Potential molecular mechanism of natural extract modulation of aflatoxin toxicity.

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Summary, conclusion and recommendation:

Aflatoxins are a group of closely related mycotoxins that are widely distributed in nature in different agricultural communities. The problem of using contaminated food with toxigenic fungi is still one of the most important stigmas in the field of nourishment of human and animals. This contamination accounts as serious problem because it reduces feeding value and hinder sales, because it is extremely poisonous to warm-blooded animals even at relatively low levels. This shown the serious effects of aflatoxins on liver, lymphocytes, macrophages, lung and kidney.

This thesis was conducted to find an *A. flavus* fungicidal and anti-aflatoxin natural extract. And to study the effect of different ethanolic extracts of natural products administration as protective agent against *A. flavus* induced hepatotoxicity at ICAM-1 and P53 expression gene and biochemical level.

In *in vitro* experiments, we studied the effect of ethanolic extracts of plants (*Thymus vulgaris, Cinnamuom zeilanicum, menthe pulegium, adiantum capillus* and *berberis vulgaris*) and algae (*Ulva lactuca, Jania ruben* and *Pterocladiella capillacea*) on *A. flavus* growth. The concentration of aflatoxin in the presence of examined extracts was measured by HPLC. Our results showed that *Cinnamuom zeilanicum* extract caused complete *A.flavus* growth inhibition and therefore it resulted on undetected concentration of aflatoxin, followed by *thymus vulgaris* and *menthe plugium* which they caused relative inhibition of *A.flavus* but they increased aflatoxin B1 concentration (4324.85 ppm, 1868.04 ppm) respectively than control (81.306 ppm) while decreased aflatoxin B2 concentration (62.0360 ppm, 33.5802 ppm) than control (373.56 ppm). On the other side, *berberis vulgaris* (0.01%) increased aflatoxin B1 concentration (457.85 ppm) while concentration of 1% decreased aflatoxin B1 concentration (1.3283ppm) than control (81.306 ppm) and the same happened for aflatoxin B2 as *berberis vulgaris* at concentrations of 0.01% and 1% decreased B2 concentration (122.5140 ppm, 41.0661ppm) than control (373.56 ppm).

Algae ethanolic extracts caused increase of aflatoxin B1 concentration than control while on the opposite side; it decreased the concentration of aflatoxin B2 till reached the undetected level.

The phytochemical investigation and bioscreening assays were done for all natural products extracts of plants. Dried plants and algae were phytochemically screened for alkaloids, phlobatannins, saponnins, flavonoids, steroids, terpenoids and cardiac glycosides and it was found that these plants were rich sources for polyphenolic compounds. The obtained data showed that *berberis vulgaris* had antifungal and antibacterial effect this could be returned to *Berberis vulgaris* and *cinnamon zeilanicum* had high concentrations of saponins, flavenoids. Also cholinergic effect of natural plants was examined which showed that *Berberis vulgaris* had the highest activity ($34*10^{-4}$ mols/mint) than control ($6.51*10^{-4}$mols/mint) so we concluded that *cinnamon, berberis, thymus, menthe* and *adiantum* act as activator for Ach E. When we determined the effect of tested plants on alpha glucosidase activity, we found that *menthe plugium, berbeis vulgaris* and *thymus vulgaris* increased the enzyme activities by (29.04, 8.504 and 6.8 %, respectively) than that of control one.

Differential display showed up regulated and down regulated bands between *A.flavus* and natural product (NP) treated *A.flavus* especially that treated with Cinnamon and berberis as these extracts caused genetically changes at *A.flavus* genome. These results explained our fungicidal and anti-aflatoxin effect of these extracts.

In *in vivo* study, rats were orally administrated ethanolic extracts of (*Berberis vulgaris, Cinnamon zeilanicum* , *Ulva lactuca* and *DMSO*) of concentration (20 mg/ 100 g body weight) for two weeks that acting as protective agents against the side effects that occurred due to the administration *A. flavus* (0.15 mL) for four weeks on those groups of rats. Liver function tests (ALT, AST and albumin), also kidney function tests (creatinine, urea and glucose), antioxidant enzymes (SOD and GPX) and oxidative radicals (TBARS and NO) were sepectrophotometrically determined. Hepatic ICAM-1 and P53 expression were detected by using real time PCR technique.

The routine liver function tests showed the serious effect of aflatoxin administration on hepatocytes where it elevated liver enzymes, on the other hand, only cinnamon and berberis administrations re-adjusted the enzymes and approached the normal enzymatic level. So we can conclude that those plants contain active gradients which inhibit aflatoxin toxicity.

Plasma albumin level elevated than control (4.88±0.41g/dl) on group which administrated *A.flavus* (42.97±0.88 g/dl) and DMSO (43.81±0.3 g/dl) which reflected their adverse effect on liver and/ or kidney. On the other hand, all other naturally protected groups were almost not affected with *A.flavus* induction as these groups showed normal albumin level.

Furthermore, plasma creatinine, urea and glucose levels had been changed after aflatoxin toxicity as *A.flavus* administration increased serum creatinine and glucose while decreased urea level than those of control groups. On the other, the protective groups with all extracts showed normal parameters levels.

Antioxidant enzymes activities in plasma were affected with *A.flavus* administration as GPX (0.083±0.002 IU/g) decreased than control (0.198±0.036 IU/g) while SOD (258.8±0.153 ng) was almost near to control level (252.8±0.144 ng), but for groups which administrated natural products especially cinnamon and DMSO level of GPX was highly elevated than control and induced groups as cinnamon (11.13±3.25 IU) and DMSO was (7.47 ±0.7 IU).while for SOD also naturally administrated groups were almost like control except cinnamon administrated group which elevated than control and induced groups as it was (265.15±0.18 ng) and DMSO administrated groups was (262.8±0.10 ng).

This elevation of antioxidant enzymes activity were due to high production of oxidant molecules as NO and TBARS on those groups, as NO was elevated than control level as (5460±346.1umol/l) and control was (3008.9±308.7umol/l) while cinnamon,berberis,ulva and DMSO were (6966.6±211, 8542.9±425.4, 4804.8±634.7, 6421.4±274umol/l) so it reflects the cause of increasing enzymes level, on the other TBARS level increased on induced group while decreased on other naturally administrated groups.

It's known that *P53* gene (249^{ser}) is commonly found in HCC from patients in regions with dietary aflatoxin exposure, in agreement with this findings group which administrated *A.flavus* had 87% increase in P53 gene expression. On the other side, cinnamon and berberis administration increased the P53 gene expression 410.5% and 269.3% respectively. Therefore, we conclude that those plants up-regulated p53 so they could be act as anticancer agents.

While ICAM-1 which is expressed on the surface membrane of cells of multiple lineages at sites of inflammation and immune reactivity were gene expression increased by 65.3% after *A. flavus* administration, while for groups which administrated cinnamon and berberis increased ICAM gene expression by 100.03% and 139.05% respectively.

Conclusion:

From these results, we concluded that when we used *cinnamon zeilanicum* and *berberis vulgaris* as protective compounds against hepatocytes - aflatoxin toxicity, they increased antioxidants enzymes level, returned liver enzymes into normal level and also increased p53 and ICAM-1 expression.

Recommendation:

So we recommended the uses of *cinnamon zeilanicum* and *berberis vulgaris* as protective compounds against aflatoxin toxicity beside they contains a lot of beneficial effect for humans as can act as antioxidant agents.