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EFFICACY OF CHITOSAN-PROPOLIS NANOCOMPOSITE ON BACTERIAL INFECTION IN RABBITS

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LIST OF ABBREVIATIONS

Abbreviation	Expression
ALP	Alkaline Phosphatase.
ALT	Alanine Aminotransferase.
AST	Aspartate Aminotransferase.
AvrA	A virulence gene A
bp	Base pairs
BUN	Blood urea nitrogen
CAT	Catalase
CFU	Colony forming unit.
CNPs	Chitosan nonoparticles
CPNP	Chitosan propolis nanocomposite
DDS	Drug delivery system
DLS	Dynamic light scattering.
DNA	Deoxyribonucleic acid.
E.coli	*Escherichia coli.*
EPEC	Enteropathogenic *E. coli.*
FDA	Food and Drug Administration
FT-IR	Fourier transmittance Infrared
GPx	Glutathione peroxidase.
GR	Glutathione reductase
HPLC	High Performance Liquid Chromatography.

Abbreviation	Expression
HRTEM	High-resolution transmission electron microscopy.
invA	Invasive gene A
MDA	Malondialdehyde.
MEM	Minimal essential medium.
MIC	Minimum inhibitory concentration
mV	millivolt
nm	nanometer
NPs	Nanoparticles.
PdI	polydispersity index
ROS	Reactive oxygen species
rpm	Round per minute.
S. aureus	*Staphylococcus aureus.*
S. typhimurium	*Salmonella typhimurium.*
SOD	Superoxide dismutase.
SopB	*Salmonella* outer protein B
SPSS	Statistical and Package for Social
stn	*Salmonella* enterotoxin
TEM	Transmission electron microscope.
TPP	Tripolyphosphate.
ZS	Zeta sizer.

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SUMMARY

Gastrointestinal diseases and particularly the diarrheic syndrome as a result of either colibacillosis or salmonellosis are the principal problems causing mortality in industrial fattening rabbit farms.

The current study was planned to assess the application of nanotechnology in the veterinary field and as a result of the emergence of multiple antibiotic resistance due to over and misuse of antibiotics, our study planned to evaluate the antibacterial activity of natural-based nanoparticles (chitosan-propolis-nanocomposite) against *S. typhimurium* and *E. coli* infections and their adverse effects as oxidative stress and hepato-nephrotoxicity in rabbits.

***In vitro* studies:**

The smallest size, spherical in shape and stable nano particles were obtained through the characterization of chitosan nanoparticle and nanocomposite by FTIR fingerprint spectroscopy, zeta potential and TEM microscopies. Also, safety of these particles was achieved through the culture on Vero cell line. Nanomedicine against three pathogens in veterinary medicine could be approved *in vitro* by MIC demonstrating the

applicability of chitosan nanoparticles and chitosan-propolis nanocomposite as a promising alternative antibacterial.

The results of sensitivity test showed that apramycin had more potent inhibitory effect on *S. typhimurium* and *E. coli* than other antibiotics tested.

***In vivo* studies:**

The first experimental protocol

In our study, A total number of ninety New Zealand rabbits, with average (1.5-2 kg) were randomly allocated into 6 equal groups (each of 15 rabbit) which were fasted 12h before bacterial inoculation as the following:

Group 1 (G1): were orally administered 5 mL sterilized saline (control group).

Group 2 (G2): were orally administered 5 ml suspension of *S. typhimurium* (5×10^9 CFU) (infected-non treated group).

Group 3 (G3): *S. typhimurium* infected rabbits were orally given 10 mg apramycin/kg b.wt/daily for 8 successive days (infected-apramycin-treated group).

Group 4 (G4): *S. typhimurium* infected rabbits were orally given 50 mg propolis/kg b.wt for 8 successive days (infected-propolis-treated group).

Group 5 (G5): *S. typhimurium* infected rabbits were orally given 100 mg CNPs/kg b.wt for 8 successive days (infected-CNPs-treated group).

Group 6 (G6): *S. typhimurium* infected rabbits were orally given 100 mg CPNP/kg b.wt for 8 successive days (infected-CPNP-treated group).

The second experimental protocol

A total number of ninety New Zealand rabbits, with average body weight (1.5-2 kg) were randomly allocated into 6 equal groups (each of 15 rabbit) which were fasted 12h before bacterial inoculation as the following:

Group 1 (G1): were orally administered 5 mL sterilized saline (control group).

Group 2 (G2): were orally administered 5 ml suspension of *E. coli* serotype (O26) (3×10^7 CFU) (infected-non treated group).

Group 3 (G3): *E. coli* infected rabbits were orally given 10 mg apramycin/kg b.wt/daily (infected-apramycin-treated group).

Group 4 (G4): *E. coli* infected rabbits were orally given 50 mg propolis/kg b.wt (infected-propolis-treated group).

Group 5 (G5): *E. coli* infected rabbits were orally given 100 mg CNPs/kg b.wt (infected-CNPs-treated group).

Group 6 (G6): *E. coli* infected rabbits were orally given 100 mg CPNP/kg b.wt (infected-CPNP-treated group).

Clinical symptoms, post mortem changes and mortality rate of rabbits in response to *S. typhimurium* infection and administration of different treatments

Rabbits infected with *S. typhimurium* suffered from lethargy, off- food, weight loss, diarrhea with or without mucus and fever after 48hr post inoculation. Post mortem examination of rabbits showed a congestion of small intestine which appeared flaccid and filled with a watery diarrhea, congestion of large intestine and filled with feces, congested and friable liver with necrotic foci and enlarged spleen.

Rabbits treated with apramycin (G3), propolis (G4) and CNPs (G5) revealed less serious clinical signs and post mortem lesions than did group 2. Rabbits in infected CPNP-treated (G6) were nearly healthy with much less severe clinical signs and post mortem lesions.

During the experimental period, 5 rabbits in the infected non treated group, 2 rabbits in the infected- apramycin or CNPs treated groups, 3 rabbits in the infected propolis-treated group and 1 rabbit in the infected CPNP-treated group were died.

Effect of oral administration of different treatments on *Salmonella typhimurium* re-isolation rates

Salmonella was cultured from all examined organs of non-infected and non-treated animals showed no bacterial isolation compared to the infected group which showed 100% *salmonella* re-isolation from various organs. Medication with apramycin significantly reduced the frequency of isolation of *salmonella* to 60% on 6^{th} day and 0% on 10^{th} day of the experiment. In addition, treatment with propolis reduced the isolation to 80% on 6^{th} day and 20% on 10^{th} day of the experiment. Treatment with chitosan nanoparticles significantly reduced the bacterial isolation to 60% on 6^{th} day and 0% on 10^{th} day of the experiment compared to chitosan-propolis nanocomposite treated group which showed 20% on 6^{th} day and no bacterial growth on 10^{th} day of the experiment considering it the best anti *salmonella* drug**.**

Effects of oral administration of different treatments on some biochemical parameters in *S. typhimurium* experimentally infected rabbits

On the 1^{st} day post-treatment, a significant ($p < 0.05$) increase in the levels of serum ALT, AST, ALP, CK-MB and creatinine were reported in the infected non–treated group compared to control group. Meanwhile, the oral therapy of

infected rabbits with apramycin for 8 days significantly ($p < 0.05$) decreased the serum levels of ALT, AST, ALP and CK-MB compared to the infected-non treated group. The oral treatment of infected rabbits with propolis, CNPs, or CPNP for 8 days significantly ($p < 0.05$) decreased the levels of serum ALT, AST, ALP, CK-MB and creatinine compared to the infected-non treated group. The oral treatment of infected rabbits with propolis, CNPs, or CPNP for 8 days significantly ($p < 0.05$) decreased the levels of serum ALT, AST, ALP and creatinine compared with the infected –apramycin treated group. The oral treatment of infected rabbits with CPNP for 8 days not only decreased the levels of serum ALT, AST, ALP, CK-MB and creatinine significantly ($p < 0.05$) in comparing with the other treated groups but also, the levels returned to relative normal levels.

Effects of oral administration of different treatments on oxidant/antioxidant status in *S. typhimurium* experimentally infected rabbits

S. typhimurium infection revealed a significant ($p < 0.05$) decrease in the levels of serum SOD, GSH-Px and CAT and a significant increase of serum MDA were reported in the infected non–treated group in comparing with control group on the 1st day post-treatment. Meanwhile, oral treatment of

infected rabbits with apramycin, propolis, CNPs, or CPNP for 8 days significantly ($p < 0.05$) increased the levels of serum SOD, GSH-Px and CAT and significantly reduced serum MDA compared to infected group. The oral treatment of infected rabbits with propolis, CNPs, or CPNP for 8 days significantly ($p < 0.05$) increased the levels of serum SOD, GSH-Px and CAT and significantly decreased serum MDA compared to infected-apramycin treated group. The oral treatment of infected rabbits with CPNP for 8 days not only improved the oxidant/antioxidant status significantly ($p < 0.05$) in comparing with other treated groups but also, the status returned to relative normal levels.

The second experiment:

Clinical symptoms, post mortem changes and mortality rate of rabbits in response to *E. coli* infection and administration of different treatments

Rabbits infected with *E. coli* suffered from depression, lack of appetite, diarrhea, weight loss, congestion of mucous membrane and respiratory manifestation 24 hrs post inoculation. Post mortem examination of rabbits showed pericarditis, myocarditis, congestion of the lung and intestinal inflammation and ulceration which filled with a watery diarrhea.

However, rabbits treated with apramycin (G3), propolis (G4) and CNPs (G5) revealed less serious clinical signs and post mortem lesions than did infected group. Rabbits in infected CPNP-treated (G6) were nearly healthy with much less severe clinical signs and post mortem lesions.

Mortality rates were recorded throughout the experiment in each group and calculated as a percent. High mortality rate by *E. coli* infection (26.67%) occurred in non-treated rabbits. Medication with apramycin reduced the mortality to 0% compared to 13.33% for propolis-treated and 6.67% for chitosan nanoparticles-treated groups and 0% for chitosan propolis nanocomposite treatment.

Effect of oral administration different treatments on *E. coli* re-isolation rates

E. coli was cultured from all examined organs (liver, heart, lung, spleen and kidney) of non-infected and non-treated animals which showed no bacterial isolation compared to the infected group (G2) which showed 100%. Medication with apramycin (G3) significantly reduced the frequency of isolation of *E. coli* to 40% on 6^{th} day and 0% on 10^{th} day of the experiment.

In addition, treatment with propolis reduced the re-isolation to 60% on 6th day and 20% on 10th day of the experiment. While, treatment with chitosan nanoparticles reduced the bacterial re-isolation to 60% on 6th day and 0% on 10th day of the experiment. Treatment with chitosan-propolis nanocomposite treated group showed no bacterial growth at all.

Effects of oral administration of different treatments on some biochemical parameters in *E. coli* experimentally infected rabbits

On the 1st day post-treatment, a significant ($p < 0.05$) increase in the levels of serum ALT, AST, ALP, CK-MB and creatinine were reported in the infected non–treated group in comparing with control group.

Meanwhile, the oral therapy of infected rabbits with apramycin for 8 days significantly ($p < 0.05$) decreased the serum levels of ALT, AST, ALP and CK-MB compared to the infected-non treated group.

The oral treatment of infected rabbits with propolis, CNPs, or CPNP for 8 days significantly ($p < 0.05$) decreased the levels of serum ALT, AST, ALP, CK-MB and creatinine compared with the infected-non treated group.

The oral treatment of infected rabbits with CPNP for 8 days not only reduced the levels of serum ALT, AST, ALP, CK-MB and creatinine significantly ($p < 0.05$) in comparing with the other treated groups but also, the levels returned to nearly the normal levels.

Effects of oral administration of different treatments on oxidant/antioxidant status in *E-coli* experimentally infected rabbits

E-coli infection revealed a significant ($p < 0.05$) reduction in the levels of serum SOD, GSH-Px and CAT and a significant elevation of serum MDA in the infected non–treated group compared to control group on the 1st day post-treatment.

Meanwhile, the oral treatment of infected rabbits with apramycin, propolis, CNPs, or CPNP for 8 days significantly ($p < 0.05$) increased the levels of serum SOD, GSH-Px and CAT and significantly decreased serum MDA compared to infected group.

The oral treatment of infected rabbits with CNPs, or CPNP for 8 days significantly ($p < 0.05$) increased the levels of serum SOD, GSH-Px and CAT and significantly decreased serum MDA compared to infected-apramycin treated group.

The oral treatment of infected rabbits with CPNP for 8 days not only improved the oxidant/antioxidant status significantly ($p < 0.05$) in comparing with other treated groups but also, the status returned to nearly the normal levels.

Chitosan-propolis nanocomposite showed greater efficacy against adverse effects of *E. coli* on oxidative stress and hepato-cardio-nephrotoxicity than *S. typhimurium*.