

Role of Poultry Star and Alcoholic *Myristica fragrans* extract on calf rotavirus diarrhea

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Abstract

This study was carried out to investigate the effect of using alcoholic *Myristica fragrans* extract and Poultry Star Probiotic on rotavirus infection in newly born calves. The results showed that the extract of *Myristica fragrans* inhibited the virus at (90-100%) during the average of continuous diarrhea (2.4) days, which showed that the detection of virus at beginning of diarrhea and the recovery by using Latex Agglutination (LA) was (73.3%), but in Haemagglutination (HA) and ELISA tests the detection of virus at beginning was (128-256), (73.3%) and in recovery was (64-128), (66.6%) respectively. In Probiotic group the inhibition of virus was (50-76.6%) during (4) days. The percentage of positive causes by using LA & ELISA tests showed the first samples were (80%), (86.6%) and at healing the results were (40%), (66.6%) respectively, but by the method of detection of virus by HA test showed the virus titration at beginning was (32-64) which was less than the recovery (256-512). In control group the inhibition of virus was (36.5-40%) during (5.5) days, which showed that the detection of virus by using LA and ELISA tests in percentage of (66.6%) & (73.3%) at beginning of diarrhea and (26.6%) and (26.6%) respectively in healing, but by using HA test the virus titration of samples at beginning of diarrhea was (32-64) which was less than recovery samples (1024-2048). The data presented in this study contribute; that the *Myristica fragrans* given with colostrums to calves from one day of age for 14 days inhibit the virus during (2.4) days.

دور المعزز الحيوي بولتري ستار والمستخلص الكحولي لجوزة الطيب في الإسهال الناجم من

فايروسات الروتا في العجول

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الخلاصة

أُجريت هذه الدراسة لمعرفة تأثير المستخلص الكحولي لجوزة الطيب والمعزز الحيوي بولتري ستار في الإصابة الفايروسية الناتجة من فايروس الروتا في العجول حديثة الولادة. وقد أظهرت النتائج ان لهذا المستخلص النباتي تأثيراً تثبيطياً في نمو وتكاثر فايروس الروتا، إذ أظهر نسبة تثبيط (90-100%) من فايروس الروتا خلال (2.4) يوم، معدل استمرار الإسهال، حيث كانت نسبة تشخيص الفايروس بواسطة اختبار اللاتكس (73.3%) في كل من نموذج بداية الإسهال ونموذج انتهاء الإسهال (الشفاء)، ولكن بالنسبة لأختباري التلازن الدموي والاليزا كانت في العينات الأولى (128-256) (73.3%) وأما عينات الشفاء كانت (64-128) (66.6%) على التوالي. في حين كانت نسبة تثبيط فايروس الروتا في المجموعة التي جُرعت المعزز الحيوي بولتري ستار (50-76.9%) خلال (4) أيام، حيث كانت نسبة تشخيص الفايروس بواسطة اختباري اللاتكس والاليزا (80%) (86.6%) في نماذج بداية الإسهال أما في نماذج الشفاء كانت (40%) (66.6%) على التوالي. في حين العينة الأولى كانت (32-64) اقل من العينة الثانية (256-512) عندما شخصت بواسطة اختبار التلازن الدموي. أما نتائج مجموعة السيطرة اظهرت نسبة تثبيط (36.5-40%) خلال (5.05) أيام، التي أظهرت نسبة (66.6%) (73.3%) في العينات التي تم جمعها في بداية الإسهال أما العينات التي أخذت في يوم

الشفاء من الإسهال أظهرت (26.6%) (26.6%) بواسطة اختباري اللاتكس والاليزا على التوالي. بينما في اختبار التلازن الدموي كانت العينات الأولى (23-64) أقل من العينات الثانية (1024-2048). الإثباتات التي توصل إليها البحث؛ أن أعطاء المستخلص النباتي مع اللبأ للعجول ابتداء من اليوم الأول من العمر لمدة 14 يوم أدى إلى تثبيط الفايروس خلال مدة لا تقل عن (2.4) يوم.

Introduction

Bovine Rotavirus is one of the causative agents of neonatal diarrhea in calves beside Enterotoxogenic *E. coli* (1), coronavirus (2) and Cryptosporidea. The rotaviral diarrhea in calves caused by group B rotavirus started early between 3-7 days old new born calves (3). The disease is characterized by sudden onset and rapid spread, the symptoms are anorexia, mildly depressed, watery, pale yellow diarrhea, sometimes with mucus and blood flecks. Calf rotavirus is endemic in Iraq mainly spreading in dairy cattle station. Several studies have been conducted on the agent including isolation and identification beside experimental vaccines and epidemiology of the disease (4, 5, 6). The method of controlling diarrhea caused by this viral agent depend on vaccination of pregnant dams with inactivated vaccine (7) or vaccination of newly born calves directly after birth with living vaccine or using immune colostrums (8) and also several report have shown on using probiotics as a method to enhance host immunity and increase resistance to viral diarrhea disease (rotavirus) by feeding the probiotic orally mixed with colostrums to newly born calves (9, 10). Although a number of recent studies have analyzed the useful of *Mysitrica fragrans* extract in the treatment of diarrhea, if the etiologic agent is a rotavirus (11). Grover *et al.* (2002) have studied the pharmacological effects of nutmeg and found that the extracts of nutmeg show a good antidiarrhoeal effect, with a significant sedative property. The extracts also possess a weak analgesic effect, with no harmful effects on blood pressure. This study was aimed to study the effect of using Probiotics Poultry Star® and alcoholic *Myristica fragrans* extract on rotavirus infection in newly born calves and the response of the treated calves on the problem of the diarrhea caused by calf rotavirus.

Materials and Methods

Preparation of methanolic extract of *Myristica fragrans* and detection of phytochemical components of *Myristica fragrans* by using standard procedures (26) and making the LD50 for detection the toxic dose. Probiotics Poultry Star® was obtained from Al-Nasser station. Forty five newborn calves in Al-Nasser station (Al-Swera City) were divided into three groups, the first group was fed 15 mg extract/ kg/ B.W. for 14 days, the second group was fed probiotic 6 gm/ animals for 16 days and the third group as control fed colostrums only. Two fecal samples were collected from each calf, at the beginning of diarrhea and at recovery from diarrhea, and examined by using Latex agglutination, Haemagglutination and ELISA tests.

Results

The results of extraction of *Myristicas fragrans powder* with 70% methanol gave a dark brown oily sticky extract with plant powder yield percentage of 10.39%. The results of the phytpchemicals detection of methanolic extract shows the presence of flavenoids, alkaloids, tannins, terpenes, phenols, glycosides, resins and saponins, these results are agreement with (13). The results of LD50 of acute toxicity revealed that no mortality and changes in the behavior were observed in all the treated and control groups of mice up to a dose of 4500 mg/kg. The percentage of positive causes for rotavirus infecting which detected by using Latex agglutination (LA), Haemagglutination (HA) and ELISA tests taken at begging of diarrhea and at healing day.

The Extract group, the results of the percentage of infected calves with rotavirus diarrhea resulting at the beginning of diarrhea by using LA and ELISA tests were (73.3%) and (73.3%) respectively, and the virus titration by using HA test was (128-256), but noted the positive causes at healing samples which detected by using LA and ELISA tests were (73.3%) and (66.6%) respectively, and the virus titration by using HA test was (64-128) table (1).

The Probiotics group, the results of the percentage of infected calves with rotavirus diarrhea resulting at the beginning of diarrhea by using LA and ELISA tests were (80%) and (86.6%) respectively, and the virus titration by using HA test was (32-64), but noted the positive causes at healing samples which detected by using LA and ELISA tests were (40%) and (66.6%) respectively, and the virus titration by using HA test was (256-512) table (1).

The control group, the results of the percentage of infected calves with rotavirus diarrhea resulting at the beginning of diarrhea by using LA and ELISA tests were (66.6%) and (73.3%) respectively, and the virus titration by using HA test was (32-64), but noted the positive causes at healing samples which detected by using LA and ELISA tests were (26.6%) and (26.6%) respectively, and the virus titration by using HA test was (1024-2048) table (1).

Table (1) Rotavirus detection in infected calves by several tests

Group	Test	Percentage (%) of infected animals			
		LA	HA	HI	ELISA
Extract	Beginning of diarrhea	73.3	128-256	53.3	73.3
	Recovery	73.3	64-128	53.3	66.6
Probiotics	Beginning of diarrhea	80	32-64	46.6	86.6
	Recovery	40	256-512	46.6	66.6
Control	Beginning of diarrhea	66.6	32-64	46.6	73.3
	Recovery	26.6	1024-2048	20	26.6

Group calf no. = 15

Discussions

The results of extraction of *Myristica fragrans* different with remainder researcher, these differences may be due to the difference in the origin and quality of the seeds or different parts of the plant, or due to the use of different proportions of water-alcohol mixture, and time for extraction. The results of LD50 of acute toxicity due to nutmeg, a well know spice and an herbal drug is widely used in Unani medicine without any known or recorded toxicity in the management of male sexual disorders. This suggests that its short term uses methanolic extract of nutmeg is relatively safe (14). The table (1) was showed the results of the extract of *Myristica fragrans* inhibited the virus was (90-100%) during 2.4 days, but in Probiotic group the inhibition of virus was (50-76.6%) during 4 days, While in control group the inhibition of virus was (36.5-40%) during 5.05 days. These results due to:

The Extract group, These results due to some of phytochemical component of *Myristica fragrans* include Phenolic & Flavonoid compounds have ability to inhibition of viral replication of rotavirus (15, 16). Tannins have inhibited viral cytopathic effect (17) Alkaloids have ability to inhibit protein synthesis during viral replication (18) and Saponin which is more important for induction of effective immunity at a mucosal site of the intestine (19). These results agree with the results of (20) who reported that the *Myristica fragrans* extract inhibited rotavirus was (99.2%).

The Probiotics group, These results due to the probiotic bacteria members of the genera *Lactobacillus* and *Bifidobacterium* have been modulation of local and systemic immune response (21, 22), and had a significantly higher magnitude of IgA and IgG antibody secreting cell responses in ilium, and serum IgM, IgA and IgG antibody and virus neutralizing antibody titers, and is a strong Th1 cytokin (IL-12) inducer (23).

The control group, These results were explained by the presence of high levels of antibodies in the colostrums, which begin to decline gradually and remain IgA only a low level fixed at low months after birth (24) and lead to multiple activities of the virus, malabsorption occurs because of the destruction of gut cells called enterocytes (25).

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