جامعة الإسكندرية
ALEXANDRIA
UNIVERSITY
Faculty of Science

Faculty of Science

Department of Biochemistry

Molecular and immunological investigation of neuro-inflammation induced by mycotoxines: therapeutically trial with natural product extracts.

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

In

Biochemistry

Presented by

Nadia Fouad Esmail Elyas

B.Sc. 2006

M.Sc. 2012

2019

Summary and conclusion

The brain is an organ that serves as the centre of the nervous system in all vertebrate and most invertebrate animals. The function of the brain is to exert centralized control over the other organs of the body.

Neuroinflammation represents the coordinated cellular response to tissue damage. While the appropriate regulation of this process facilitates recovery, uncontrolled neuroinflammation can induce secondary injury.

Mycotoxins are low-molecular-weight natural products (i.e., small molecules) produced as secondary metabolites by filamentous fungi. The toxic effect of mycotoxins on animal and human health is referred to as mycotoxicosis, the severity of which depends on the toxicity of the mycotoxin, the extent of exposure, age and nutritional status of the individual and possible synergistic effects of other chemicals to which the individual is exposed.

Examples of mycotoxins of greatest public health and agro-economic significance include aflatoxins (AF), ochratoxins (OT), trichothecenes, zearalenone (ZEN), fumonisins (F), tremorgenic toxins, and ergot alkaloids.

Fusarium species are the main pathogenic fungi causing maize ear and kernel rot worldwide, including *F. verticillioides*, *F. graminearum* species complex (FGSC), *F. oxysporum*, F. equiseti, *F. subglutinans*. These pathogens not only cause grain rot, but also produce a variety of mycotoxins that are a direct threat to human and animal health, *F. proliferatum* mainly produce fumonisin B (FB) that contaminate grains and grain products, these mycotoxins act as phytotoxins and virulence factors, interact with their hosts.

Many drugs currently available in Western medicine were originally isolated from plants. The flavonoids with the most potent complement inhibitory activity found in this plant are afzelin and quercitrin. The phenylpropanoid eugenol is a substance present in essential oils of various plants, and it is part of a phenolic group with a recognized antioxidant capacity. Eugenol can prevent lipid peroxidation and also inhibit the formation of superoxide radicals from xanthine oxidase system and the generation of hydroxyl radical.

In this study, fungi were isolated from corn seeds and ethanolic natural product crude extracts were used as antifungal compounds. Antifungal effects of three extracts were assessed in vitro on toxin production and different toxin concentrations (in vitro study). Furthermore, the

antiafusarium efficacy against neuroinflammatory induced by *fusarium species* was carried out at biochemical and molecular levels (in vivo study) through targeting oxidative stress, inflammatory, transporter and dementia progression markers.

A 909 nucleotide bases of the 18S ribosomal RNA isolated from our strain was sequenced and the obtained sequence was got the accession number (KX808233), version 1 (KX808233.1) by GenBank. This alignment revealed a newly identified sequence that was registered to our study group and was given a new accession number by GenBank (KX808233), version 1 (KX808233.1) and named as *Fusarium sporotrichioides*.

In vitro study, there was no differences detected in fungi growth either in (-ve) control or (+ve) control (in which different concentration of DMSO were used). *Calluna vulgaris* L. (Ericaceae) (0.5%, 1%, 1.5% and 2%) and *Berberis vulgaris* showed no inhibitory effect toward fungi growth. *Cinnamuom zeilanicum* was extremely acted as potent inhibitor for *Fusarium sporotrichioides* growth as its high concentrations (1%, 1.5% and 2%) completely inhibited the fungi growth.

Deoxynivalenol and Aflatoxin were not detected in growth media of *F. sporotrichioides*, On the other hand, Zearalenone level produced by *F. proliferatum* was highly reduced by treatment with 2% *Cinnamuom zeilanicum* and moderately reduced by 2% *Berberise vulgaris* while *Calluna vulgaris* had almost no effect on Zearalenone level. The inhibitoriest effect for the production of fumonisin B_1 was for *Cinnamuom zeilanicum* followed by *Berberise vulgaris* and finally *Calluna vulgaris*.

In vivo study, for liver tests enzymes level, the toxin fed rats showed highest AST activity (94.0±5.48U/L), and showed the highest ALT activity (61.67±3.27U/L) at $p<0.05$, while celebrex post treated group showed the lowest enzymatic AST activity (55.50±4.89U/L). All other treated groups showed AST activities lower than that of both sham control (79.50±2.88U/L) and toxin fed groups, at $p<0.05$.While, Cinnamon control treated rats had the same sham control ALT activity (42.67±2.25U/L), at $p<0.05$., Either the pretreatment with cinnamon or celebrex significantly normalized the ALT activity, at $p<0.05$. The post-treatment with cinnamon or celebrex significantly decreased ALT activity than that of toxin one.

Our results has a significant lower level in brain total protein in media toxin group (8.4±0.03 mg/mg wet tissue) compared to sham control (11.93±0.55 mg/g wet tissue). Cinnamon

control treated rats showed a significant lower and higher protein levels when compared to sham control and media toxin groups respectively, so cinnamon extract has increased the levels of serum total protein towards the respective normal value, which indicates hepatoprotective activity.

Effect of ethanolic *Cinnamoun zeilanicum* crude extract on immunological function tests in brain homogenate during prevention of *F. sporotrichioides* induced neuroinflammation in our study, toxin-fed (30.4±0.61pg/mg protein) and cinnamon post-treated groups showed the highest brain IL-6 level as an inflammatory response, while sham control level (14.0±0.26 pg/mg protein) was the lowest, at $p<0.05$. All treatments except cinnamon post-treated group showed IL-6 levels lower than that of toxin fed group level, at $p<0.05$. Also, Toxin-fed group showed the highest brain IL-12 level while the sham control and cinnamon control groups showed the lowest level, at $p<0.05$.and this because of the induction of inflammatory factors.

Gene expression by Real time PCR the fold change calculated values for the assessment of IL-1β gene expression in different studied groups showed the highest levels of IL-1β gene expression was observed in both media toxin as well as cinnamon pre-treated and celebrex post-treated groups showed IL-1β expression level lower than that of sham control ones, at $p<0.05$. The highest levels of Cox II gene expression was also observed in media toxin and Celebrex pre-treated groups; all studied groups had COX II expression level higher than that of sham control but lower than that of media toxin.

Unlike IL-1β or Cox-II, high level of TNF gene expression was observed only in media toxin group that showed a mean fold change of (239.43±71.64). All other studied groups had lower TNF gene expression levels than that of sham control one.

Finally, for iNOS expression level all groups showed higher than that of control one. The highest expression was showed in media toxin groups while the lowest one was found in cinnamon control group, at $P<0.05$.those activated genes were a response of neuroinflammatory effect induced by toxins FB1 production.

Alkaloids and organophosphoric compounds are well known inhibitors of AchE activity, but numerous molecules are moderators of this enzyme as well. Inhibited activity of AchE results in increased concentration of acetylcholine, which reflects on the experimental animal behaviour. In our study there was a significant increase in AchE specific activity in media toxin (64.67±8.85 μmoles/min/ mg protein) and control cinnamon fed rats groups when

compared to sham control group (51.17±7.14 µmoles/min/ mg protein). Cinnamon pre-treated group and celebrex pre-treated groups showed high significant difference specific activities of AchE when compared to sham control group. Cinnamon post-treated group showed a significant decrease in Ach specific activities compared to media toxin group, while cinnamon and celebrex pre-treated groups showed a significant increase in AchE specific activities compared to media toxin group, at $p \leq 0.05$.

For ethanolic *Cinnamoun zeilanicum* crude extract heat shock protein (Elisa Hsp) level in brain homogenate during prevention of *F. sporotrichioides* induced neuroinflammation there was a significant increase in Hsp levels in media toxin and control cinnamon fed rats groups when compared to sham control group. Cinnamon and celebrex pre-treated and post-treated groups showed high significant difference levels compared to sham control group. On the other hand, Cinnamon and celebrex pre-treated and post treated groups didn't show any significant difference in Hsp levels compared to media toxin induced group.

Effect of ethanolic *Cinnamoun zeilanicum* crude extract on beta-amyloid-42 concentration in brain homogenate during prevention of *F. sporotrichioides* induced neuroinflammation, there was a significant increase in β-amyloid-42 levels, in media toxin fed rats group when compared to sham control group. But there was non-significant increase in β-amyloid-42 level in cinnamon control group compared to sham control. Only post-treated cinnamon group showed significant decrease in β-amyloid-42 level compared to sham control group.

Paraffin sections were stained with haematoxylin and eosin for morphological changes in rat brain tissues for normal and different exposed to treatment, The histopathological photographe of brain tissue were studied in the area of hippocmapus and cortex region rat brain toxin induced group, showed neuronal degeneration in the cerebral cortex where many of the nerve cells appeared shrunken, containing dark-stained hyperchromatic nuclei called red neurons and other neurons showed central chromatolysis with pale eosinophilic cytoplasm, rat brain control group, showed the histo-architecture of hippocampus rounded clear nuclei with prominent nucleolus of neuron cells and healthy glial cells (PC), there are normal morphology of cortical cells and few pyknotic cells. pre-treated cinammon group of rat brain showed showing the shrunked nerve cells surrounded the halo empty spaces in addition to the presence the normal shaped nerve cells; the mild vacuolation in the neuropil reflect the recovery of the brain tissue, post-treated celebrex-induced toxin group of rat brain Light micrograph cerebral cortex of mice post treated drug showed the appearance of many

pyknotic nuclei of the neuronal cells that contain an eosinophilic cytoplasm, while post-treated cinammon group of rat brain Showed that that most of the neurons had fragmented nuclei and they are surrounded by large vacuolated spaces

Conclusion:

From these results, we concluded that when we used 18S ribosomal RNA we determined the presence of new strain of fusarium and its named *F. sporotrichioides*, which then used as a neuro-inflammatory inducer. Then we concluded that when we used ethanolic crude *Cinnamon zeilanicum* extract against fungi growth and different toxin production it showed the highest inhibitory effect. And when we used *Cinnamon zeilanicum* as a natural product protective compound and also we used celebrex as an anti-inflammatory commercial drug (as treating compound) against neuroinflammation induced by *F. sporotrichioides* – Fumonisin B_1 toxicity, they increased antioxidants enzymes level, returned liver enzymes into normal level and also decreased inflammatory genes expression and caused protection and recovery for histopathological neuro-cells against inflammation. While when we used both of them *Cinnamon zeilanicum* as a treatment and celebrex as a protective compound we noticed that there were no obvious effect as anti-inflammatory agents.

Recommendation:

So we recommended the uses of *Cinnamon zeilanicum* as a natural product protective agent against inflammation induced by Fumonisin B_1 beside they contains a lot of beneficial effect for humans as can act as antioxidant, antifungal and anti-inflammatory agent. And using celebrex as treatment drug against inflammation but it may have some side effects.