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A Comparative Study of the Effect of the Aqueous Extract of Some Gulf Environmental Plants on Hyperuricemic Rats

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ABSTRACT

Background and objective: This study aimed to investigate the effect of the aqueous extract of some environmental plants in the gulf area from Kuwait and Saudi Arabia, which are Al-Arfaj (Rhanterium), Sageya Salvia officinalis, and chrysanthemum on kidney function in experimentally hyperuricemic rats. **Materials and Methods:** Fifth groups (35 rats) of Sprague Dawley rats fed on basal diet supplemented with potassium oxonate (uricase inhibitor) and one group was fed on basal diet only which used as a negative control group. While the other group had raised the level of uric acid in their blood, then these rats were divided into four groups, the second group with high uric acid in the blood (the positive control group) and the third group on food supplemented with Al-Arfaj drink 10%, and the fourth group included supplemented with 10% support for sage drink, and the fifth group received 10% supplemented with chrysanthemum for a period of four weeks. **Results:** From the study showed that feeding rats infected with high uric acid blood on a food supplemented with a sage drink 10% for a period led to an increase in body weight and the percentage of food use. It also led to a significant decrease in the concentrations of uric acid, urea and creatinine in the serum than other groups. It also produced a good effect on renal histology as it ameliorated the renal damage caused by elevated serum uric acid. **Conclusion:** This study suggests that the aqueous extract of study plantsat 10% may be beneficial to patients suffering from hyperuricemia and kidney disease.

INTRODUCTION

Plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to prevent diseases. The knowledge of their healing properties has been transmitted over centuries within and among human communities. Active secondary metabolites are usually responsible for the biological properties of some plant species used throughout the globe for various purposes, including treatment of infectious diseases (Silva & Fernandes., 2010).

Rhanterium species are globally distributed over Western, North Africa, Afro–Asian countries, the Arabian Peninsula, Iraq and Iran (Kala *et al*., 2009 & Shama *et al*., 2008)**.** In Saudi Arabia, genus Rhanterium is represented by *R.epapposum*, which is a perennial dwarf shrub, with richly branched pale stem, up to 70 cm height, tiny narrow leaves and yellow flowers 1.5 cm wide, cupped in a soft-spiny involucre (Chaudhary., 2001). *R. epapposum* is distributed in Saudi Arabia mainly in northern region (Migahid., 1996), Kuwait (Omar & Bhat., 2008), north-eastern parts of the United Arab Emirates (Hellyer and Aspinall., 2005), Iran (Kala *et al*., 2009) and Sudan (Shama *et al.,* 2008).

Rhanterium eppaposum Oliv. (Arfaj) the national plant of Kuwait is a perennial bushy shrub and the national flower of Kuwait and Saudi Arabia. It is one of the most palatable forage plants and an excellent performer for desert rehabilitation. Due to several anthropogenic activities and climate change, this native plant has suffered severe degradation. (Fadila *et al.,* 2020)

Rhanterium epapposum (Asteraceae), locally known as Al-Arfaj is prevalent in various parts of Kuwait, Saudi Arabia and other Afro-Asian countries. The Arfaj flower is also the national flower of Kuwait. It is a bushy shrub approximately 80 cm height (Marwa and, Abdelrhman., 2016). The leaves are small and narrow, and in late spring it will start flowering (April–May). It is considered one of the main desert forage plants for camels, sheep and used in folk medicine by people in rural areas as a remedy for skin infections and gastrointestinal disturbances and as an insecticide. Phytochemical analysis of the aerial parts of *R. epapposum* showed the presence of flavonoids, tannins, sterols, triterpenes and volatile oils (AL-Yahya *et al*., 1990).

The sage *Salvia officinalis* L. (Lamiaceae), locally called meramiya, is a perennial sub-shrub native to the Mediterranean area but is known all over the world. (Ahmad and Mahdi, 2017). The plant grows in various locations in Jordan and is used locally in folk medicine and in

cosmetics. *S. officinalis* has been an important medicinal plant since earliest times (Perry *et al*., 1999). This plant species is very rich in biologically active compounds and many studies have indicated its increasing medicinal importance; it is used for the treatment of various ailments, including the relief of menstrual problems such as cramp, as well as regulation of the menstrual cycle in amenorrhea due to its estrogenic substances (De Leo *et al*., 1998).

Salvia officinalis L. (Sage), a member of the family of Lamiaceae, has been reported to have a wide range of biological activities, such as antioxidant, antibacterial, hypoglycemic and anti-inflammatory properties. Recent studies have found that the Sage has positively physiological effects on heart, liver, kidney and testes (Abd El Fattah *et al*., 2013).

The plant *Chrysanthemum indicum* is the subspecies of *Afro americanium* commonly known as a wild daisy in English, is a natural herbaceous plant which is part of Asteraceae family. It is widely consumed in Northcentral and Northeastern part of Nigeria as herbal tea that helps in indigestion and gastrointestinal tract disturbance (Turner., 1965). *Chrysanthemum indicum* extract has been used for a number of medicinal conditions including recovery from influenza, for treating yellow fever. According to folklore medicine, Chrysanthemum tea is used for various veins and atherosclerosis treatment. It was also said to be effective for inhibiting the agglutination of blood platelets and promote myocardial blood circulation and white blood phagocytosis and it is used in treating many diseases such as furuncle (Hussaini *et al.,*2018). This plant has been also used as an herbal medicine used as anti-inflammatory, analgesic, antipyretic purposes and the treatment of eye diseases. It is also known to showed inhibitory activity against rat's aldose reductase and against nitric oxide production in lipopolysaccharides activates macrophages (Ghani., 2003).

From the previous concept, the present work was a trial to spot the light on the beneficial effects of Rhanterium, Salvia and Chrysanthemum beverages on the body weight, food efficiency ratio, and renal function in hyperuricemic rats.

MATERIALS AND METHODS

Materials:

Plants: Rhanterium, Salvia and Chrysanthemum leaves were obtained from a local market of Herbs and Medicinal plants, Mubarkya, Kuwait. The selected plant materials were air-dried, grinded in an electrical blender into a fine powder which packed in plastic bags till use for beverages supplementation. The chemical composition for plants is given in table 1.

Potassium oxonate: It was obtained from Al-Saudi Company & Chem., Riyadh as a pure white powder packed in bottles each containing 25 g.

Rats: Thirty-five adult male albino rats of Sprague Dawley strain weighing 150-165 g body weight were used in this study. Rats were obtained from Laboratory Animal Colony, of College of Science, Kuwait University, fed on basal diet and provided with water *ad libitum*.

Chemicals: Casein, all vitamins, minerals, cellulose and choline chloride were obtained from Al-Saudi Company & Chem., Riyadh.

Methods:

Plant extraction: The plant was shade dried and pulverized to dry powder using wood mortar and pestle with continual pounding and sieving. About 100 g of the powder plant material was macerated in 400 ml of distilled water for 24 hours and was filtered using Whatman No 1 filter paper. The filtrate was evaporated at 40-50^{o}C on a water bath to get a solid extract and was kept in the refrigerator until when the need arises water extracts of Plants. The Plants water extracts were prepared as the method described by (chan *et al;* 1999).

Preparation of basal diet:

Basal diet was prepared according to (Reeves *et al;* 1993). It consists of 20 % protein (casein), 10% sucrose, 4.7% corn oil, 2% choline chloride, 1% vitamin mixture, 3.5 % salt mixture and 5% fibers (cellulose). The remainder was corn starch.

Induction of hyperuricemia:

It was induced by feeding rats on the basal diet containing 2 % potassium oxonate (salt of oxonic acid) which is uricase inhibitor for 6 weeks before start of the experiment according to the method described by (Stavric & Nera; 1978). After feeding period, a random blood sample was withdrawn from the orbital sinus of the eye and serum uric acid was measured to insure incidence of sustained hyperuricemia.

Experiments and grouping of rats:

Thirty-five male albino rats were used in this experiment. Rats were divided into 5 groups of 7 rats. Group (1) was fed on the basal diet and kept as a negative control, while the other groups were fed on 2 % potassium oxonate supplemented diet for 6 weeks for induction of hyperuricemia in rats. Group (2) was left as a positive (hyperuricemic) control, while groups (3), (4) and (5) were fed on the same experimental diets supplemented with 10 % of plant beverages. After the end of experiment period (4 weeks), at end of the experimental period, rats were fasted overnight, then anaesthetized & incised longitudinally and blood samples were collected from the aorta. The blood samples were centrifuged and serum was separated to estimate some biochemical parameters, i.e. urea, creatinine and uric acid concentrations.

Biochemical analyses:

The collected serum samples were used for estimation of uric acid using enzymatic colorimetric method described by (Fossati *et al*; 1980), urea nitrogen using BioMerieux kits according to (Fawcett & Scott; 1960) and creatinine using kits of Bio Merieux by the Jaffe-reaction according to the method of (Husdan & Rapoport; 1968).

Histopathological examination:

Kidneys of the scarified rats were dissected, removed and fixed in 10% formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. These specimens were cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness and stained with Hematoxylen and Eosin (H & E) then examined microscopically according to (Carleton; 1979).

Statistical analysis:

Data are presented as means ± SD and the analysis was conducted using SPSS program, Version 16.0 (2007).

RESULTS AND DISCUSSION

As shown in Table (2), feeding normal rats on diet supplemented with 2% potassium oxonate for 4 weeks significantly increased body weight gain (BWG%) and feed efficiency ratio (FER). Feeding hyperuricemic rats on diet supplemented with 10 % Rhanterium beverage decreased both BWG% and FER, while diets supplemented with 10% Chrysanthemum beverage and 10% Salvia beverage significantly increased both BWG% and FER. The decrease in body weight by Rhanterium beverage, in the present study, was similar to that reported by (Chanda *et al*; 2015) who concluded that rhanterium beverage is valuable in weight loss diets as it provides low calorie content.

On the other side, the increase in body weight obtained by Chrysanthemum beverage was nearly similar to that reported by (Wojcikowski *et al;* 2004) for Chrysanthemum extract and by (Nandy and Datta; 2012) for Salvia extract.

Rhanterium, Salvia and Chrysanthemum

The biochemical results demonstrated in Table (3) showed that feeding diets supplemented with either Rhanterium, Salvia and Chrysanthemum beverages 10 % significantly decreased the concentrations of uric acid, urea nitrogen and creatinine in the serum. These findings are in agreement with those reported by (Ashafa *et al*; 2011) who concluded that Salvia was effective as a mild diuretic, Salvia is considered to eliminate excess water accumulation in the body. This can help in reducing the blood urea and uric acid levels, thereby maintaining the nitrogen balance and markedly improves kidney function. Salvia is purported to have several health benefits, including possibly aiding in digestion (Bozin *et al*; 2006).

On the other side, Salvia is also known as a diuretic. It is believed that it can help clear the kidneys with its anti-oxidant and anti-inflammatory properties. The potassium and sodium in Chrysanthemum are an excellent means for balancing fluid in the body. Also, the diuretic effect of Chrysanthemum juice also aids the breaking and elimination of urinary and gall bladder

stones. (Jung; 2009), In addition, (Hamia *et al*; 2013) suggested that the beneficial property of Rhanterium beverage on the kidney is due to its high content of chromium reported that feeding raw and processed Rhanterium to rats with acute renal failure significantly improved renal function as it decreased the elevated levels of uric acid, urea and creatinine in serum of these rats. These findings agree with those reported by (Salah *et al*; 2016) for Salvia and by (Ivana *et al;* 2011) for Rhanterium. The improvement in renal function with the reductions in levels of uric acid, urea and creatinine in serum of hyperuricemic rats and this confirms the improved renal function reported in the present study.

The biochemical observations were supplemented by histopathological examination of kidney sections of hyperuricemic rats. Histopathological examination of the kidney of normal (negative control) rats revealed normal histological picture of renal parenchyma as shown in Fig. (1). Examination of kidney of positive control (hyperuricemic) rats showed severe degeneration of renal parenchyma, vacuolation of endothelial lining glomerular tufts and epithelial lining renal tubules with presence of protein material in Bowman's capsules (Fig.2). The kidney of rats fed on basal diet supplemented with 10% rhanterium beverage showed only vacuolar degeneration of renal parenchyma (Fig.3). Rats fed on diet supplemented with 10% salvia beverage had almost normal histology of renal parenchyma as demonstrated in Fig. (4). Examination of the kidney of hyperuricemic rats fed on basal diet supplemented with 10% chrysanthemum beverages revealed improvement of renal parenchyma as shown in Fig. (5). These findings agree with those reported by (Amel *et al*; 2020) for rhanterium and by (Po-Jung *et al;* 2017) for chrysanthemum and (Fatma *et al;* 2017) for salvia. The improvement in renal histology goes parallel with the reductions in levels of uric acid, urea and creatinine in serum of hyperuricemic rats and this confirms the improved renal function reported in the present study.

CONCLUSION

This study suggests that the aqueous extract of Rhanterium, Salvia and Chrysanthemum at 10 % for 4 weeks may be beneficial for patients suffering from hyperuricemia and kidney disease as it lowers the levels of uric acid, urea nitrogen and creatinine in serum of hyperuricemic rats. Moreover, it has a good effect on the renal damage caused by elevated serum uric acid in hyperuricemic rats. Moreover, it has an excellent effect on the histology of kidney as it ameliorates the renal damage caused by elevated serum uric acid in hyperuricemic rats.

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Figures:

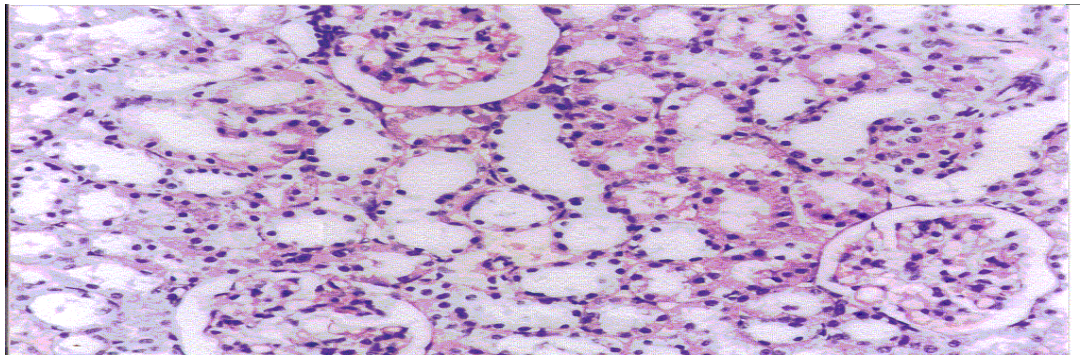

Fig.1: Kidney section from control negative rats showing apparently healthy renal tissue, (H+E X200).

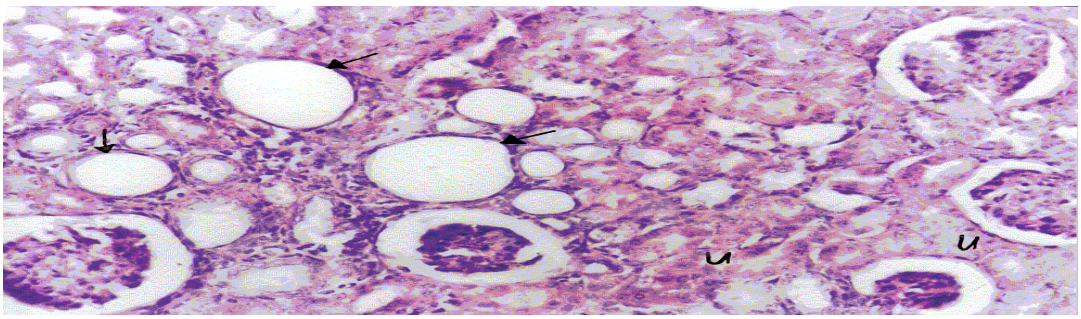

Fig.2: Kidney section from rats suffered from kidney failure (control +ve) demonstrating cystically dilated tubules (arrows) compensating the other necrosed ones, (n) (H+E X200).

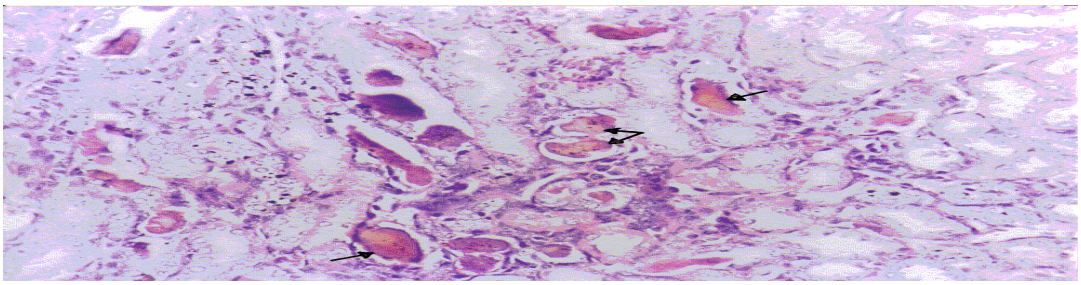

Fig.3: Kidney section from rats suffered from kidney failure (control +ve) displaying hemoglobin tubular nephrosis, (arrows), (H+E X200).

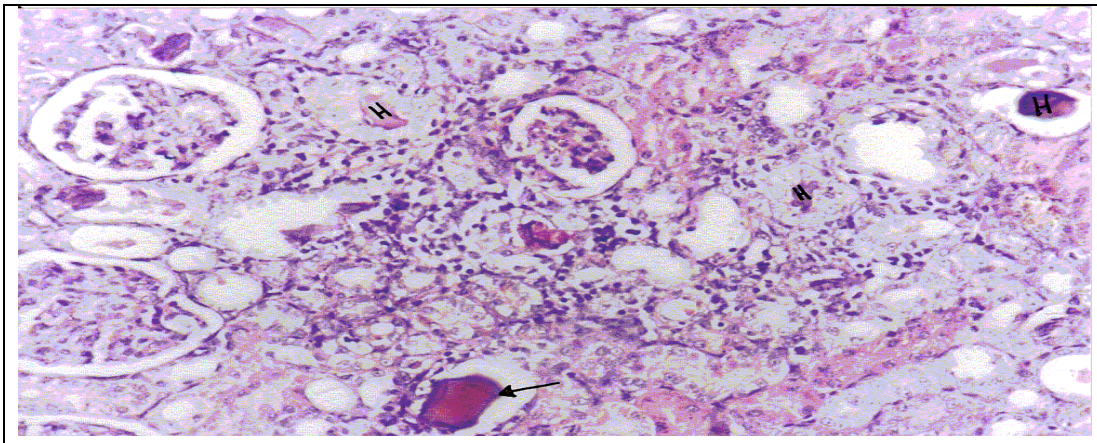

Fig.4: Kidney section from diseased rats given Rhanterium showing diffuse glomerulo-tubular nephrosis accompanied with both hyaline (H), and hemoglobin casts, (arrow), (H+E X200).

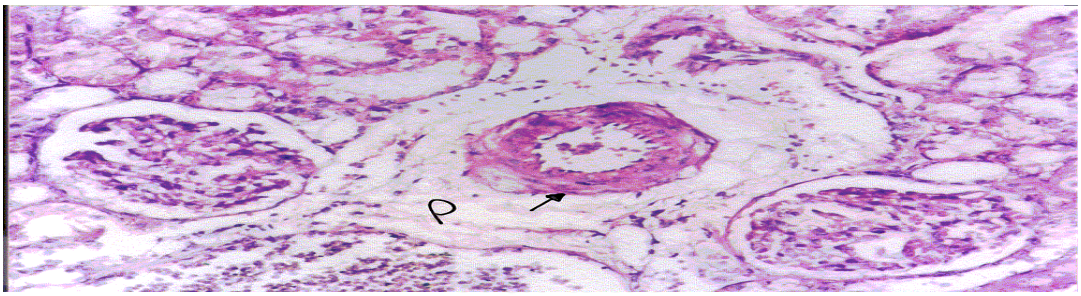

Fig.5: Kidney section from rats given Chrysanthemum displaying moderate vasculitis (arrow) perivasculitis (P) (arrows), (H+E X200).

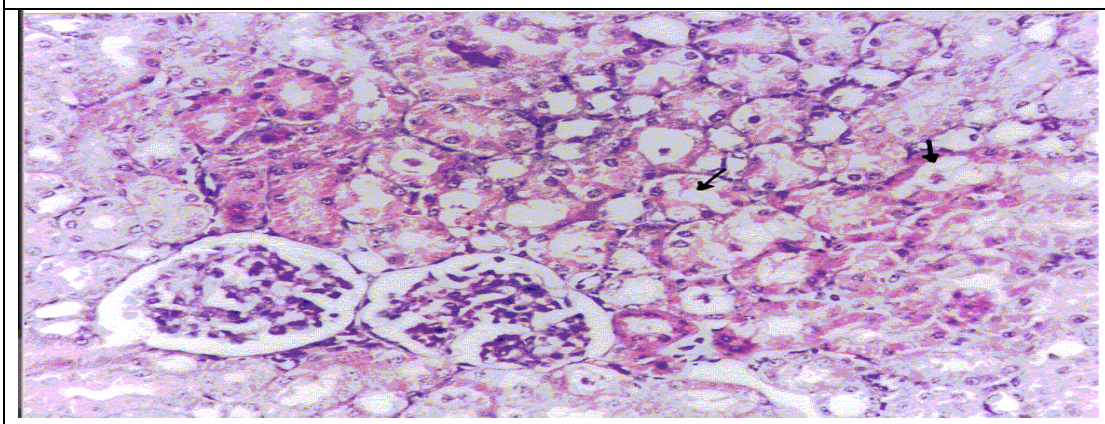

Fig.6: Kidney section from diseased rats treated by Salvia demonstrating mild tubular vacuolation, (arrow), (H+E X200)

Tables:

Table No. 1: Nutritional value of Rhanterium, Salvia and Chrysanthemum per 100 g

Nutrient	Amount in plants		
	Rhanterium	**Salvia**	***Chrysanthemum***
Energy	241.2 kcal	315	90 kcal
Carbohydrate	44.2 g	63.2g	22 g
Protein	4.5	07.63 g	0.0g
Ashe	9.6 g	2.6 g	0.0 g
Fats	3.5	1.270 g	0.0g
vitamin C	8.40 mg	0.0 mg	200.0 mg
vitaminB6 (pyridoxine)	0.10 mg	0.3 mg	Traces
vitamin B1 (thiamin)	0.06 mg	0.2 mg	Traces
VitaminB2 (riboflavin)	0.05 mg	0.1 mg	Traces
vitamin A	160.80 IU	Traces	Traces
vitamin K	35.26 mcg	Traces	Traces
potassium	344.4 mg	280 mg	136 mg
manganese	0.12 mg	Traces	Traces
Calcium	48.00 mg	29.0 mg	200.0 mg
phosphorus	30.00 mg	221 mg	Traces
magnesium	13.20 mg	79.0 mg	Traces
Iron	0.48 mg	2.5 mg	Traces

Table No. 2: Effect of aqua supplementation with Rhanterium, Salvia and Chrysanthemum on food intake (FI), body weight gain (BWG %) and food efficiency ratio (FER) in hyperuricemic rats

Groups	FI (g)	BWG (%)	FER
Negative Control	9.11 ± 0.39	11.07 ± 0.22	1.07
Positive Control	12.92 ± 0.51**	15.77 ± 0.29 **	1.22
Rhanterium **(10%)**	11.32 ± 0.09**	14.08 ± 0.11**	1.15
Salvia **(10%)**	13.97 ± 0.78*	17.88 ± 0.09*	1.25
Chrysanthemum **(10%)**	13.17 ± 0.11*	18.04 ± 0.33*	1.27

Values denote means ± SD. ***** Significant at $p < 0.05$ ****** Significant at $p < 0.01$

Table No. 3: Effect of diet supplementation with some plant materials on serum levels of uric acid, urea nitrogen and creatinine in hyperuricemic rats. (n= 7 rats)

Groups	Uric acid ((mg / dL)	Urea nitrogen (mg / dL)	Creatinine (mg / dL)
Negative Control	3.07 ± 0.02	1.72 ± 0.05	18.93 ± 0.04
Positive Control	6.18 ± 0.04**	3.31 ± 0.06**	22.76 ± 0.09 **
Rhanterium **(10%)**	4.57 ± 0.04*	2.11 ± 0.07*	19.65 ± 0.05*
Salvia **(10%)**	3.87 ± 0.03 *	1.93 ± 0.03*	19.03 ± 0.03*
Chrysanthemum **(10%)**	5.11 ± 0.03 *	2.09 ± 0.06 *	18.97 ± 0.07*

Values denote means ± SD. ***** Significant at $p < 0.05$ ****** Significant at $p < 0.01$

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