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EVALUATION OF SOME PLANTS USED IN FOLK MEDICINE IN SAUDI ARABIA

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ABSTRACT

A polyherbal formulation (PHF)consisting of *Boswellia sacra* gum, *Nigella sativa* L. seeds and *Hordium vulgare* is used in folk medicine in Kingdom of Saudi Arabia for treatment of diabetes. The acute toxicity, median lethal dose (LD_{50}), antidiabetic activity, liver and kidney function markers of each component as well as the PHF were evaluated. The highest antidiabetic activity was noticed with *Boswellia sacra* gum and the PHF. While *Nigella sativa* L. afforded moderate activity followed by *Hordium vulgare*. Liver and kidney functions did not altered by administration of any of the tested samples. Furthermore, some quality control parameters were conducted in order to establish the identity of the tested drugs.

Keywords: Folk Medicine, Diabetes Mellitus, Quality Control, Biological Activity

INTRODUCTION

During the past decade, traditional medicine have become a topic of global importance. A large proportion of the population relies heavily on medicinal plants to meet primary health care needs. Herbal medicines (phytomedicines) have often maintained

popularity for historical and cultural reasons. Few plant species that provide medicinal herbs have been scientifically evaluated for their possible medical application, safety and efficacy. Furthermore, the herbal medicines market is poorly regulated, and herbal products are often neither registered nor controlled. There is a great need up-to-date, authoritative information on the safety and efficacy of medicinal plants.

A common traditional herbal preparation, used for treatment of diabetes, consisted of *Boswellia sacra* gum, *Nigella sativa* L. and *Hordium vulgare* was chosen to evaluate its safety and efficacy and to validateits correspondence with the established quality measures of the individual components of the preparation. *Boswellia sacra* gumalso known as Frankincense or olibanum is a natural oleo-gum-resin obtained from the incisions made in the bark of the trees of genus *Boswellia* (Family Burseraceae) **[1]**. It has been used for variety of therapeutic purposes, including cancer **[2]**, inflammation **[3]**, arthritis **[4]**, asthma **[5]**, colitis **[6]**, and hyperlipidemia **[7]**. *Nigella sativa* L. family Ranunculaceae, Also Known As *Nigella cretica*, Black cumin, Black seeds, has being used in countries bordering the Mediterranean Sea, Pakistan, India and Iran, as a natural remedy for over 2000 years **[8]**. Black seed components display a remarkable array of biochemical, immunological and pharmacological actions, including anti-inflammatory **[9]**, antibacterial **[10]**, hypoglycaemic and immunopotentiating effects **[11]**. Many active compounds have been isolated, identified and reported so far in different varieties of black seeds. The most important active compounds are thymoquinone (30%-48%), thymohydroquinone, dithymoquinone, p-cymene (7%-15%), carvacrol (6%-12%), 4-terpineol (2%-7%), t-anethol (1%-4%), sesquiterpenelongifolene (1%-8%) α-pinene and thymol etc. Black seeds also contain some other compounds in trace amounts. Seeds contain two different types of alkaloids; i.e. isoquinoline alkaloids e.g. nigellicimine and nigellicimine- N-oxide, and pyrazol alkaloids or indazole ring bearing alkaloids which include nigellidine and nigellicine. Moreover, *N. sativa* seeds also contain alpha-hederin, a water solublepentacyclictriterpene and saponin, a potential anticancer agent **[12, 13]**. *Hordeum vulgare* L. (barley) is one of three species of genus Hordeum belonging to the tribe Triticeae of Poaceae family **[14]**. Its Cultivation as domestic food crop started in the beginning of agriculture 10,000 years ago **[15, 16]**. Phenolics have been identified and quantified in nine varieties of barley and their corresponding malts as flavan-3-ols,

flavonols, phenolic acids and a polar esters. Flavan-3-ols are monomers, (+)-catechin and (−)-epicatechin, and polymers constituted mainly by units of (+)-catechin and (+)-gallocatechin **[17]**.

The present study includes the evaluation of herbal products in folk medicine in KSA by conducting some quality control analysis and biological studies on these herbal products.

MATERIAL AND METHODS

Plant Material

Products and plant materials (*Boswellia sacra* gum, *Nigella sativa*, and *Hordium vulgare)* were collected from the local markets and places which offers herbal products (two places).

Methods used in Quality Control Evaluation

Ashand Moisture Content

The ash remaining following ignition of medicinal plant materials is determined. Three type of ash (total ash, acid-insoluble ash and water-soluble ash) were determined following the procedure of the Association of Analytical Communities **[18]**. The moisture content was manually determined by drying method in an oven at 105°C for 6 hours **[18]**.

Determination of Extractable Matter

For determination of water-extractable matter, distilled water (100 ml) was added to the flask containing 4 g sample and weighed to obtain the total weight including the flask. The contents were shaken well and allowed to stand for 1 h. A reflux condenser was attached to the flask and boiled for 1 h, after that cooled and weighed. The flask was readjusted to the original weight with distilled water. The mixture was shaken well and filtered rapidly. After that, 25 ml of the filtrate was transferred to a flask and evaporated to dryness on a water bath. Finally, it was dried at 105°C for 6 h, cooled in a desiccator, and immediately weighed. The same procedure was followed using ethanol instead of distilled water to determine ethanol extractable matter. The extractable matter was calculated as mg/g of air-dried material **[19]**.

Biological Activity

Animals

Male albino mice (30-35 g) were maintained in the Laboratory Animal Unite of the College of Pharmacy, Salman Bin Abdulaziz University. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum*. Male mice were used because of their constant metabolism compared to the variation in the female physiology. Animals were allowed to adapt to the laboratory environment for one week before experimentation. The care and handling of the animals were in accordance with the internationally accepted standard

guidelines and was approved by an institutional review board.

Preparation of the Extract for Biological Studies

Herbal product *Nigella sativa*, *Boswellia sacra*, *Hordium vulgare* and PHF of these plants were prepared accordingly to the methods described by the seller in the herbal product market (in which boiling the components in tap water and after that were used). Ten g of each product was, separately, boiled in 100 tap water then cooled and filtered. The extracts were orally administered to mice at a dose of 20 ml/kg.

Acute Toxicity and Determination of Median Lethal Dose (LD_{50})

Acute toxicity of the studied plants was conducted in albino mice **[20]**. The animals were fasted overnight and the herbal preparation and the separate components were administered orally at three dose levels (1000, 2000 and 4000mg/kg body weight). The vehicle control group received 3% v/v Tween 80 in distilled water. Animals were observed individually after dosing for a total of 14 days for any clinical sign of mortalities.

Sub-Chronic Toxicity

Twenty-four wistar albino mice were randomly divided into 4 groups of 6 animals. The 1st group was kept as control (5 mL/kg of 3 % Tween 80), while 2nd, 3rd and 4th groups were administered *Nigella sativa*, *Boswellia scara* gum, *Hordium vulgare* in dose of 400 mg/ kg, respectively. All medications were administered orally with the aid of an orogastric cannula for 35 consecutive days. Micewere maintained under identical conditions with food and water *ad libitum* for the entire period with close observation. At the end of the experimental period, blood samples (2 mL) were drawn by puncturing retro-orbital venous sinus of each rat (under ether anesthesia) and centrifuged at 10000 rpm for 5 min. Sera were separated to be used for the biochemical estimations.

Antidiabetic Activity

Sixty male albino mice were randomly divided into ten groups (6 animals, each). The 1st group was kept as normal control, while 2nd diabetic control (diabetic induced using STZ, in which mice received a single intraperitoneal injection of 40mg/kg of STZ (Sigma, St. Louis, MO, USA), 3rd, 4th, 5th and6th were administered diabetic and extracts of Nigella sativa, *Boswellia sacra*, *Hordium vulgare* and PHF of the previous three plants respectively. While groups from 7th and 10th (Normal mice) were administered, only extracts of *Nigella sativa*, *Boswellia sacra* (frankincense), *Hordium vulgare* and PHF of the studied plants respectively. Mice were maintained under identical conditions with food and water ad

libitum for the entire period with close observation. At the end of the experimental period, blood samples were drawn by puncturing retro-orbital venous sinus of each mouse (under ether anesthesia).Plasma glucose levels were measured with an Accu-Check Go strip test in a glucometer.

Measurement of Liver and Kidney Function Markers

Liver functions were evaluated for normal mice by measuring the serum activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) following the method of **[21]**. Serum levels of total bilirubin **[22]**, total proteins **[23]** and albumin **[24]** were also assayed. Serum concentrations of urea **[25]** and creatinine **[26]** were determined calorimetrically as measures of kidney functions.

Statistical Analysis

The values were expressed as mean± standard error of six observations in each group. All groups were subjected to one-way analysis of variance (ANOVA), which was followed by Bonferoni's test to determine the intergroup variability by using SPSS ver. 14.0.

RESULTS AND DISCUSSION

Acute Toxicity and Determination of Median Lethal Dose (LD_{50})

Nigella sativa, *Boswellia scara* gum, *Hordium vulgare* in oral doses up to 4000 mg/kg b.wt. did not produce any demonstrable acute toxic effects or deaths in all groups of mice.

Quality Control Assessments

For this purpose ash value, water-soluble ash acid insoluble ash and extractable mater were determined.

The quality control parameters of *Boswellia sacra* gum, *Nigella sativa* seeds, *Hordium vulgare*, used in this study were shown in **Table 1**. The results revealed thatthe ash content was (4.62, 4.56), (2.32, 2.35), (3.38, 3.34) and (3.41, 3.43) for *N. sativa* seeds, *B. sacra* gum, *H. vulgare* and the PHF respectively for the two places. Acid insoluble ash was (0.41, 0.39), (0.61, 0.63), (0.68, 0.63), and (0.65, 0.66), for *N. sativa* seeds, *B. sacra* gum, *H. vulgare* and the PHF respectively for the two places. While water-soluble ash was (1.22, 1.24), (1.12, 1.13), (1.20, 1.21) and (1.19, 1.19) for *N. sativa* seeds, *B. sacra* gum, *H. vulgare* and the PHF respectively for the two places.

Water extractable matter (mg/g) was (136.5, 135.3), (475.3, 477.2), for *N. sativa*, *B. sacra* gum respectively for the two places. In addition, ethanol extractable matter was (172.5, 173.2), (485.2, 488.2) for *N. sativa*, *B. sacra* respectively for the two places.

Moisture content was (3.22, 3.25), (6.82, 7.31), (8.95, 9.12) and (10.44, 10.22) for *N.*

sativa seeds, *B. sacra* gum, *H. vulgare*, and the PHF respectively for the two places.

The above results for *B. sacra* gum are generally agreed with the published normal values **[27]**, in which the ash content of *B. scara* resin and leaves ranged from 1.02 to 6.66%, moisture 5.41 to 11.54%, also ethanol extractable mater in the same trend.

The published ash content of *N. sativa* (4%) by **[28]**, which also in agreement with our results, moisture content of our results was slightly lower than that described by the same author, which may be due to the high aridity in central Saudi Arabia.

Sub-chronic Toxicity

In the current study, no significant changes were detected in the biochemical parameters of mice after 35 days of treatment with *Boswellia sacra* gum, *Nigella sativa* seeds, *Hordium vulgare*. Oral adminstration of the tested extract in dose of 400 mg/ kg to mice for 35 days did not show any significant effect on the levels of ALT, AST,TB, TP, Alb, urea and creatinine **(Table 2)** as compared to control animals.

Anti-diabetic Activity

The present results indicated that, the highest activity as anti-diabetic activity was achieved by using *Boswellia sacra* and PHF followed by *N. sativa* after that H. vulgare in the last position. This results indicated that the effects of the herbal products may be due to the presence of *Boswellia sacra* as an active principle of the PHF.

In normal mice, also Frankincense (*Boswellia sacra)* showed significant reduction in blood glucose level followed by PHF. Boswellia contains oils, terpenoids, sugars, and volatile oils. Up to 16 percent of the resin is essential oil, the majority being alpha-thujene and p-cymene. Four pentacyclictriterpene acids are also present, with beta-boswellic acid being the major constituent **[28]**.

The popularity of *Boswellia sacra*, a botanical medicine, discovered over three millennia ago, is now experiencing resurgence. Long recognized for its anti-inflammatory benefits, this oleogum resin also has anti-cancer and immunomodulatory properties **[29]**. *H. vulgare* seeds possessed the hepatoprotective activity as evidenced from the functional, physical, biochemical and histological parameters **[30]**. Barley is a rich source of magnesium, a mineral that acts as a co-factor for more than 300 enzymes, including enzymes involved in the body's use of glucose and insulin secretion. It is also a very good source of fibers and selenium and a good source of phosphorus and copper. It was found that frequently consumption of whole grains reduced the risk of type II diabetes by 31 % indicating that whole grains offer special benefits in promoting healthy blood sugar control **[31]**.

CONCLUSION

In conclusion, the PHF of *Boswellia sacra* gum, *Nigella sativa* and *Hordium vulgare* showed good ant diabetic activity. In addition quality control parameters indicated that these plants with in the normal limits.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Table 1: Quality Control Parameters for the Studied Plants

Plant Name	Ash content (%)	Acid insoluble ash (%)	Water Soluble Ash (%)	Water extractable maters (mg/g)	Ethanol extractable maters (mg/g)	Moisture Content (%)
N. sativa (1)	4.62 ±0.61	0.41±0.01	1.22 ± 0.06	136.5 ± 2.16	172.5 ± 3.15	3.22 ± 0.22
N. sativa (2)	4.56 ± 0.52	0.39± 0.01	1.24 ± 0.05	135.3 ± 2.22	173.2 ± 3.20	3.25 ± 0.18
B. scara(1)	2.32 ± 0.21	0.61± 0.01	1.12 ± 0.05	475.3 ± 4.50	485.2 ± 5.50	6.82 ± 0.22
B. scara(2)	2.35 ± 0.24	0.63± 0.01	1.13 ± 0.02	477.2 ± 4.30	488.2 ± 6.50	7.31 ± 0.44
H. vulgare(1)	3.38 ± 0.16	0.68± 0.03	1.20 ± 0.04	NA	NA	8.95 ± 0.88
H. vulgare(2)	3.34 ± 0.16	0.63± 0.03	1.21 ± 0.05	NA	NA	9.12 ± 0.82
PHF (1)	3.41 ± 0.84	0.65± 0.01	1.19 ± 0.06	NA	NA	NA
PHF (2)	3.43 ± 0.78	0.66± 0.03	1.19 ± 0.03	NA	NA	NA

Table 2: Sub-chronic Toxicity of Water Extract of *Nigella sativa*, Boswellia, *Hordium vulgare* for 35 Days on the Serum Activity of ALT and AST and Serum Levels of Total Bilirubin, Total Protein, Albumin in Mice, (n=6)

Groups	ALT (UL^{-1})	AST (UL^{-1})	T. bilirubin ($mg\ dL^{-1}$)	T. protein ($g\ dL^{-1}$)	Albumin ($g\ dL^{-1}$)
Control	64.28±2.56	130.10±5.27	1.52±0.11	8.5±0.33	3.7±0.16
Nigella sativa	66.11±1.77	131.52±5.44	1.61±0.11	8.2±0.31	3.4±0.14
B. sacra	63.55±1.90	121.24±2.77	1.51±0.09	8.1±0.31	3.1±0.13
Hordium vulgare	64.12±2.83	130.16±4.88	1.63±0.14	8.0±0.22	3.7±0.16

Table 3: Antidiabetic Activity of the Studied Plants in Diabetic Mice (a) and Hypoglycemic Activity in Normal mice (b) STZ Injection

a)

Group	Treatment	Before treatment	After treatment
1	Control	135.21±3.32	133.12±3.43
2	Diabetic Control (STZ 40mg/kg; i.p.)	232±3.66	225.32±4.11
3	D+ *Nigella sativa*	276.33±3.11	221.12±3.45*
4	D+ *B. sacra*	271.12±3.22	198.35±3.16**
5	D+ *Hordeum vulgare* L	232.12±3.11	205.22±4.55*
6	D+ PHF	219.23±3.10	178.66±3.12**

D: Diabetic, *, Indicate Significance Compared to CCl_4 Group ($p< 0.05$). ** Indicate Significance Compared to CCl_4 Group ($p< 0.01$)

b)

Group	Treatment	Date: 14th may 2013	Date: 2nd June 2013
7	*N. sativa*	152±3.66	150±3.66
8	*B. sacra*	152±3.66	141±3.66*
9	*H.vulgare* L	153±3.66	150±3.66
10	PHF	137±3.66	132±3.66

***, Indicate Significance Compared to CCl_4 Group ($p< 0.05$)**