EVALUATION OF THE POSSIBLE EFFECT OF BERBERINE NANOPARTICLES AND /OR CISPLATIN AGAINST TOXICITY INDUCED BY A CARCINOGENIC AGENT IN RAT LIVER

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6. SUMMARY AND CONCLUSION

Despite advances in diagnosis and treatment using slandered therapies such as surgery, radiation and chemotherapy, hepatotoxicity is still the major formidable challenge for clinical treatment. The main strategy of chemotherapy drugs based on the phenomenon that these drugs selectively target the tumor cells, largely by the means of genotoxicity partially caused by the production of reactive oxygen species, which does not specifically damages the cancer cells but also the normal cells.

The most common type of chemotherapy drug is platinum drugs, cisplatin, is an inorganic platinum compound with broad-spectrum anti-neoplastic activity against different types of human tumors, particularly solid tumors. However, severe side effects of cisplatin such as nephrotoxicity, neurotoxicity, ototoxicity, greatly hamper its chemotherapeutic efficacy.

Designing drugs with greater degree of cell specificity to ameliorate efficacy and minimizes side effects is considered to be an important issue. Nanoparticles are being applied extensively to trigger targeted drug therapy.

In the present study Chitosan (CS) -based nanoparticles were prepared for the sustained release of berberine (BBR). Berberine loaded chitosan nanoparticles were successfully synthesized by the ionic cross-linking method. Berberine loaded chitosan, exhibited good stability and had ideal releasing profile after intra-articular injection of BBR-loaded.

The present study aimed to investigate:

1- The possible effect of Berberine nanoparticles (BBR-NPs) against liver toxicity induced by *N*-Nitroso-diethylamine (DENA) and carbon tetrachloride (CCl_4) as a new approach in treatment.

2- The possible beneficial effect of combination of cisplatin as a chemotherapeutic drug and berberine as a (nanoparticle drug) against induced hepatic toxicity.

Animals were divided into five groups each of 10 rats as follows:

Group I: The rats of this group were treated orally with olive oil (0.5 ml/kg/day) for 60 days and considered as a control group.

Group II: The rats of this group were treated intraperitoneally with the *N*-Nitroso-diethyl amine (DENA) (200 mg/kg) as a single dose and followed by carbon tetrachloride (CCl_4) (1.5 ml/kg/day) for 30 days (~~Ramanathan *et al.*, 2011~~).

Group III: The rats of this group were treated intraperitoneally with the *N*-Nitroso-diethyl amine (DENA) (200 mg/kg) as a single dose followed by carbon tetrachloride (CCl_4) (1.5 ml/kg/) for 30 days then treated intraperitoneally with cisplatin (8 mg/kg/week) for 30 days (~~Vermorken *et al.*, 1982~~).

Group IV: The rats of this group treated intraperitoneally with the *N*-Nitroso-diethyl amine (DENA) (200 mg/kg) as a single dose and followed by carbon tetrachloride (CCl_4) (1.5 ml/kg/day) for 30 days then treated orally with berberine nanoparticle (BBR-NPs) (1mg/ml/day) for 30 days

Group V: The rats of this group were treated intraperitoneally with the *N*-Nitroso-diethylamine (DENA) (200 mg/kg) as a single dose followed by carbon tetrachloride(CCl_4) (1.5 ml/kg) for 30 days then treated intraperitoneally with cisplatin (8 mg/kg/week) and berberine nanoparticles(BBR-NPs) (1mg/ml/day) for 30 days.

In the present study, the induction of DENA/CCl_4 leaded to significant increase in white blood cell counts (WBCs) and decrease in the other blood elements in comparison to the control group include red blood cell counts (RBCs), hemoglobin content (Hb), hematocrit (Hct), and platelets. In contrast, treatment by berberine nanoparticles (BBR-NPs) and/or showed significant increase in the values of RBCs, Hb, Hct and platelet counts however, it was found that cisplatin treatment alone caused a significant decrease in RBCs, platelet counts. Treatment with BBR-NPs, cisplatin after DENA/CCl_4 and their combination leaded to decrease the level of WBCs when compared to the induced group DENA/CCl_4.

Induction of DENA/CCl_4 leaded to elevation in liver markers (ALT, AST, ALK, bilirubin, LDH, GGT, 5NT and AFP) meanwhile, decreased in the level TP and ALB. Post treatment by BBR-NPS and cisplatin and their combination after DENA/CCl_4 decreased

the elevation levels of ALT, AST, ALK, bilirubin, LDH, GGT, 5`NT and AFP and increased in TP and ALB level.

In the current study induction of DENA/CCl_4 caused elevation in the lipid peroxidation level determined by measured the Malondialdehyde (MDA) moreover, decrease in the antioxidant enzyme activities superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH). Treatment with BBR-NPs, cisplatin and their combination leaded to decrease in the MDA level and increase in antioxidants enzymes (SOD, GPX and CAT) and GSH.

The best results were clearly present in the combination group (BBR-NPs + cisplatin + DENA/CCl4) in all measured parameters.

In the current study induction of the carcinogenic agent **DENA/CCl_4** leads to severe hepatic injury manifested by lose of normal hepatic architecture, congested portal vein, proliferation of bile ductule, hepatocytes with irregular hyper chromatic nuclei, and distribution of lipid droplet and formation of fibrous capsule.

With the usage of electron microscope, glycogen loss, clusters of swelling mitochondria with loss of cristae, short profile of endoplasmic reticula, bile ductule with partial detachment microvilli and kupffer cell with variable shape can be detected.

Treatment with **DENA/CCl_4 & cisplatin** showed some hepatic lobules with well-formed central veins and intact hepatic cells. In other area congested sinusoids or dilated sinusoids with hypertrophic Kupffer cells were detected. Ultrastructure examination showed signs of improvement in which the nucleus chromatin is mainly distinguished into electron dense peripheral heterochromatin and dispersed inner euchromatin, the cytoplasm possessed wide areas of glycogen granules and numerous scattered rounded or elongated mitochondria with two clear membranes.

Treatment by **DENA/CCl_4 & BBR-NPs** showed signs of improvement through a well arrangement of hepatocytes with normal central vein but there is less improved area with collapsed sinusoid. Transmission electron microscope showed almost normal nucleus and cytoplasm is embedded by round or elongated mitochondria, glycogen droplets and rough endoplasmic reticulum.

Treatment by **DENA/CCl_4 group & cisplatin/BBR-NPs** showed highly improvement in the histological structure where cells are normal with round nuclei arranged in cords with no cytoplasmic vacuoles and separated by regular blood sinusoids. Regeneration activity is detected by the presence of dividing stages as prophase and metaphase. The ultrastructural picture showed a highly improved appearance of well-organized cytoplasm containing noticeable glycogen, oval shaped mitochondria with tubular cristae bounded by rough endoplasmic reticulum and bile ductule with well-formed microvilli.

The molecular examination involved the assessment of ADAM metallopeptidase domain 17 (ADAM 17), tumor necrosis factor α (TNF-α), protein 53(P53) against glyceraldehyde 3- phosphate dehydrogenase GAPDH as housekeeping gene in rats.

The administration of DENA/CCl_4 increased the relative expression of ADAM17&TNF-α which associated with down regulation of P53 when compared to control relative gene expression levels which indicating the toxic effect and oxidative stress of the DENA/CCl_4.

The treatment by BBR-NPs alone improved the genetic expression of testing genes (ADAM 17, TNF-α & p53) but didn't normalize them. Cisplatin administration down regulates ADAM 17& TNF-α and P53expression.

The best effect was obtained by using both drugs cisplatin and berberine nanoparticles that leads to regulation in the relative gene expression by down regulated the inflammatory molecule expression ADAM17&TNF-α and up regulate p53 expression when compared to the induced level .

Conclusions

From the above mentioned results, we can conclude that:

1- DENA is a potent carcinogen is used for hepacellular forming and CCl_4 is used for its initiation

2- Treatment by berberine nanoparticles led to signs of improvement in the biochemical, histology and molecular studies with the absence of side effects

3- Treatment with cisplatin drug led to signs of improvement in the biochemical, histology and molecular study with the presence of some side effects include anemia and inflammation

4- The combination treatment between BBR/NPs as herbal medicine and cispatin as chemotherapeutic drug has the best curative effect with no side effects.

Recommendation:

Further studies and investigations must be done to study the possible evaluation way of treatment by using BBR-NPs alone or in combination with different chemotherapeutic drugs. Moreover, reasearches needed to done to study the mechanism of BBR-NPs in treatment of different types of cancers.