

Zagazig University
Faculty of Veterinary Medicine
Biochemistry Department



Some biochemical studies on ammonia intoxication in rabbits

By

Lamiaa Mohamed Zakaria Mohamed Alghzzawy
B. V. Sc. Benha University (2007)

Under supervision of

Prof. Dr.

Medhat Mohamed Fawzy
Emeritus professor of Biochemistry
Faculty of Vet. Medicine
Zagazig University

Prof. Dr.

Mahmoud Mohamed Arafa
Head of Research
Animal Health Institute
Doqi

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LIST OF ABBREVIATIONS

AC	Ammonium chloride
ACTH	Adrenocorticotropic hormone
ADP	Adenosine diphosphate
ALT	Alanine aminotransferase
AMP	Adenosine monophosphate
AQP	Aquaporin
AREs	Antioxidant response elements
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BBB	Blood-brain-barrier
Bw	Body weight
CA	Carnosic acid
Ca⁺²	Calcium ion
Ca²⁺-CM	Calcium camodulin
CAT	Catalase
CCL4	Carbon tetrachloride
cGMP	Cyclic guanosine monophosphate
CM	Calmodulin
CORT	Corticosterone
COX2	Cyclooxygenase 2
CPS1	Carbamoylphosphate synthetase-1
CRF	Corticotropin Releasing Factor
DNA	Deoxy ribonucleic acid
DPPH	1, 1-diphenyl-2-picrylhydrazyl
ELISA	Enzyme linked –Immuno-Sorbent Assay
EOs	Essential oils
FAD	Flavin adenine dinucleotide

List of Abbreviations

Fig	Figure
G/dl	Gram per deciliter
GC	Guanylate cyclase
GDH	Glutamate dehydrogenase
GH	Growth hormone
GHRH	Growth hormone releasing hormone
GLS	Glutaminase
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
GST	Glutathione S-transferase
H₂O₂	Hydrogen peroxide
HA	Hyperammonemia
HE	Hepatic encephalopathy
HPA	Hypothalamus pituitary adrenal axis
IGF-1	Insulin –like growth factor 1
IKK-β	Inhibitor of nuclear factor kappa-B
IL-1	Interleukin-1
IL-10	Interleukin-10
IL-1b	Interleukin 1b
IL-6	Interleukin-6
iNOS	Inducible nitric oxide synthase
IP	Intraperitoneal
K⁺	Potassium ion
KG	Kilogram
LPS	Lipopolysaccharide
LSD	Least significant difference
MDA	Malondialdehyde
MG	Milligrams

List of Abbreviations

Mg/dl	Milligram per deciliter
Mmol/ml	Millimole per mille
mRNA	Messenger Ribonucleic acid
Na⁺	Sodium ion
NAD(P)	Nicotinamide adenine dinucleotide (phosphate)
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NAG	N-acetylglutamate
NAGS	N-Acetylglutamate synthase
NFκB	Nuclear factor
NF-κB	Nuclear factor kappa beta
NH₃	Un-ionized ammonia
NH₄⁺	Ionized ammonia
NMDA	N-methyl-D-aspartate
NMDA receptor	N-methyl-D-aspartate receptor glutamate receptor
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
Nrf-2	Nuclear factor erythroid 2-related factor 2
OAT	Ornithine aminotransferase
OM	Origanum majorana
OMEE	Origanum majorana ethanol extract
OMP	Orotidine monophosphate
ONOO[□]	Peroxynitrite
P5CR	Pyrroline-5-carboxylate reductase
P5CS	Δ ¹ -pyrroline-5-carboxylate synthase
PAG	Phosphate activated glutaminase
Pg/ml	Pico gram per milliliter
PH	Hydrogen ion concentration

List of Abbreviations

PKC	Protein kinase C
Ppm	Part per million
PTM	post-translational modification
RA	Rosmarinic acid
RNOS	Reactive nitrogen oxide species
RNS	Reactive nitrogen species
ROEE	Rosemarinus officinalis ethanol extract
ROS	Reactive oxygen species
SD	Standard deviation
sGC	Soluble guanylate cyclase
SOD	Superoxide dismutase
Suc-CoA	Succinyl coenzyme A
TBARS	Thiobarbituric acid reactive substances
TCA	Tricarboxylic acid cycle
TNFα	Tumor necrosis factor- α
U/L	Unit per liter
UCD	Urea cycle defect
UMP	Uridine monophosphate
XO	Xanthine oxidase
α-KG	α -ketoglutarate
α-KGDH	α -ketoglutarate dehydrogenase
μM	Micromole
iNOS	Inducible nitric oxide synthase
Hr	Hour

SUMMARY

Hyperammonemia is a major contributing factor to neurological abnormalities observed in hepatic encephalopathy and in congenital defect of ammonia detoxification. In addition, Ammonia toxicity was reported to cause hepatic dysfunction and failure. Recently, attention has been drawn to the health promoting activity of plant food and its active components.

The present study was conducted to evaluate the possible protective effect of *Origanum majorana* and *Rosemarinus officinalis* ethanol extract and their combination on ammonia intoxication induced by ammonium chloride (NH₄CL) in rabbits. In order to achieve this goal healthy adult male rabbits of average body weight 1.5 to 2 kg were used. The animals were divided into seven groups, each group contain 16 rabbits as the following:

Group 1: Served as control the animals were fed standard diet and supplied with fresh water for 4 weeks.

Group 2: Was received ethanol extract of *Origanum majorana* (250 mg/kg bw) orally daily for 4 weeks

Group 3: Was administered ethanol extract of *Rosemarinus officinalis* (250 mg/kg bw) orally daily for 4 weeks

Group 4: Was injected intraperitoneally with NH₄CL (100 mg/kg) 3 times/week for 4 weeks

Group 5: Was injected intraperitoneally with NH₄CL (100 mg/kg) 3 times/week and orally administered ethanol extract of *Origanum majorana* (250 mg/kg bw) daily for 4 weeks

Summary

Group 6: Was injected intraperitoneally with NH₄CL (100 mg/kg bw) 3 times/week and orally administered ethanol extract of *Origanum majorana* and *Rosemarinus officinalis* (250 mg/kg bw) for 4 weeks

Group 7: Was injected intraperitoneally with NH₄CL (100 mg/kg) 3 times/week and orally administered ethanol extract of both Marjoram and *Rosemarinus officinalis* (250 mg/kg bw) for 4 weeks

Random blood samples were taken after 2 weeks and at the end of the experiment. Blood serum was kept in a deep freeze at -20°C until used for subsequent biochemical analysis (Ammonia, Cortisol, Growth hormone, Total bilirubin ,Albumin, ALT, AST , Urea, Creatinine, Uric acid , MDA,NO). In addition, samples from the liver tissue were used for the determination of the following parameters: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), glutathione reductase (GR), reduced glutathione (GSH).

The presented study showed that:

- 1- Serum ammonia was significantly increased in ammonia group as compared to the control group, while groups (5, 6) revealed a significant decrease in ammonia level as compared with NH₄CL-treated group. Moreover, Group 7 significantly reduced ammonia levels as compared with ammonia group and ammonia group treated with Marjoram and Rosemary alone.
- 2- Serum cortisol was significantly increased in ammonia group as compared to the control group, while groups (5, 6)

Summary

showed a significant decrease in cortisol level as compared with NH₄CL-treated group. Meanwhile, Group 7 significantly reduced cortisol levels as compared with ammonia group and ammonia group treated with Marjoram and Rosemary alone.

- 3- Serum GH was significantly decreased in ammonia group as compared to the control group, while groups (5, 6) displayed a significant increase in GH level as compared with NH₄CL-treated group. Furthermore, Group 7 significantly elevated GH levels as compared with ammonia group and ammonia group treated with Marjoram and Rosemary alone.
- 4- Concerning the results of some liver function tests and albumin, ammonium chloride treated group showed a significant increase in liver enzymes (ALT and AST), bilirubin levels with decrement in albumin serum levels along the experiment. Meanwhile, groups (5, 6, and 7) displayed a significant reduction in serum ALT, AST, and bilirubin. On the other hand, there was a significant increase in albumin as compared to intoxicated group (NH₄CL).
- 5- The results of kidney function tests declared a significant increase in serum urea, creatinine, and uric acid levels in NH₄CL-treated group as compared to control group while groups (5 and 6) showed a significant decrease kidney function tests as compared with NH₄CL-treated group. Moreover, Group 7 showed a significant decrease in kidney function tests as compared with ammonia group and ammonia group treated with Marjoram and Rosemary alone.

Summary

- 6- Serum MDA was significantly provoked in ammonia group as compared to the control group. On the other hand, groups (5, 6) revealed a significant decline in MDA level as compared with NH₄CL-treated group. Further, Group 7 significantly decreased MDA levels as compared with ammonia group and ammonia group treated with Marjoram and Rosemary alone.
- 7- Serum NO was significantly increased in ammonia group as compared to the control group. In contrast, groups (5, 6) displayed a significant reduction in NO level as compared with NH₄CL-treated group. Additionally, Group 7 significantly reduced NO levels as compared with ammonia group and ammonia group treated with Marjoram and Rosemary alone.
- 8- The obtained results of liver tissue antioxidant enzymes displayed a significant decrease in SOD, Catalase, glutathione reductase and a significant increase in GPx in ammonia group. In contrast, groups (5 and 6) revealed a significant increase in (SOD, catalase, GR, GSH) and significant decrease in GPx enzymes. Furthermore, group 7 displayed a significant increase in (SOD, catalase, GR, GSH) and a significant decrease in GPx enzymes as compared with ammonia group and ammonia group treated with Marjoram and Rosemary alone.