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MEDICINAL POTENTIALS OF WATERMELON SEEDS AQUEOUS EXTRACT FOR THE TREATMENT OF HELICOBACTER PYLORI ULCER IN RATS

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ABSTRACT

The inhibitory activity of watermelon seeds extracts was determined in vivo, using rats. Rats were divided into 3 groups of 5, 10 and 10 (n=25). Group **A** comprising of 5 rats was neither infected nor treated with the extract and was used as control. Group **B** comprising of 10 rats were orally challenged with 0.1 ml (3.0 x 10^4 CFU) of *Helicobacter pylori* and received no extract treatment and Group **C** comprising of 10 rats were orally challenged with 0.1 ml (3.0 x 10^4 CFU) of *Helicobacter pylori* and were orally treated twice daily with 0.5 ml of extract containing 200 mg/kg body weight of the extract at 12 hours interval for six (6) days, respectively. In group A average body weight of the rats ranges from 150 ± 1 to 154 ± 1, average body temperature of the rats ranges between 31±2 to 33±4, average food consumption of the rats ranges from 34±2g to 36±5g and *Helicobacter pylori* count in rats feaces was found to be zero. In group B average body weight of the rats ranges from 152 ± 1 to 138 ± 0, average body temperature of the rats ranges between 33±6 to 45±1, average food consumption of the rats ranges from 33±1g to 26±5g and *Helicobacter pylori* count in rats feaces was found to be raising from $7.8x10^5$ to $9.8x10^5$ between day-1 to day-5 after which (day 6-10) death of the rats continue to occurred as a result of no treatment. In group C average body weight of the rats ranges from 150 ± 0 to 175 ± 4, average body temperature of the rats ranges between 35±9 to 32±3, average food consumption of the rats ranges from 32±4g to 39±3g and *Helicobacter pylori* count in rats feaces was found to be decreasing from 9.8x105 to 1.3x195 and no death of any rat observed, suggestive the presence of bioactive compound in the watermelon seeds which could be used to treat *H. pylori* ulcer.

KEYWORDS: Watermelon seeds extracts, *Helicobacter pylori*, Rats, Antibacterial.

INTRODUCTION

Herbal medicine is getting growing attention in the field of medicine and about 60% of rural populations depend on it for their primary health care (Sevin *et al*., 2000). This could be attributed to affordability, accessibility, in the economic sense and socially and availability of the herbs in the localities. Traditional medical practitioners in Nigeria use a variety of herbal preparations to treat different kinds of diseases such as pyloric ulcer. Information are accumulation that pathogenic microorganisms are becoming increasingly resistant to existing antibiotics at alarming rates which creates problems in health care delivery in relation to microbial infections in man Attentions have been directed towards medicinal research to substantiate the claims of cure made by traditional healers and thus provide scientific basis for their efficacy. Several important drugs have been discovered in plants and are now synthesized chemically for commercial purposes. However, the search for new ones continues especially in the tropical plants. The research for new drugs is on course hence the need to investigate the potentials of watermelon seeds used traditionally in the treatment of *helicobacter pylori* ulcer.

MATERIALS AND METHODS

Collection of plant materials

Fresh mature *water melons* were purchased at onion market Aliero Kebbi State, Nigeria. The plant was identified in the Botany department, kebbi state university of science and technology Aliero.

Preparation of seed extract

The water melons were gently cleaned and washed under running tap water to remove dirt after which the flesh part of the water melon was separated from the seeds and the seeds were air-dried at room temperature in the absence of sunlight for two weeks and then ground into powdered form using clean laboratory motor and pestle and then stored in a sterile plastic container. 20g of the seeds powder was soaked in 200ml of 95% ethanol in a conical flask for two weeks with regular shaking at room temperature. This was then filtered and the solvent evaporated using Rotary evaporator and kept at 4^0C before used (Allen *et al*., 2007).

Phytochemical Screening

The crude seeds extract obtained was subjected to preliminary phytochemical screening, to identify the chemical constituents. The methods of analysis employed were those described by (Sofowora, 1993).

Isolation of test organism

Stock cultures of *Helicobacter pylori* used in this study were obtained from federal medical center birnin kebbi, Nigeria; the organism was further confirmed by gram staining and subjecting it to biochemical tests which includes: production of catalase, oxidase, urease, and H2S, nitrate reduction, growing in 3.5% NaCl, growing with 1% glycine, and growing at different temperatures (Debongnie, 1998).

16SrRNA Identification of *Helicobacter pylori*

Following identification of isolate by biochemical tests as *H. pylori* it was further confirmed by using primers specifically designed for the identification of *H. pylori* based on 16SrRNA sequence. The primers for 500 bp product of the 16SrRNA sequence are represented by the forward primer sequence: 5 GCT AAG AGA TCA GCC TAT GTC C3 and the reverse one: 5 TGG CAA TCA GCG TCA GGT AAT G3 (Elyse, 2002).

Source of albino rats

Twenty five (25) healthy albino rats (*Rattus norvegicus*) were used in this study, Male and female sexes, aged 3 months and weighing between 100–150g. The rats were obtained from Animal House of the Usman Danfodiyo University Sokoto Nigeria and were assigned randomly and individually in micro-isolated cages in the same room on 12hrs light-dark cycle. The rats were allowed to acclimatize to their new environment for 1 week before inoculation and were tested four times over the 1-week period to ensure that they were negative for *Helicobacter pylori* pathogen free food and deionized water were autoclaved and provided them from the day the rats were procured until the completion of the experiment.

Preparation of Inoculum

The inoculum was prepared from a stock culture of *Helicobacter pylori* initially identified. A loopful of the organism stored in nutrient agar slant was transferred onto test tubes containing 10 mls of sterilized peptone water and incubated at 37^{O}C for 24 hrs. (Bigard, 2004).

Infection and treatment of rats

A loopful of the organism stored in nutrient agar slant was transferred into test tube containing 10mls of sterilized peptone water and incubated at 37^{O}C for 24hrs. Rats were divided into 3 groups of 5, 10 and 10 (n=25). Group A comprising of 5 rats was neither infected nor treated with the extract and was used as control except that the animals were given equal volume of distilled water. Group B comprising of 10 rats were orally challenged with 0.1 ml (3.0×10^4 CFU) of *Helicobacter pylori* and received no extract treatment and Group C comprising of 10 rats were orally challenged with 0.1 ml (3.0×10^4 CFU) of *Helicobacter pylori* and were orally treated twice daily with 0.5 ml of extract containing, 200 mg/kg body weight of the extract at 12 hours interval for six (6) days, respectively. All animals were allowed to freely access sterile food and distilled water throughout the experiment (Malfertheiner *et al*., 2012).

Detection of *Helicobacter pylori* from rat's faeces

Stool samples were collected from anal swab of the rats and were serially diluted and were then cultured on *Helicobacter pylori* agar for helicobacter detection and number of colonies appearing were counted using colony counter (Thomas *et al*., 1992).

Measurement of body temperature

Body temperature in degree Celsius (^{o}C) of the rats were taken daily to check for any change in body temperature due to infection for the whole period of the study and for the whole mice in three groups by inserting clinical thermometer in to the anus of the mice for ten minutes (Sevin *et al*., 2000).

Measurement of food consumption of the rats

Food consumption by the rats was measured by measuring the amount of food in grams given to each rats in its micro cage per day and subtracting the amount of food remaining in the next day (Megraud and Lehours, 2007).

Identification of *H. pylori*

Small round colonies, bulging and glossy, under the microscope the isolate appeared gram negative curve shape bacteria, intense urease positive, oxidase and catalase positive.

RESULTS

GROUP A (CONTROL)

Table 1: Showing results in mean and standard deviation of average distribution of rat's Body weight, Body temperatures, Food consumption and *Helicobacter pylori* count in the rat's faeces in the group A.

Days	Average Body Weight (g)	Average Body Temperature (^{O}C)	Average Food Consumption (g)	*Helicobacter pylori* Dosage (ml)	Extract treatment Dosage (ml)	*Helicobacter pylori* Count
1.	150 ± 1	32 ± 5	34 ± 3	0	0	0
2.	150 ± 4	31 ± 7	34 ± 2	0	0	0
3.	151 ± 2	33 ± 4	34 ± 5	0	0	0
4.	153 ± 2	32 ± 2	35 ± 2	0	0	0
5.	153 ± 5	32 ± 3	35 ± 3	0	0	0
6.	153 ± 7	31 ± 6	35 ± 7	0	0	O
7.	153 ± 9	33 ± 0	35 ± 8	0	0	0
8.	154 ± 7	32 ± 2	35 ± 9	0	0	0
9.	155 ± 0	32 ± 6	36 ± 1	0	0	0
10.	156 ± 1	31 ± 2	36 ± 5	0	0	0

Group B (Rats Infected Without Treatment)

Table 2: Showing results in mean and standard deviation of average distribution of rat's Body weight, Body temperatures, Food consumption and *Helicobacter pylori* count in rat feaces in the group B.

Days	Body Weight (g)	Body Temperature (^{O}C)	Food Consumption (g)	*Helicobacter pylori* Dosage (ml)	Extract Dosage (ml)	*Helicobacter pylori* Count
1.	152 ± 1	33 ± 6	33 ± 1	0.1	0	7.8×10^{5}
2.	151 ± 0	38 ± 5	31 ± 0	_	0	8.3×10^{5}
3.	150 ± 1	40 ± 2	30 ± 2	_	0	9.5×10^{5}
4.	140 ± 0	42 ± 0	29 ± 5	_	0	9.6×10^{5}
5.	138 ± 3	45 ± 1	26 ± 5	_	0	9.8 x 105
6.	Death	-	-	-	-	-
7.	Death	-	-	-	-	-
8.	Death	-	-	-	-	-
9.	Death	-	-	-	-	-
10.	Death	-	-	-	-	-

GROUP C (RATS INFECTED WITH TREATMENT)

Table 3: Showing results in mean and standard deviation of average distribution of rat's Body weight, Body temperatures, Food consumption and *Helicobacter pylori count* in rat feaces in the group C.

Days	Average Body Weight (g)	Average Body Temperature (^{O}C)	Average Food Consumption (g)	*Helicobacter pylori* Dosage (ml)	Extract Dosage (ml)	*Helicobacter pylori* Count
1.	150 ± 0	33 ± 2	32 ± 4	0.1	0.5	9.5 x 105
2.	151 ± 1	35 ± 6	34 ± 3	-	0.5	9.8 x 10^{5}
3.	150 ± 3	35 ± 9	36 ± 3	-	0.5	9.3 x 105
4.	149 ± 1	35 ± 0	38 ± 1	-	0.5	9.3 x 105
5.	155 ± 5	34 ± 1	38 ± 5	-	0.5	7.8 x 105
6.	175 ± 4	34 ± 1	37 ± 9	-	0.5	6.6 x 105
7.	174 ± 6	33 ±1	38 ± 9	-	0.5	5.3 x 105
8.	175 ± 9	33 ± 2	39 ± 0	-	0.5	2.8 x 105
9.	175 ± 3	33 ± 3	38 ± 9	-	0.5	1.8 x 105
10.	175 ± 4	32 ± 3	39 ± 3	-	0.5	1.3 x 105

DISCUSSION AND CONCLUSIONS

The present study was conducted to determine preliminary information on the antibacterial activity of watermelon seeds aqueous extracts. The inhibitory activities were determined by measuring parameters such as *Helicobater pylori* detection in rat's feaces, measurement of changes in rat's body temperature and food consumption of the rats before and during the study (Faik and Raiss, 1998). The phytochemical screening of aqueous seed extract of watermelon revealed the presence of some secondary metabolites such as Flavonoid, Terpenoid, Saponin, Tannin and Anthraquine while Steroid absent. All the rats were found negative for *Helicobacter pylori* in fasces before inoculation and treatment with plants extracts.

The results revealed that there was a significant difference in the percentage of the albino rats that shed the organisms in faeces during the experiments between the groups A, B and C and also a significant association between the treatment with the extract and *Helicobacter pylori* isolation in faeces Table- 1, 2 and 3. In group A average body weight of the rats ranges from 150 ± 1 to 154 ± 1, average body temperature of the rats ranges between 31±2 to 33±4, average food consumption of the rats ranges from 34±2g to 36±5g and *Helicobacter pylori* count in rats feaces was found to be zero. In group B average body weight of the rats ranges from 152 ± 1 to 138 ± 0, average body temperature of the rats ranges between 33±6 to 45±1, average food consumption of the rats ranges from 33±1g to 26±5g and *Helicobacter pylori* count in rats feaces was found to be raising from $7.8x10^{5}$ to $9.8x10^{5}$ between day-1 to day-5 after which (day 6-10) death of the rats continue to occurred as a result of no treatment. In

group C average body weight of the rats ranges from 150 ± 0 to 175 ± 4, average body temperature of the rats ranges between 35±9 to 32±3, average food consumption of the rats ranges from 32±4g to 39±3g and *Helicobacter pylori* count in rats feaces was found to be decreasing from 9.8x105 to 1.3x195 and no death of any rat observed. This result is interesting because in the traditional method of treating a bacterial Ulcer, decoction of the plant parts or boiling the plant in water is employed whereas, according to present study, preparing of seeds extract with an organic solvent was shown to provide a better antibacterial activity as detection of *Helicobacter pylori* in feaces decreases. Suggestive the presence of bioactive compound in the watermelon seeds which could be used to treat *H. pylori* ulcer.

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