



**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

**EVALUATION OF DIURETIC ACTIVITY OF METHANOLIC EXTRACTS OF
MUSTARD SPECIES IN NORMAL MICE**

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ABSTRACT

In present study, methanolic extracts of *Brassica nigra* L. and *Sinapis alba* L. (Brassicaceae) were prepared and administered orally to Swiss albino mice at doses of 150 mg/Kg and 5 mg/Kg respectively, to evaluate the diuretic activity. The diuretic effect of the extracts was evaluated by measuring the urine volume, pH & excretion of sodium, potassium, and chloride ions in urine. Diuretic activity was confirmed by an increase in urine volume in *B. nigra* (1.52 fold) and *S. alba* (1.66 fold) extracts treated group as compared to control mice. The urinary electrolytes (Na⁺, K⁺ and Cl⁻) excretion was also found to be increased in drug treated groups. On the basis of above results, we can conclude that the methanolic extracts of *B. nigra* and *S. alba* produced notable diuretic effect and provides a quantitative basis for explaining the folkloric use of *B. nigra* and *S. alba* as a diuretic agents.

Keywords: Brassicaceae; *Brassica nigra*; *Sinapis alba*; Sodium Ion;

INTRODUCTION

Diuretics are the drugs that increase the urine flow rate, and excretion of sodium. Diuretics are used to maintain the volume and composition of body fluids in several clinical manifestations such as congestive heart failure, nephritic syndrome, cirrhosis, kidney failure, hypertension, and pregnancy [1]. Most diuretic drugs have side effect including impotence, fatigue, weakness, frequent urination, electrolyte disturbance, headache, muscle cramps, increased thirst, irregular menstruation, high blood sugar levels, hyponatremia, abnormal heart rhythm, dizziness, skin rashes, anorexia, nausea etc. Naturally occurring diuretics include caffeine, tea, and cola; inhibit Na^+ re-absorption while alcohol in beer, wine and mixed drinks; inhibit secretion of ADH [2,3]. Herbal medicines derived from the plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, but still their mechanism of action is unknown. *Sinapis alba* L. and *Brassica nigra* L. are commonly called white or yellow mustard and black or true mustard respectively. Mustard is used as a food flavoring, for forage, as an emetic, and diuretic, as well as a topical treatment for inflammatory conditions such as arthritis and rheumatism. Derivatives of the mustard chemical

constituent allyl isothiocyanate form the basis for toxic agents such as mustard gases and antineoplastic drugs (e.g., bendamustine). Mustard seed and its components have been demonstrated to possess antineoplastic activity [4,5]. Experimental reports have shown the antioxidant [6], hypoglycemic [7], anticancer [8] and antimicrobial effect [9] of *B. nigra* seed.

A bibliographic survey showed that there are no systematic studies have been reported for diuretic activities of *B. nigra* and *S. alba* species. Therefore, the present study was designed to investigate the diuretic activity of the methanolic extracts of black and yellow mustard seeds in mice.

MATERIALS AND METHODS

Plants collection and extract preparation

The black and yellow mustard seeds were purchased from local market in Al-Kharj, Kingdom of Saudi Arabia. Taxonomic identification was made by an expert taxonomist. For the preparation of extract, air dried black and yellow mustard seeds were powdered and packed into Soxhlet's apparatus and successively extracted with methanol at room temperature for 7 days. The extract was evaporated to dryness in rotary evaporator till further use.

Animals

Adult Swiss albino mice (body weight = 20–25 gm, both sex) bred in the animal house facility of College of Pharmacy, Sattam Bin Abdulaziz University, KSA were used for the experiment. The animals were housed in propylene cages (6 mice per cage) under standard environmental conditions ($25 \pm 1^{\circ}\text{C}$, $55 \pm 5\%$ humidity and 12 h/12 h light/dark cycle), for at least one week for acclimatization and had free access to food and drinking water.

Acute Toxicity Test

Overnight fasted mice ($n=6$) were treated orally with methanolic extracts of black and yellow mustard at different doses (50–5000 mg/kg). Another control group received the vehicle (3% v/v Tween 80 in distilled water), and kept under the same conditions. Animals were observed for gross behavioral changes, and mortalities till 48 h and the LD_{50} were calculated [10].

Grouping of animals

Animals were randomly assigned into four groups each consisting of 6 mice for diuretic test. Negative controls were treated with the vehicle used for reconstitution (2 ml/100 g of body weight). Positive controls were treated with reference drug, furosemide 10 mg/kg. Two treatment groups were treated with

methanolic extracts of *B. nigra* (150 mg/Kg) and *S. alba* (5 mg/Kg) respectively.

Diuretic activity

Diuretic activity was determined following the methods used by Lahlou et al. [11], with slight modification. Each mice was placed in an individual metabolic cage (Metabolic cage for mice, TECHNIPLAST, Italy) 24 h prior to commencement of the experiment for adaptation and then fasted overnight with free access to water. The animals were pretreated with physiological saline (0.9% NaCl) at an oral dose of 0.15 ml/10 g body weight, to impose a uniform water and salt load [12]. Each group was then treated as described in grouping section orally by gavage. Immediately after administration, the mice were individually placed in a metabolic cage. The volume of urine excreted was measured at the end of 24 hr. The urine was then filtered and finally stored at -20°C for electrolyte analyses [13]. The total concentrations of sodium, potassium, and chloride ions were estimated in urine [14, 15]. Urine pH was measured with a digital pH meter of fresh urine sample.

Analytical procedures

Sodium, potassium and chloride levels of urine and the plant extract were analyzed. Sodium and potassium concentrations were determined by making use of flame

photometry, and chloride concentration was quantified using Ion Selective Electrode (ISE) analyzer (AVL 9181 Electrolyte Analyzer, Roche, USA). The flame photometer worked by flame production when the atom changed from its excited state to the ground state, while the ISE analyzer contains software which permits electrolyte parameter configuration. A calibration was performed automatically in both cases prior to analysis with different levels of standards. Ratios of electrolytes; Na^+/K^+ and $\text{Cl}^-/\text{K}^+ + \text{Na}^+$ were calculated to evaluate the saluretic activity of the different extracts. In addition, pH was directly determined on fresh urine samples using a pH meter. Moreover, the salt content of the extract was also determined to rule out its contribution on urinary electrolyte concentration.

Statistical Analysis

All data obtained were expressed as the mean \pm standard error of mean (SEM). Statistical differences between the treatments and the control groups were analyzed by using one-way analysis of variance (ANOVA) followed by post hoc Tukey's test for comparisons in different treatment groups. $P < 0.05$ was considered to be statistically significant. Statistical analysis was performed using SPSS program (version 8) software package (SPSS_ Inc., USA).

RESULTS

No mortalities were observed with oral administration of methanolic mustard extracts during 48h of observation even at the highest dose (5000 mg/kg). The tested extract did not produce any symptom of acute toxicity and none of the mice exhibit hyperactivity, convulsions, sedation, hypothermia, and respiratory distress. Accordingly, it suggested that oral LD_{50} of methanolic extracts of both yellow and black mustard is higher than 5 g/kg b.wt.

The results of the diuretic activity evaluations carried out on the methanolic extracts are listed in Tables 1 - 3. Table 1, shows the urinary volume, pH and diuretic action, while Table 2 shows the excretion of electrolytes (sodium, potassium and chloride ion) in urine obtained from the mice of different treated groups. Table 3 shows the saluretic effect, natriuretic effect and Carbonic Anhydrase Inhibition (CAI).

Table 1 showed that, urine volume was significantly found to be increased in all drug treated groups and the maximum effect was found with *S. alba*.

Methanolic extracts of both mustard seeds produced significant increase in electrolytes concentrations (Na^+ , K^+ and Cl^-) when compared to normal control group. *B. nigra* found to be have good effect on sodium

excretion ($120.25 \text{ mmol/L} \pm 2.60$), while potassium ($83.50 \text{ mmol/L} \pm 3.76$) and chloride ($89.75 \text{ mmol/L} \pm 2.30$) ion excretion was found to be maximum in *S. alba* treated group.

Urinary pH measurement revealed that the different treatment groups of methanolic extracts of mustard seeds had produced relatively acidic urine (Table 1). The pH of urine treated with *B. nigra* and *S. alba* extracts had shown an increase from normal control (6.08) to 6.43 and 6.68 respectively. The normal control group produced the lowest pH and the furosemide group an intermediate pH (6.55).

DISCUSSION

According to previous literature survey, the leaves and seeds of plants, widely used for the treatment of hypertension and renal disease, but to the best of our knowledge, no previous pharmacological or clinical study has been done to test the diuretic activity of these plants. Since diuretics are employed clinically in the treatment of edema, it would be highly important to demonstrate effectiveness in the presence of electrolyte and water [16].

In the present study, *S. alba* found to produce more diuresis (1.66 fold), potassium excretion (1.78 fold) and chloride excretion (1.60 fold) while *B. nigra* produced the

highest sodium excretion (1.21 fold), that was in agreement with the effect noted on urine excretion, as well as being statistically significant (* $p < 0.05$). And for potassium excretion, the data showed that only *S. alba* produced a significant increase, which was very close or higher than the value obtained using the furosemide, used as reference diuretics.

Thus, in the present study, the above K^+ - sparing effect was not observed. It is probable that the substances responsible for the powerful K^+ - sparing effect were not found in sufficient concentrations in the extracts.

In the present study, the effect of the methanolic extracts of mustard seeds showed significant increase in urine volume and electrolyte excretion, as compared to the control group, it supports that the diuretic effect of *B. nigra* and *S. alba* was of the saluretic type in contrast to aquaretic type, which is a typical feature of most herbal diuretic agents [17]. Both extracts did have an interesting natriuretic effect and thus, could have a beneficial effect in different edematous conditions. The ratio Na^+/K^+ was also calculated as indicator of natriuretic activity and resulted in values of 1.61, 1.31 and 2.01 for *B. nigra*, *S. alba*, and furosemide respectively. This indicates that

the extracts increase sodium excretion more than potassium, which is considered as a very good safety profile of diuretic agents, as hypokalemia is one of the potential adverse effects of synthetic diuretics, such as furosemide.

The extent of carbonic anhydrase inhibitory activity was calculated by using the formula $\text{Cl}^- / \text{Na}^+ + \text{K}^+$. Carbonic anhydrase inhibition can be excluded at ratios between 0.8 and 1.0 and with decreasing ratios; slight to strong inhibition can be assumed [18]. The $\text{Cl}^- / \text{Na}^+ + \text{K}^+$ ratio for *S. alba*, showed the strongest inhibitory effect with value of 0.47. Hence, it is possible to assume that one of the possible mechanisms of action of these extracts could be carbonic anhydrase inhibition.

Thus, the diuretic effect of extracts indicated by increase in both water and sodium ion excretion, which proved its strong diuretic activity, but active constituents responsible for the diuretic activity cannot be concluded on the basis of the present study.

Plant-based food contains numerous non-nutritive, bioactive compounds known as 'phytochemicals'. Experimental researches suggest the existence of flavonoids with antioxidant effects in the hydro-alcoholic *B. nigra* seed [19]. Several studies have earlier reported the use of isothiocyanates from

plants belonging to the family of Brassicaceae as colon-cancer-preventing agents [20]. The preliminary phytochemical investigation revealed the presence of phytosterol and alkaloids in methanolic extract which can be responsible for diuretic activity but need to confirm by further study.

CONCLUSION

Methanolic extracts of mustard species (*B. nigra* and *S. alba*) seeds have significant effect on urine volume and excretion of Na^+ and other electrolytes in urine output, thus, the results obtained in the present study provides a quantitative basis to explain the traditional ethno-pharmacological use of seeds as a diuretic agent for the treatment of hypertension. Indeed, multiple mode of action had been reported with some herbal medications [21]. The safety profile of the extract is an added advantage that calls for conducting further research to ascertain the findings reported in this study.

Conflict of interest statement

We declare that we have no conflict of interest.

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Table 1: Effect of Mustard species (*Brassica nigra* & *Sinapis alba*) on urine volume, diuretic action, diuretic activity and pH in Swiss albino mice

Parameters	Normal control	Furosemide	<i>B. nigra</i>	<i>S. alba</i>
	2 ml/kg	10 mg/Kg	150 mg/Kg	5 mg/Kg
Urine Volume (ml)	2.2±0.029	4.63±0.060*	3.35±0.046*	3.70±0.065*
Diuretic action	1.00	2.10	1.52	1.66
Diuretic Activity	-	1.00	0.72	0.79
Urinary pH value	6.08±0.034	6.55±0.089	6.43±0.127	6.68±0.113

- Diuretic action = urine volume of test group/urine volume of control group
- Diuretic activity = urine volume of test group/urine volume of furosemide group
 - * $P < 0.05$ compared with normal control group (n = 6)

Table 2: Effect of Mustard species (*Brassica nigra* & *Sinapis alba*) on electrolytic excretion index in 24 h of urine collection of Swiss albino mice

Parameters	Normal control	Furosemide	<i>B. nigra</i>	<i>S. alba</i>
	2 ml/kg	10 mg/Kg	150 mg/Kg	5 mg/Kg
Urinary Na ⁺ (mmol/L)	99.50±2.11	165.75±3.50*	120.25±2.60*	108.25±2.19
Urinary K ⁺ (mmol/L)	47.00±1.29	82.50±1.79*	75.00±2.08*	83.50±3.76*
Urinary Cl ⁻ (mmol/L)	56.00±4.71	94.75±2.57*	83.75±3.50*	89.75±2.30*
Na ⁺ Index	1.00	1.67	1.21	1.09
K ⁺ Index	1.00	1.75	1.59	1.78
Cl ⁻ Index	1.00	1.69	1.49	1.60

- Na⁺ index = sodium excretion in test group/sodium excretion in control group; K⁺ index = potassium excretion in test group/potassium excretion in control group; Cl⁻ index = chloride excretion in test group/chloride excretion in control group
 - * $P < 0.05$ compared with normal control group (n = 6)

Table 3: Effect of Mustard species (*Brassica nigra* & *Sinapis alba*) on saluretic, natriuretic and Carbonic Anhydrase Inhibition (CAI) activity in 24 h of urine collection of Swiss albino mice.

Parameters	Normal control	Furosemide	<i>B. nigra</i>	<i>S. alba</i>
	2 ml/kg	10 mg/Kg	150 mg/Kg	5 mg/Kg
Saluretic effect (Na+ Cl)	155.50±5.11	260.50±5.50*	204.00±4.97*	198.00±3.80*
Natriuretic effect (Na/K)	2.12±0.04	2.01±0.04	1.61±0.05	1.31±0.07
CAI{Cl/(Na+K)}	0.38±0.03	0.38±0.01	0.43±0.01	0.47±0.02
Saluretic Index	1.00	1.67	1.31	1.27
Natriuretic Index	1.00	0.95	0.76	0.62
CAI Index	1.00	1.00	1.13	1.24

- CAI= Carbonic Anhydrase Inhibition; Saluretic Index = saluretic activity in test group/saluretic activity in control group; Natriuretic Index = natriuretic activity in test group/natriuretic activity in control group; CAI activity index = CAI activity in test group/CAI activity in control group.
 - * $P < 0.05$ compared with normal control group (n = 6).