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Research Article

EFFECT OF SOPHORETIN ON EXPERIMENTAL INFECTION OF RABBITS WITH *Pseudomonas aeruginosa* AND *Klebsiella pneumoniae*

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Abstract

Forty mature male rabbits were employed in the investigation, and they were separated into 5 equal groups of 8 each. The rabbits in the first group, which served as the control group, were fed a regular diet throughout the trial, the second and third groups were provided a conventional ration, whereas the fourth and fifth groups were fed a food enhanced with Sophoretin. The tested organisms were dissolved in Phosphate buffer solution (PBS), and the CFU count was performed using 10 folds serial dilutions. Thereafter, intramuscular injections of the drug were administered to the animals in the second and fourth groups 22 × 10^5 (CFU) of *Pseudomonas aeruginosa*, while the third and fifth groups' animals received intramuscular injections 18 × 10^6 (CFU) of *Klebsiella pneumoniae*. Following a 24 hours period, all of the 3rd group's rabbits and three of the 5th group's rabbits died, their stomachs, lungs, spleens, livers, and thigh muscles were removed, weighed, and homogenized with Phosphate buffer solution (PBS) to count the number of Colony forming units (CFU) in all of these organs. Two days later, the remainder fiverabbits of the 5th group were executed, and the prior procedures were carried out. For the 2nd and 4th groups, the prior procedures were carried out for the bacterial count after 48 hours following the bacterial injection, when all of the rabbits in the 2nd group and four rabbits in 4th group died. The remaining 4 rabbits in the fourth group were killed for the bacterial count 2 days later. The findings showed that the addition of Sophoretin to the ration boosted rabbit survival, and that the organ bacterial count of rabbits receiving Sophoretin considerably decreased as compared to rabbits receiving fat-free food at (P≤0.05).

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1. Introduction

Pseudomonas aeruginosa represents problems as an opportunistic bacteria in medicine (Yutaka Tamura and Shozo Tanaka, 1985). *Klebsiella pneumoniae* is one of the isolated bacteria from samples of infected hospitalized patients and it consider a serious complication in patients with malignancies (Cross, 1991).

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There have been reports of genetic and age-related host resistance to *P. aeruginosa* (Berk *et al.*, 1981). The majority of adult rodents show some level of resistance to *P. aeruginosa.* Thus, unless virulence-enhancing drugs are utilized, a high challenge dosage must be used in infection experiment (Feller *et al.*, 1964). The properties anti-inflammatory and immunomodulatory activitieshave been reported in Sophoretin (Jabbar *et al.*, 2018; Pandey and Ibrahim, 2009; De Groot

and Rauen, 1998). Sophoretin has an important role in immune function and host antioxidant defense (Al-Uboody and Al-Diwan, 2015). Sophoretin is a strong scavenger of peroxyl radicals that stops lipid peroxidation (Moskuag *et al.*, 2004).

2. Materials and Methods

Experimental animals and Diets

Animals and diets: Forty adult (male) albino rabbits (1700 - 1800 grams weights). Animals received standard diet for 1 week, and then they weredivided into five groups of 8 rabbits each as follows:

a) Standard rations were given to the control group.
b) Second group, received standard ration.
c) Third group received standard ration.
d) 4th group, received sophoretin in addition to the regular ration (250 mg/kg of diet).
e) 5th group, received sophoretin in addition to the regular ration (250 mg/kg of diet).

Challenge experiments

After five weeks on the fat-focused diets, rabbits of both 2nd and 4th groups were gently injected with 22 × 10^5 (CFU) intramuscularly of *Pseudomonas aeruginosa* bacteria (Provided by Al-Hussein teaching hospital). Lungs, spleens, livers, and thigh muscles were removed, weighed and homogenized with sterile PBS. Placing the samples on Cetrimide agar plates allowed researchers to count the bacteria that were present in the experimented organs. CFU/g of tissue was used as a measure of the results.

The rabbbits of the 3rdand 5th groups were gently injected with 18 × 10^6 (CFU) of *Klebsiella pneumoniae* (Provided by Al-Hussein teaching hospital). Thigh muscle, lung, kidney, liver and spleen were removed, weighed and homogenized with sterile PBS. The number of viable bacteria was calculated in the organs and determined by plating on brain heart infusion agar. CFU/g of tissue was used as a measure of the results. Plates were incubated for 24 hrs at 37 °C.

Cetrimide Agar Preparation

The medium containing 0.03 % Cetrimide was prepared in the procedure of Brown and Lowbury in 1965. About 20 g of Proteose peptone made up the base medium, New Zealand agar, 15 g; Glycerol, 10 g with 1000 ml of water (Distilled water). The media was adjusted to pH 7.2 and autoclaved for 15 minutes at 121 °C. A volume of 100 ml of the melted base were combined with the following ingredients: 1 ml of a 15 % solution of K_2HPO_4 (anhydrous) and 1 ml of a 15 %. Solution of $MgSO_4$, 2 % Cetrimide solution was added to the Basal medium.

Statistical analysis

Using the SPSS analysis program version 19, the results of this study were statistically evaluated to identify significant differences between the treatment groups using the (t) test with a probability of ($P \leq 0.05$).

3. Results and Discussion

The findings demonstrated that the addition of Sophoretin to the rabbits' diet enhanced the quantity and percentage of rabbits that survived after being intramuscularly injected with 22 × 10^5 (CFU) of *P. aeruginosa* (4thgroup) and compared to those fed with Sophoretin free ration (2nd group) where all of those rabbits died (Table - 1 shows the results). The findings also demonstrated that the addition of Sophoretin to the rabbits' diets enhanced the quantity and percentage of animals that survived after being intramuscularly injected with 18 × 10^6 (CFU) of *Klebsiella pneumoniae* (5thgroup) as compared to rabbits which fed with sophoretinfree ration (3rdgroup) where all of those rabbits died (Table - 2 show the results).

The results also showed that, in both cases of injections of *P. aeruginosa* or *K. pneumoniae,* the numbers of the cultured bacteria that were isolated from various organs of the animals were significantly higher in the groups that did not receive Sophoretin than in rabbits which did, as the results showed in Tables 3 and 4, respectively.

Sophoretin's effect in reducing the amounts of bacteria in the body's essential organs, as indicated in the tables, and subsequently in increasing the number of animals that survived, can be attributed to this flavonol's role in immunity.

According to reports, Sophoretin antibacterial properties include changing the bacterial cell membrane permeability, rupturing their cell walls, limiting the production of nucleic acids, which has an impact on proprotein synthesis and expression, and decreasing enzyme activity. The results showed that Sophoretin has properties including broad spectrum antibacterial activity, Moreover, it has a significant inhibitory effect on fungi as well as bacteria. Moreover, sophoretin effectively inhibits the development of pathogenic bacteria, such as *Aspergillus* spp., *P. aeruginosa*, *E. coli*, *S. aureus*, and *S. enterica* (Wang *et al.*, 2018). Moreover, Sophoretin inhibits the growth of *E. coli* through changing the way adenosine triphosphate functions (Plaper *et al.*, 2022). Moreover, TEM image demonstrated that Sophoretin (50 MIC) treatment finally caused *E. coli* to cavitate and die, and that the same treatment can damage the cell membrane and cell wall of *S. aureus* (10 MIC) in an identical study (Wang *et al.*, 2019), *E. coli*, *P. aeruginosa,* and *S. aureus* growth were found to be strongly inhibited by a novel synthetic sophoretin acyl glucoside that had been chemically created. Moreover, *Listeria monocytogenes*, *Shigella* spp., and *Salmonella* spp. were all significantly inhibited by the poplar plum extract (which contains Sophoretin), with MIC values ranging from (2.07 - 8.28) mg/ml and also Sophoretin could protect rabbits from Catheter - associated *S. aureus* infection by preventing the activity of Thrombin (Zhao *et al.*, 2015).

Moreover, it was discovered that sugarcane bagasse extract (with 470 mg Sophoretin/g polyphenol) prevented the multiplication of *S. aureus, L. monocytogenes*, *S. typhi* and *E. coli* (Liang *et al.*, 2020). Furthermore, it was discovered that sophoretin, at concentrations below the MIC, inhibited *L. monocytogenes* abiotic surface colonization genes (Xin Hua Zhu *et al.*, 2012). The researchers demonstrated under Scanning electron microscope and Confocal laser scanning microscopy, Sophoretin (MIC) continued to be inhibitory to *E. faecalis* MTCC 2729 at MIC 256 g/ml (Qayyum *et al.*, 2019). The study introduced by Lee *et al.* (2013) confirmed that Sophoretin can prevent *Streptococcus hepatitis* biofilm forming, Sophoretin also disrupts or affects cellular membranes, inhibits population intervention pathways, inhibits bacterial adhesion, and inhibits efflux pumps. According to a study by Torovic *et al.* (2017), sophoretin inhibits the expression of genes involved in bacterial adhesion.

Table - 1: Demonstrates how Sophoretin affected the survival of rabbits after they were exposed to *Pseudomonas aeruginosa*

Group	Total rabbits number	Number of died rabbits	Number of survived rabbits	Ratio of survived rabbits %
Control	8	0	8	100
2nd	8	8	0	0
4th	8	4	4	50

Table – 2: Represents the effect of Sophoretin on rabbits` survival after being challenged with *Klebsiella pneumoniae*

Group	Total rabbits number	Number of died rabbits	Number of survived rabbits	Ratio of survived rabbits %
Control	8	0	8	100
3rd	8	8	0	0
5th	8	3	5	62.5

Table – 3: The effect of Sophoretinon the numbers of cultured *Klebsiella pneumoniae* bacteria isolated from different organs. The numbers represent the means. The letters on the numbers refer to the significant difference among groups at (P≤0.05) (vertical comparison).

Group	Organs					
	Muscle	Lung	Kidney	Liver	Spleen	Stomach
Control	C 0	C 0	C 0	C 0	C 0	C 0
Klebsiella (2nd group)	A 35×10^7	A 20×10^7	A 30×10^7	A 8×10^7	A 19×10^7	A 17×10^7
Klebsiella + Sophoretin (4th group)	B 57×10^6	B 12×10^6	B 17×10^6	B 32×10^6	B 41×10^6	B 36×10^6
DLS	2.18	547.03	670.46	327.72	516.45	488.84

Table – 4: The impact of sophoretin on the quantity of *P. aeruginosa* bacteria isolated from various organs and grown. The means are shown by the numbers. The letters next to the numbers denote the group differences that are statistically significant at (P≤0.05) (vertical comparison).

Group	Organs					
	Muscle	Lung	Kidney	Liver	Spleen	Stomach
Control	C 0	C 0	C 0	C 0	C 0	C 0
Pseudomonas (3rd group)	A 27×10^7	A 22×10^7	A 20×10^7	A 30×10^7	A 33×10^7	A 29×10^7
Pseudomonas + Sophoretin (5th group)	B 31×10^6	B 14×10^6	B 12×10^6	B 29×10^6	B 33×10^6	B 30×10^6
L.S.D.	1854.32	1693.06	1615.66	1962.24	6.46	1926.51

4. Conclusion

The findings showed that the addition of Sophoretin to the ration boosted rabbit survival, and that the organ bacterial count of rabbits receiving Sophoretin considerably decreased as compared to rabbits receiving fat-free food at (P≤0.05).

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