Original Research Article

ANTIDIABETIC, ANALGESIC, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACTS OF FRUITS AND SHOOTS OF *BRASSICA OLERACEA VAR. ITALICA Linn*.

ABSTRACT

Background: *Brassica oleracea var* italica conjointly known as "broccoli" is a crucial ayurvedic medication in traditional medicine mostly cultivated in Italy, France, England, California,The Southern American States and Spain and employed in opposing cancer (prostate cancer) , anti aging, helps digestion and management of diabetes, preventing anemia, protects against ultraviolet radiation, reducing the chance of alzheimer's disease, reducing the risk of cardiopathy cholesterol and high blood pressure. The plant principally contains indol-3-carbinol, sulforaphane, diindolylmethane, selenium and glucoraphanin. Also contains great deal of vitamin C and multiple nutrients.

Methods: In this study antidiabetic activity was investigated by alloxan induced diabetic model. Analgesic activity was manifested by using acetic acid-induced writhing. Antioxidant activity was evaluated by DPPH scavenging method whereas antimicrobial activity screening was carried out by disc diffusion method

Results: Qualitative analysis of *Brassica oleracea var italica* extracts assured the existence of flavonoids and tannins etc. Moreover, it contains mild antibacterial and antioxident activity where IC_{50} of the extraction is 1424.30 µg/ml. In addition, it also contains slightly analgesic and antidiabetic activity.

Conclusion: Our results recommend that presence of flavonoids and tannins render *Brassica oleracea var italica* with therapeutic potential for oxidative stress and inflammation connected disorders. It may even be a possible candidate for brand spanking new antibacterial and antidiabetic agents.

Trial registration: For experimental clinical study on animal trial registration and permission was issued from departmental clinical ethical review committee, department of pharmacy, university of Chittagong. The trail registration reference number is ERC/DP/CU/2015/0014

Keywords: *Brassica oleracea var italica*, antidiabetic, analgesic, antioxidant and antimicrobial activity.

Background

The role of medicinal plants in healing diseases is increasing because of the presence of versatile compounds that have the flexibility to cure a spread of diseases and serving to physicians to influence increasing quantitative relation of ailments recently [1]. Medicinal plants contain bioactive compounds with the ability to heal. These embrace saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds found as secondary metabolites in plants [2]. Plant secondary metabolites square measure for the most part viewed as potential supply of novel antibiotics, insecticides and herbicides. This is often attributable to their biological significance and potential health advantages akin to antioxidant, anti-aging, anti-atherosclerotic, antimicrobial and anti-inflammatory activities [3]. Regular intakes of plant products wealthy in phenolics are reportable to scale back risks of developing chronic diseases similar to cancer, heart diseases and diabetes [4].

Diabetes is evolving in concert of the foremost fatal diseases endeavor humanity right behind cancer and cardiovascular diseases. Existing databases indicate its high prevalence, morbidity and mortality rate [5-6]. About 4 % population worldwide is dying by this deadly malady and this toll is probably going to swell by 5.4 % in the year 2025 [7]. Poor management of blood glucose levels is that the key conducive issue to the associated complications and treatment of hyperglycemia is thus, the most targets within the interference of those diabetes connected complications [8-9]. Hyperglycemia plays a crucial role in the development and progression of diabetic complications by various mechanisms together with exaggerated oxidative stress, minimized nitric oxide bioavailability, glucose autoxidation and non-enzymatic protein glycation [10].The global exponential growth of diabetes has led to a synchronous rise within the usage of herbal remedies to treat diabetes due to their natural origin, free accessibility and lesser side effects [11].It is also well renowned that oxidative stress develops once reactive oxygen-derived free radical production exceeds the antioxidant defense mechanism of the cell [12].Antimicrobial properties are rumored a lot of times during a wide selection of plant extracts and essential oils and natural products in a trial to discover new chemical categories of antifungal and antibacterial

drugs that might resolve strains expressing resistance to the obtainable antifungal and antibacterial drugs [13-14]. *Brassica oleracea var. italica* (Roots, leaves and Fruits) is utilized in anti cancer especially prostate cancer, Anti aging, management of diabetes, Preventing anemia ,Protects against ultraviolet radiation, Reducing the chance of heart disease cholesterol and high pressure [15-16].

MATERIALS AND METHODS:

Plant Material Collection and Identification

Fruits and shoots of *Brassica oleracea var. italica* were collected from Savar area district of Dhaka and were identified by the experts and preserved in the herbarium (Acc. No: CU/DP/PS/2015600321) department of pharmacy, University of Chittagong.

Extraction of plant material

Dried, ground Fruits and shoots of *Brassica oleracea var. italica* (900 g) was taken in a clean flat bottomed glass container and soaked in 2 l of methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by clean, white cotton then followed by a filtration through Whatmann filter paper. The filtrate was allowed to keep for 7 days to evaporate the solvent. Finally a blackish crude extract was obtained.

Phytochemical screening

All of the crude extracts were qualitatively analyzed for the presence of different chemical groups, such as Alkaloids, Glycosides, Tannins, Flavonoids and Saponins [17-18].

Antioxidant Activity

Antioxidant activity of the methanolic extract of *Brassica oleracea var. italica* was determined by DPPH free radical scavenging activity on the basis of the modified method of Gupta [19]. Stock solutions (10 mg/ml) of the plant extracts were prepared in ethanol from which serial dilutions were carried out to obtain concentrations of 1, 5, 10, 50, 100 and 500 µg/ml. Diluted solutions (2 ml) were added to 2 ml of a 0.004% ethanol solution of DPPH, mixed and allowed to stand for 30 min for reaction to occur. The absorbance was determined at 517 nm using a double beam UV-visible spectrophotometer and from these values corresponding percentage of

inhibitions were calculated. Then % inhibitions were plotted against log concentration and from the graph IC_{50} was calculated. The experiment was performed in triplicate and average absorption was noted for each concentration. Ascorbic acid was used as positive control. Radical scavenging activity was expressed as the inhibition percentage (I %) and calculated as per the following equation:

%inhibition= [(Blank absorbance - Sample absorbance)/Blank absorbance] × 100

Antibacterial Activity

Antibacterial activity of the methanolic extract of *Brassica oleracea var. italica* was assessed by the disc diffusion method according to the previously described method [20-21]. Bacteria used as test organisms for the antibacterial activity test is listed in table 5.

Experimental Animals

Young Swiss-albino mice aged 4-5 weeks old and average weight 20-25 g was employed for the experiment. The mice were purchased from the Animal Research Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B). They were kept in standard environmental condition (RH 55% to 60%, room temperature 25± 2oC and 12 h light/ dark cycle) for one week for adaptation after their purchase and fed ICDDRB formulated rodent food and water. The experimental study was performed under the guidelines of Institutional Animal Ethics Committee [22].

Chemicals and Drugs

The standard drug, Metformin hydrochloride was the generous gift samples from Beximco Pharmaceuticals Ltd of Bangladesh. Alloxan monohydrate was purchased from Loba Chemie, India. Carrageenan was purchased from Otto Chemika, India. Blood samples analyzed for blood glucose content by using OK meter Match glucose test meter (Hsinchu, Taiwan). Acetic acid was collected from laboratory of Bangladesh University. The standard drug Diclofenac-Na was purchased from Square Pharmaceuticals Limited of Bangladesh

Experimental induction of diabetes

Experimental induction of diabetes in mice, freshly prepared solution of alloxan monohydrate in normal saline at a dose of 120 mg/kg body weight, were injected to mice intraperitoneally. Alloxan can produce fatal hypoglycemia as a result of massive pancreatic insulin release mice were treated with 20% glucose solution (5 - 10 ml) orally after 6 h. The mice were then kept for

the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia. After 1 week, mice with moderate diabetes that exhibited glycosuria and hyperglycemia (i.e. blood glucose concentration >200 mg/dL) were taken for the experiment [22].

Experimental design for antidiabetic activity study

Fifteen mice were divided in to five groups as Group I: normal rats received only distilled water during the experimental period, Group II: diabetic control rats received only distilled water during the experimental period, Group III: diabetic mice administered 500 mg/kg sample, Group IV: diabetic mice administered 250 mg/kg sample, Group V: diabetic mice administered 0.25 mg/kg glibenclamide.

Treatment was continued for a period of 6 hours following oral administration to the experimental animals by gastric intubation, using a force - feeding needle. Blood glucose was estimated on withdrawing blood samples were from tail vein prior to dosing (0 hour) and then 1st hour, 3rd hour and 5th hour respectively from all groups of mice. Fixed amount of rat chow and fluid was given to each rat and replenished the next [23-25].

Acetic acid-induced writhing test for Analgesic activity

The analgesic activity of the samples was also studied using acetic acid-induced writhing model in mice. Test samples and vehicle were administered orally 30 munities before intra-peritoneal administration of 1% acetic acid but Diclofenac-Na was administered intraperitonially 15 mins, the mice were observed for specific contraction of body referred to as “writhing” for the next 10 munities [26-27].

RESULT AND DISCUSSION:-

Phytochemicals Screening:

Phytochemical screening of methanolic extract of *Brassica oleracea var italica* indicates the presence of tannins and flavonoids (Table 1)

Table 1. Results of different chemical group tests of *Brassica oleracea var italica*

Plant Extract	Alkaloids	Glycosides	Tannins	Flavonoids
methanolic extract of *Brassica oleracea var.italica*	-	-	+	+

+: Positive result; -: Negative result

Antioxidant activity

Antioxidant activity of *Brassica oleracea var. italica* was determined on the basis of its ability to scavenge DPPH free radicals. Methanolic extracts of leaves of *Brassica oleracea var. italica* showed potential DPPH free radical scavenging activity where the IC_{50} was **1424.30**µg/ml compared to that of ascorbic acid, used as standard, where the IC_{50} was **9.48** µg/ml results are summarized as **Table 2 and Figure1.**

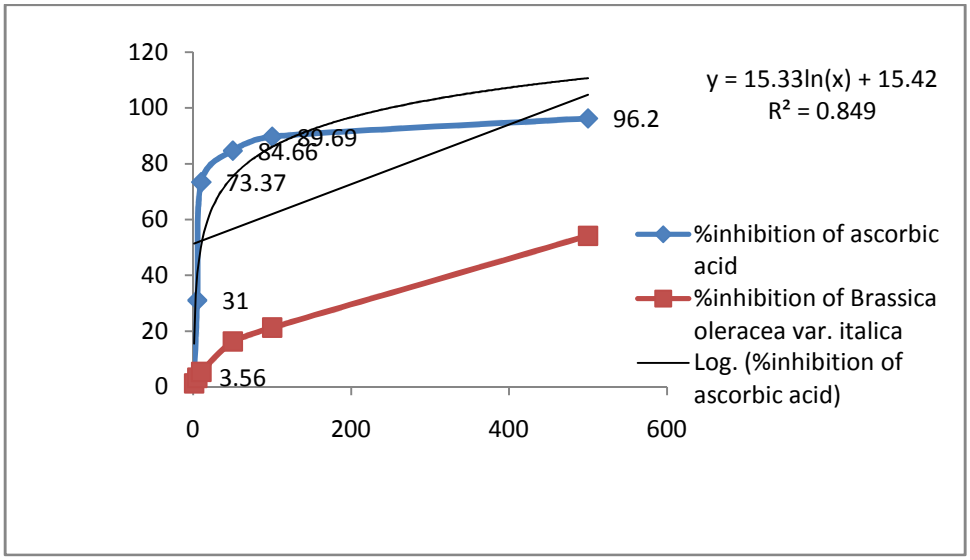
Table 2. Antioxidant activity of *Brassica oleracea var. italica*

Sample	**Concentratin (µg/ml)**	**% inhibition**	**IC_{50} (µg/ml)**
MeOH extract	1	1.2 ± 0.023	
	5	3.20 ±	
Brassica oleracea var. italica	10	5.28 ±	**1424.30**
	50	16.20 ±	
	100	21.23 ±	
	500	54.11 ±	
Ascorbic acid	1	3.56±0.011	**9.48**

5	31±0.024
10	73.37±0.03
50	84.66±0.01
100	89.69±0.02
500	96.2±0.031

Values are expressed as mean ± S.D

Figure1: Effect of the methanolic extract of *Brassica oleracea var. italica* leaf antioxident



Anti-diabetic activity

Anti-diabetic activity results are summarized as

Table-3 Effect of the methanolic extract of *Brassica oleracea* var. italica leaf on Anti-diabetic activity **in diabetic mice:**

Time	**Normal Group**	**Control Group**	**Standard Group**	**Extract Group (250 mg/kg)**	**Extract Group(500mg/kg)**
0 min	5.8±0.36	18.5±0.26	25.6±0.20	11.1±0.43	12.1±0.36
30 min	5.7±0.32	21.3±0.47	18.5±0.25	18.0±0.31	24.6±0.25
90 min	5.8±0.52	19.7±0.21	16.3±0.35	16.3±0.25	17.1±0.40

60min	5.7±0.25	16.6±0.27	12.8±0.45	11.3±0.27	13.3±0.28
120 min	5.7±0.22	15.2±0.45	05.2±0.33	09.5±0.56	09.3±0.58

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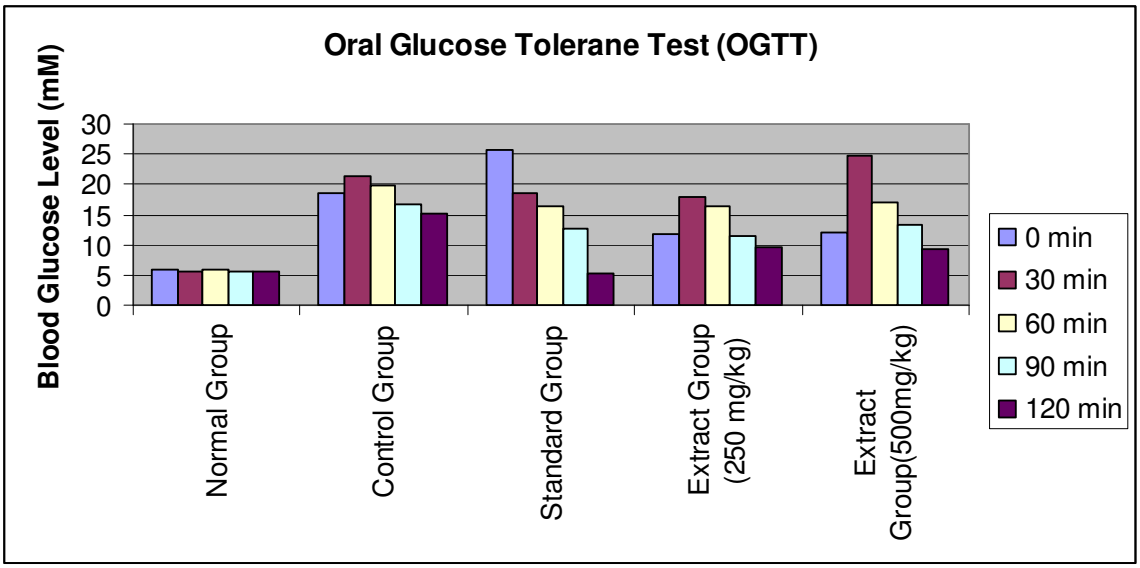
166 **Figure2:** Effect of the methanolic extract of *Brassica oleracea var. italica* leaf on oral glucose
167 tolerance test in diabetic mice.



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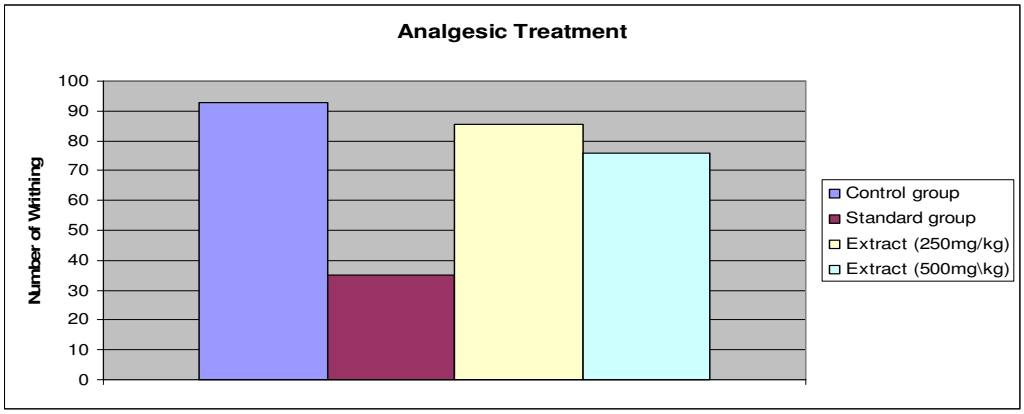
169 **Analgesic effect of *Brassica oleracea var. italica*. extract on Acetic acid-induced writhing in**
170 **mice**

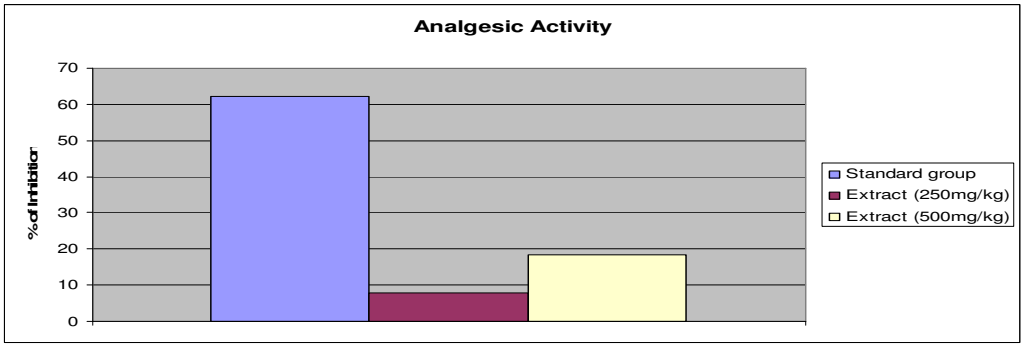
171 **Table-4. of the methanolic extract of *Brassica oleracea var. italica*. leaf on Acetic acid-**
172 **induced writhing in mice:-**

Animal Group	**Writhing Counting (Mean ± SEM)**	**Percentage of Writhing Inhibition**
Control Group	92.75±0.66	-
Standard Group	35.00±0.38	62.26
Extract Group (250mg/kg)	85.50±0.56	07.82
Extract Group (500 mg/kg)	75.75±0.32	18.32

Figure3: Effects of the methanolic extract of *Brassica oleracea var. italica*. leaf of on acetic acid–induced writhing mice



Percent of inhibition effects of the methanolic extract of leaf *Brassica oleracea var. italica.* on acetic acid-induced writhing in mice.



Antibacterial activity

Table 5 showed the antibacterial activity of *Brassica oleracea var. italica* relative to that of the standard drug Ciprofloxacin.It showed mild antibacterial activity against *Bacillus subtilis*, *Bacillus cereus, Pseudomonus aeruginosa* and *E.coli* where the zone of inhibition was about 6 mm

Table 5: Antibacterial activity of *Brassica oleracea var. italica*

Bacteria	Zone of inhibition (mm)	
	Methanol Extract (500 µg/disk)	**Ciprofloxacin (30 µg/disk)**
Gram Positive		
Bacillus subtilis	7.02 ± 0.21	31.01 ± 0.31
Bacillus cereus	6.11 ± 0.22	33.21 ± 0.33
Gram Negative		
Pseudomonas aeruginosae	5.23 ± 0.25	32.06 ± 0.36
E. coli	6.14 ± 0.23	35.04 ± 0.34

CONCLUSION:

The present experimental research work was undertaken to determine the Anti-diabetic, analgesic, antimicrobial and antioxidant effects of the methanolic extract of *Brassica oleracea var. italica* Leaf on white albino mice (male).

The present study illustrates about the hypoglycemic effect of *Brassica oleracea var. italica* Leaf was satisfactory considerable : 500 mg/kg showed mild decrease (from 12.1 mM ± SD to 9.3 mM ± SD) and 250 mg/kg showed mild decrease (from 11.1 mM ± SD to 9.5 mM ± SD) compared to standard drug metformin (from 25.6 mM ± SD to 05.2 mM ± SD).

Significant analgesic effect was monitored in dose 500 mg/kg of extract inhibited 17.2 % and dose 250 mg/kg of extract of *Brassica oleracea var. italica* inhibited 6.81 % of writhing movements compared to Control group where as standard drug diclofenac gave 62.26 % of inhibition.

The methanolic extract of *Brassica oleracea var. italica* has minor anti oxidant activity. The IC_{50} of the extraction is 1424.30 µg/ml, whereas IC_{50} of Ascorbic Acid is 9.48 µg/ml.

Finlly, we concluded from the current research work that the the methanolic extract of *Brassica oleracea var* italica possesses marked antidiabetic, analgesic, antioxidant and antimicrobial potentials. The usefulness of this plant should be confirmed through further phytochemical and toxicity analyses

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