm to determine vitamin- C from grapes. Absorbance of blank and standards was taken at  $\lambda_{\max}$  of 521 nm for construction of calibration graph to measure the concentration of vitamin – C from grapes.

### MINERAL ANALYSIS

The atomic absorption spectrophotometer was used to measure the concentration of iron, calcium, magnesium, sodium and potassium. Hollow cathode lamps of iron, calcium, magnesium, sodium and potassium were used to measure the elements of interest. Samples were handled with great care to stay away from cross contamination. All reagents were of AR grade and glassware was washed appropriately. De-ionized water was employed throughout the current work.

### STATISTICAL ANALYSIS

Statistical analysis such as minimum, maximum, mean, standard deviation was carried out with the help of SPSS software version 18 installed in the laptop computer. Microsoft excel was also used for statistical analysis.

### RESULTS & DISCUSSION

The pH or hydrogen ion concentration measures the acidic character of the citrus fruits. Grapes and other citrus fruits are suggested to consume with sign to their acid content of pH level about the effect on gastrointestinal tract, intestine and stomach (Türkyılmaz, M. 2013). Grapes were collected from ten selected markets of district Khairpur. The pH of the samples was measured by getting fruit juice from each sample with help of pH-meter (*HANNA INSTRUMENTS*). The pH range grapes collected from study area was measured as 3.8 – 4.4 whereas, moisture content was found in the range of 76.70 - 81.30% shown in Table 2. The order of pH and moisture content was found as, G8 = G6 = G4 > G1 > G2 > G5 > G9 > G3 = G10 > G7 and G8 > G6 > G3 > G1 > G2 > G4 > G10 > G7 > G9 > G5 respectively.

### VITAMIN C

The hexuronic acid, cevitamin acid or xiloascorbic acid are other names for vitamin C however, representative name is ascorbic acid (Türkyılmaz, 2013).L. ascorbic acid or antiscorbic vitamin is also used to express ascorbic acid. Enough ascorbic acid is found in grapes, lemon and orange (Du, et al., 2012). Rich source of vitamin – C include fruits and vegetables, greater than 90% of which is consumed for human food. Maximum upper level of vitamin C is 1.0 g/day, while greater than this limit may cause heart disease, affect the production of cholesterol plaque,

destruction of internal lining of arteries disease (Pehlivan, 2017). Generally, vitamin C is considered harmless for sustaining sound health and for avoidance from ordinary cold. Vitamin C should be present in large amounts in the human body (Lee, 2020). Scurvy is the disease caused due to the deficiency of vitamin C whose symptoms may include joint swelling and pain, tooth and hair loss, bruising and bleeding; poor wound healing, and increased risk of cardiovascular diseases, weakness and edema. Ascorbic acid deficiency is also related to colds, bronchial infection, poor digestion and lack of energy. These symptoms may be associated with the weakening of the bone, connective tissues and blood vessels which contain collagen (Bechara et al., 2022). Due to the lack in the hydroxylation of collagen, various deficiency symptoms may be described resulting in malfunctioning connective tissues (Tveden-Nyborg, 2021). Production of cancer-causing compounds may be slow down due to vitamin C (Caritá et al., 2020).

Samples of grapes collected from ten markets of districts Khairpur showed minimum and maximum concentration of vitamin C as, 5.23 mg/kg and 6.82 mg/kg from market G8 and G7 respectively. The order of concentration of vitamin C in grape samples collected from different markets of district Khairpur was found as, G7 > G3 > G10 > G2 > G5 > G1 > G4 = G9 > G6 > G8 shown in Table 2.

### MINERAL ELEMENTS

Minerals are highly important in human health such as calcium, magnesium and phosphorus are very important in the construction of teeth and bones. Numerous minerals control cell composition and body fluids for instance, sodium, chloride, potassium, magnesium and phosphorus (Yilmaz et al., 2020).Potassium is an important mineral which maintains acid balance and body fluids. It transmits nerve impulses to muscles and maintains muscle contraction and blood pressure. Approximately 200 mg of potassium is required for the human body on a daily basis, while its deficiency is scarce, higher Na/K ratio of consumption may cause chronic disease (Adnan et al., 2019). The concentration range of potassium in grapes of study area was found as 33.47 - 38.46 mg/kg, whereas average concentration was found as 35.94 mg/kg shown in Table 3. The order of concentration in different markets of district Khairpur was observed as, G9 > G4 > G10 > G2 > G3 > G1 > G6 > G7 > G5 > G8 shown in Table 3. Various side effects may be caused due to deficiency of magnesium in the human body because of electrolyte disturbance (Ju et al., 2014). Symptoms include tremors, poor coordination, muscle fits, loss of appetite, identity changes and nystagmus (Hansen and Bruserud, 2018).

**Table 2. Moisture, pH and Vitamin C contents of grapes collected from different markets of district Khairpur**

Sample	% Moisture	pH	Vit-C (mg/kg)
G1	78.8 ± 0.54	4.3 ± 0.33	5.52 ± 0.53
G2	78.4 ± 0.46	4.2 ± 0.32	5.76 ± 0.37
G3	79.3 ± 1.23	3.9 ± 0.34	6.75 ± 0.65
G4	77.9 ± 2.21	4.4 ± 0.42	5.45 ± 0.44
G5	76.7 ± 0.98	4.1 ± 0.28	5.65 ± 0.45
G6	80.2 ± 1.78	4.4 ± 0.38	5.34 ± 0.45
G7	76.9 ± 2.28	3.8 ± 0.36	6.82 ± 0.48
G8	81.3 ± 0.93	4.4 ± 0.41	5.23 ± 0.25
G9	76.8 ± 1.43	4.0 ± 0.39	5.45 ± 0.43
G10	77.6 ± 1.18	3.9 ± 0.28	5.88 ± 0.53
Min:	76.70	3.80	5.23
Max:	81.30	4.40	6.82
Mean	78.39	4.14	5.79
SD	1.54	0.23	0.56

Disorder may include cardiovascular diseases; those with low level of magnesium in general may have low content of potassium (Lordan et al., 2018). Deficiency may cause diabetes mellitus, weak absorption from the digestion tracts, prolonged urinary trouble, and laxity of the bowels, liquid addiction and low dietary entrance. Various drugs are responsible for Mg deficiency for instance proton siphon inhibitors (PPIs) and furosemide (Lordan et al., 2018).

The concentration range of Mg in grape samples collected from different markets was found as 73.87 – 78.26 mg/kg. The average content of Mg was observed as, 76.04 mg/kg. The concentration order of Mg in samples of grapes was found as, G7 > G2 > G8 > G1 > G3 > G4 > G6 > G5 > G10 > G9 shown in Table 3.

Hyponatremia occurs due to sodium deficiency (Liu et al., 2020), which may occur at a lower level than 135 mmolL<sup>-1</sup> (Sterns, 2018). Mild symptoms may include weakened thinking ability, poor balance, cerebral pains and sickness while severe symptoms include coma, heart failure and confusion (George, et al., 2018; Tinawi, 2020). Effects of low hyponatremia and normal hyponatremia include looseness, regurgitating, sweating and high urine, pee respectively (Liu et al., 2020). High hyponatremia volume may cause liver damage, heart attack and kidney failure (Liu et al.,

2020). Low sodium inference may cause high fat levels in blood, high blood sugar, and high protein levels (Abebe et al., 2018). Sodium content range in samples of grapes bought from different markets of Khairpur were found as, 24.23 mg/kg - 27.78 mg/kg, while the mean sodium content was determined as 25.65 mg/kg. The concentration order of sodium was found as, G8 > G5 > G3 > G10 > G7 > G1 > G2 > G6 > G4 = G9 shown in Table 3.

**Table 3. Mineral contents of grapes collected from different markets of district Khairpur**

Sample	K (mg/kg)	Mg (mg/kg)	Na (mg/kg)	Ca (mg/kg)	Fe (mg/kg)
G1	35.46 ± 2.35	76.46 ± 4.34	25.40 ± 1.45	47.9 ± 2.28	3.34 ± 0.16
G2	36.68 ± 3.2	77.43 ± 4.45	24.65 ± 1.78	48.32 ± 2.30	3.65 ± 0.18
G3	36.33 ± 3.6	76.23 ± 4.35	26.34 ± 0.46	47.21 ± 2.25	3.86 ± 0.19
G4	37.68 ± 5.7	75.98 ± 5.45	24.23 ± 0.65	46.99 ± 2.23	3.11 ± 0.15
G5	34.33 ± 6.2	74.96 ± 5.32	27.35 ± 0.85	45.98 ± 2.18	2.88 ± 0.14
G6	34.88 ± 3.5	75.46 ± 0.76	24.64 ± 0.95	48.43 ± 2.35	4.12 ± 0.21
G7	34.76 ± 1.58	78.26 ± 0.98	25.79 ± 0.99	46.96 ± 2.28	4.24 ± 0.21
G8	33.47 ± 5.45	77.12 ± 0.89	27.78 ± 2.15	49.65 ± 2.37	3.56 ± 0.17
G9	38.46 ± 2.54	73.87 ± 2.76	24.23 ± 1.36	50.23 ± 2.40	3.78 ± 0.18
G10	37.32 ± 3.46	74.67 ± 3.26	26.10 ± 1.75	47.11 ± 2.24	3.15 ± 0.15
Min:	33.47	73.87	24.23	45.98	2.88
Max:	38.46	78.26	27.78	50.23	4.24
Mean	35.94	76.04	25.65	47.88	3.57
SD	1.61	1.35	1.26	1.31	0.45

Calcium deficiency causes hypocalcemia (hypocalcemia), however calcium concentration less than 2.1mmol<sup>-1</sup> may cause hypocalcemia (Hassan-Smith and Gittoes, 2017). Calcium deficiency side effects may include heart attack, hysteria, convulsion, muscle fits and numbness (Perrotta, 2020). Vitamin D deficiency and hypoparathyroidism may be caused due to normal Calcium deficiency (Gafni and Collins, 2019). Other symptoms include calcium channel blocker overdose, tumor, rhabdomyolysis (Gafni and Collins, 2019). Changes are observed using electrocardiograms. The concentration range of Ca was found to 45.98 - 50.23 mg/kg, while average concentration of calcium was found as 47.88 mg/kg.

Concentration order in decreasing order was found as, G9 > G8 > G6 > G2 > G1 > G3 > G10 > G4 > G7 > G5 shown in Table 3. Iron is found in liver, bone marrow and spleen, and is one of the essential elements among twenty essential elements found for growth of body. Iron is necessary for proteins in the muscles and for red platelets to allow oxygen in the body. Iron plays different roles in the body such as, safety against encounter contaminants, significant for growth of human body and cerebral development (Sukhbaatar and Weichhart, 2018). Iron is very important in adults and kids' development especially for their mind growth (Pivina et al., 2019). The range of iron content in samples of grapes bought from ten different markets of district Khairpur was observed as 2.88 mg/kg - 4.24 mg/kg, whereas the mean concentration of iron was found as 3.57 mg/kg from samples of grapes. Concentration order of iron in samples of grapes was found as G7 > G6 > G3 > G9 > G2 > G8 > G1 > G10 > G4 > G5 shown in Table 3.

### CONCLUSIONS

The average moisture and pH were found to be 78.39% and 4.14 respectively from grapes collected from ten markets of district Khairpur. The average concentration of ascorbic acid from grapes samples was observed as, 5.79 mg/kg. The average concentration of minerals like K, Mg, Na, Ca and Fe was observed as 35.94, 76.04, 25.65, 47.88 and 3.57 mg/kg respectively. It can be concluded from the present work that grapes frequently consumed in study areas are good sources of vitamin C and minerals.

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### CONFLICT OF INTEREST

Authors declare that they have no conflict of interest

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### AUTHOR CONTRIBUTION

The experimental work was conducted by Aslam Khan and Atta Hussain Rind in Institute of Chemistry, Shah Abdul Latif University Khairpur, while Dr. Abdul Raheem Shar prepared the manuscript draft which further refined by Rabia Parveen Memon and Sahib Ghanghro. The statistical analysis was conducted by Aijaz Ali Shar. Prof, Dr. Ghulam Qadir Shar supervised the whole work. All authors agreed to submit the research article in Sindh University Research Journal.

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