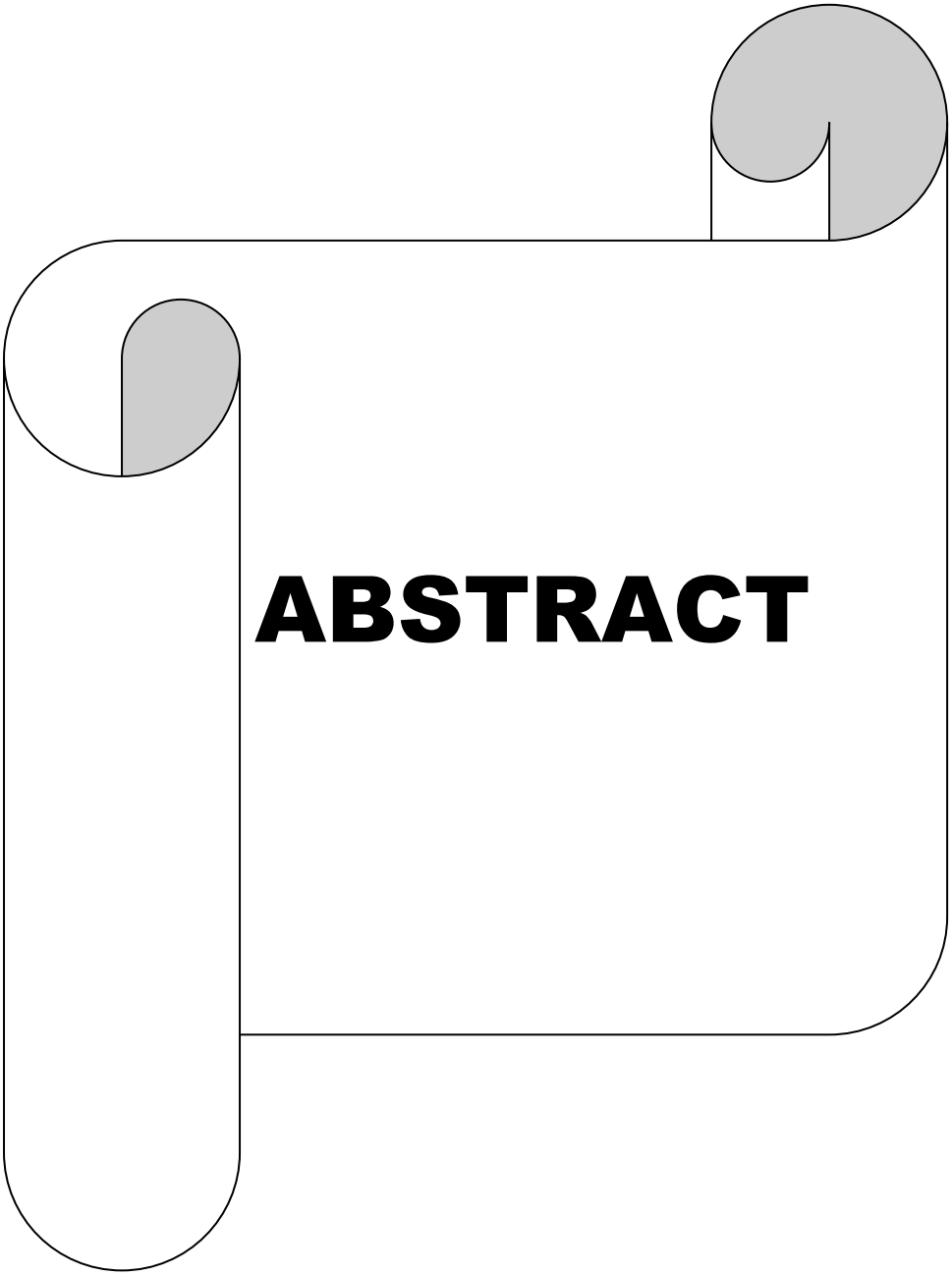

# ABSTRACT

# ABSTRACT

Cisplatin is an active cytotoxic agent that has proved to be successful in the treatment of various type of solid tumors. The drug induces nephrotoxicity has been very well documented in clinical oncology. The present study had used rats as a model to evaluate the effect of cisplatin on kidney function and antioxidant enzymes, and also evaluate the effect of natural diets such as dried black grape and / or dried hot red pepper in reducing nephrotoxicity that caused by cisplatin.

١٢٠ adult male albino rats were randomly allocated in ٨groups , each group contains ١٥ rats, weighing from (٨٠-٩٠g), the experimental period lasted for ٣٧ days . All groups injected intraperitoneal (ip) with cisplatin (١٠ mg/kg of body weight) , except negative control group( G١)injected ip by saline (١٠ mg/kg of body weight). The diets fed were :G١(basal diet negative control group )and G٢ (basal diet positive control group),G٣ and G٤ : basal diet+ (٥٪) of either dried black grape or hot red pepper. G٥ and G٦ : basal diet + (١٠٪) of either dried black grape or hot red pepper.G٧ and G٨ : basal diet and incorporated with either ٥٪ or ١٠٪ of dried black grape and dried hot red pepper respectively.

Blood samples collected after ٣٧ days for the determination of serum total protein , potassium, uric acid, urea, creatinine, nitric oxide and total antioxidant capacity, and also determination of hemoglobin ,SOD and GsHPx activities in blood . On the other hand, the kidney processed for determination of SOD, GsHPx activities, nitric oxide and lipid peroxide. After ٣٧ days, there were a significant increase in serum urea, creatinine, uric acid, potassium and nitric oxide, whilst, there were a significant decrease in serum total protein and serum total protein and total antioxidant capacity in G٢ $p < ٠,٠٠١$ when compared to G١. On the other hand, there were a significant increase in kidney tissues lipid peroxides and

nitric oxide, whilst, there were a significant decrease in kidney tissues and blood SOD, GSHPx activities and blood HB in G٢ p < ٠,٠٠١ when compared with G١. Treatment with dried black grape and / or dried hot red pepper showed a good improvement in the following descending manner (G٨ and G٧), (G٦ and G٥)and then (G٤ and G٣).

## Key Words:

Cisplatin, lipid peroxidation, enzymatic antioxidant, nephrotoxicity, black grape, hot red pepper.

# Aim of the work

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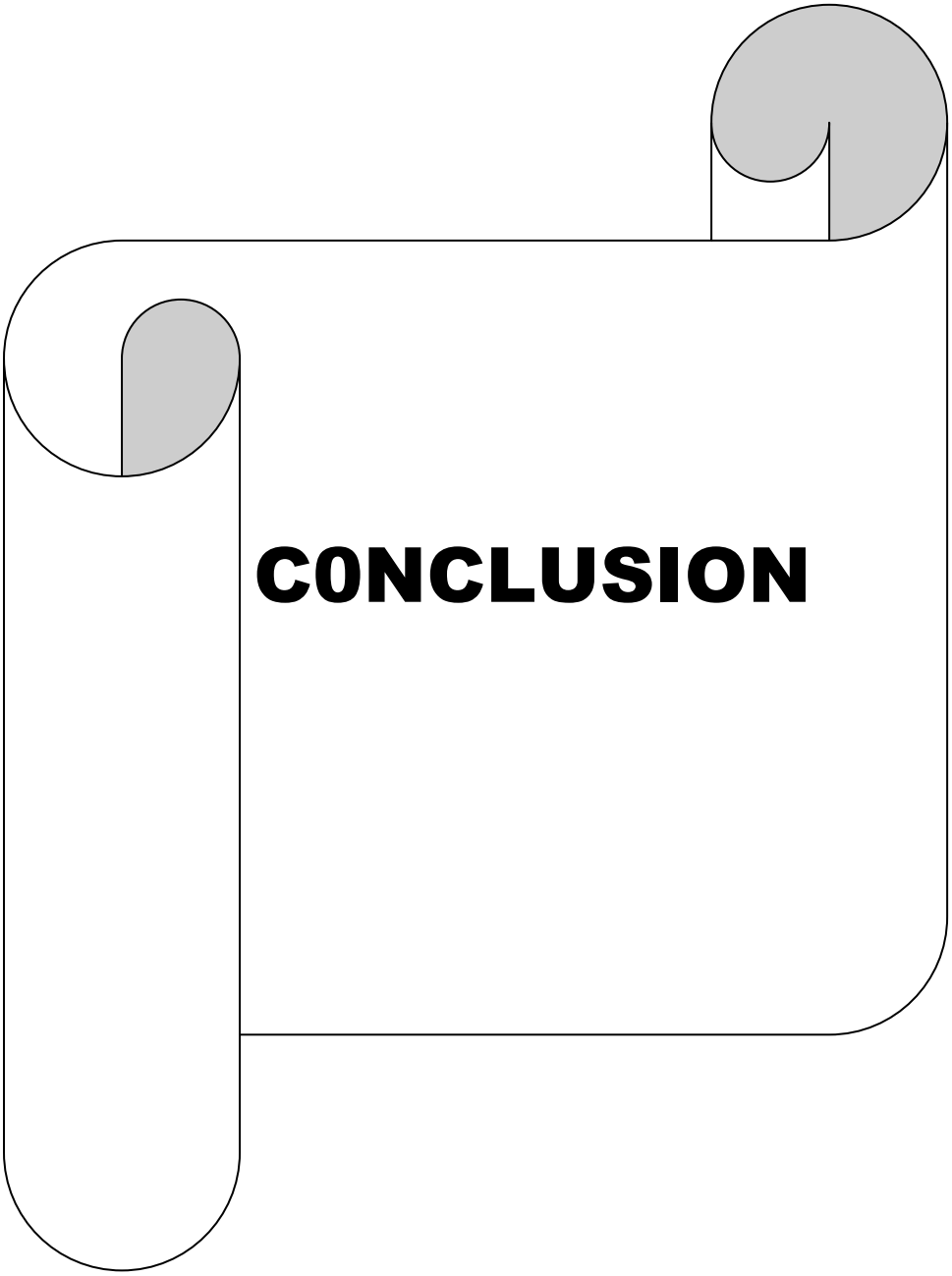
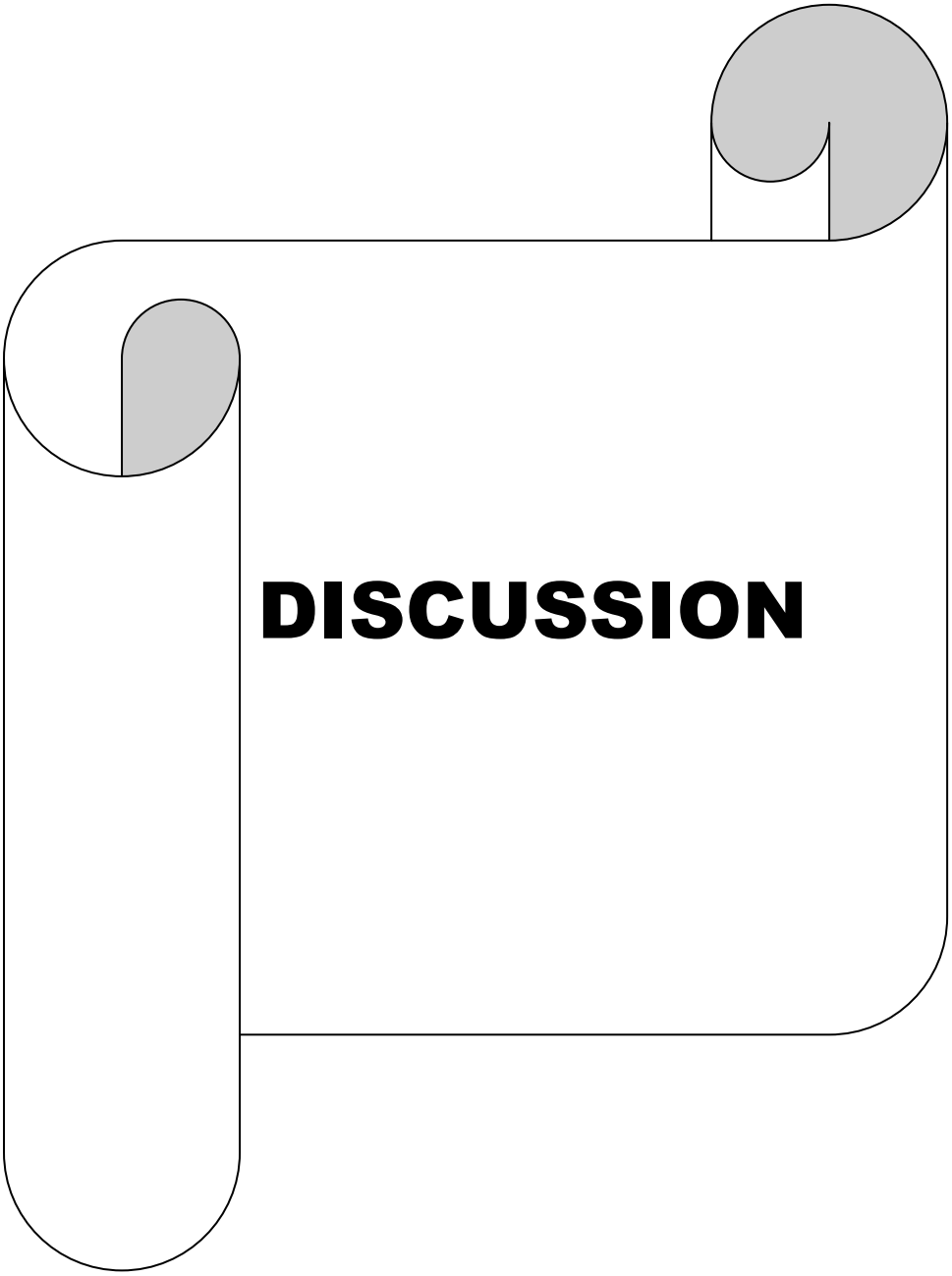
This study was conducted to elucidate the mechanism responsible for the potent inhibitory effects of dried black grape and /or dried hot red pepper with an active constituent against cisplatin induced lipid peroxidation and nephrotoxicity in experimental animals.


C0NCLUSION

# CONCLUSION

As far as we know, this is the first study that compares hot red pepper and black grape combination or alone in the prevention of CDDP-induced nephrotoxicity. Our study provides encouraging results about the protective effect of hot red pepper and black grape combination against CDDP nephro- toxicity. So combination of hot red pepper and black grape seems more effective than were fed on either hot red pepper or black grape alone.



# DISCUSSION

## Discussion :

In the present study, administration of CDDP (١٠ mg/kg body weight i.p.) produced a significant nephrotoxicity in rats. Acute nephrotoxicity , demonstrated by a significant increase in serum creatinine, urea level ,serum potassium level and serum uric acid and a significant decrease in serum total protein in positive control group when compared with negative control group .This increase in kidney function tests may be due to the decrease in glomerular filtration rate or may be secondary to increase the reactive oxygen species (ROS).This results agreeing with the result of ***Noori and Mahboob.,(2010)*** who confirmed that, cisplatin accumulated in mitochondria, leading to increase (ROS) production.

In positive control group ,there was a significant decrease in hemoglobin concentration , blood and kidney tissues superoxide dismutase (SOD) and glutathione peroxidase ( GSHPx) activities, and a significant increase $p<٠,٠٠١$ in the mean values of nitric oxide level and lipid peroxide in kidney tissues in comparison with negative control group. on the other hand, There was a highly significant increase on serum nitric oxide and a highly significant decrease on serum total antioxidant capacity in positive control group when compared with negative control group, agreeing with ***(***Yi***lmaz et al., 2005).***

The present results agree with the result of ***Mohamed,(2010)***, who explain the increase of ROS that attack the cell membrane lipids leads to increase tissue lipid peroxidation, manifested by increased MDA. Over accumulation of lipid peroxide in tissue causes over consumption and depletion of antioxidant enzymes as SOD and GSHPx activities.

Cisplatin preferentially accumulates in cells of the S٣ segment of the renal proximal tubules and is toxified to form a reactive metabolite intracellularly by hydration. The primary symptoms of cisplatin nephrotoxicity are inhibition of protein synthesis and reduce antioxidants activities , resulting in lipid peroxidation and mitochondrial damage. , which control lipid peroxidation. From these pathomechanisms of cisplatin nephrotoxicity, it is clear that, the nephrotoxicity of cisplatin involves reactive radicals. Thus the reasonable cellular-protective agents against cisplatin toxicity may have at least some antioxidant properties to scavenge the intracellular reactive oxygen species, ***(Al-Hashem, 2009).***

The results of the present study are also in accordance with those reported by ***Sogut et al., (2005),***who confirmed that, there were a significant increase in MDA and nitric oxide level in kidney tissue after treatment by cisplatin (١٠ mg/kg body weight) in comparison with negative control group.Also there were a significant decrease in antioxidant enzymes activities as SOD and GSHPx in CDDP group when compared to negative control group.

Similary***,Malini et al., (2005)*** investigated that, treatment by cisplatin low or high doses causes renal injury by increasing lipid peroxidation products and impairs antioxidants enzymes activity. On the other hand, ***Rojas et al.,(2005)*** reported that, treatment by CDDP twice per day for ١٠ weeks lead to a significant increase in serum creatinine, serum urea and a significant decrease in SOD and GSHPx activities in kidney tissue.

Another openion agree with our study, ***Eldemerdash and Saleh(2005) and Yao et al (2007),*** investigated that, cisplatin treatment causes acute nephrotoxicity which demonstrated by a marked increase in serum creatinine, serum urea, also there

were increased in the level of MDA and depletion of SOD and GSHPX activities. ***Karaoglu et al., (2005)*** go on the same line with the previous study accompaned by increasing $Na^+$ and $K^+$ level in kidneys tissues.

***Strivastava et al.,(2004),Badary et al., (2005)and Kawai et al., (2006)*** reported that, oxidative stress injury is actively involved in the pathogenesis of CDDP-induced acute kidney injury. Reactive oxygen species (ROS) directly act on th cell component, including lipids, proteins, DNA and destroy their structure. ROS are produced via xanthine-oxidase system, mitochondria, and NADPH oxidase in the cells, in the presence of CDDP, ROS are produced through all these pathways and are implicated in the pathogenesis of acute CDDP-induced renal injury. Superoxide anion, hydrogen peroxide ($H_2O_2$) and hydroxyl radical are increased and react with lipids of the cell membrane by peroxidation and denature membrane cell proteins, this explain, the high level of MDA in CDDP group, also reduced SOD and GSHPx activites, as a result of total antioxidant capacity decreased.

***Chirino et al.,(2004) and Carlström et al.,(2010)***, explained the relation between SOD and nitric oxide, NO is produced in trace quantities by neurons, endothelial cells, platelets, and esinophiles in response to homestatic stimuli. This NO is scavenged rapidly and act in a paracrine fashion to transreduce cellular signals. NO is also produced by other cells (macrophages, fibroblasts, hepatocytes) in micromolar concentration in response to inflammatory or mitogenic stimuli. The final products of NO in vivo are nitrite ($NO_2^-$) and nitrate ($NO_3^-$). The relative proportion of $NO_2^-$ and $NO_3^-$ produced from NO is variable. The exogenous source of $NO_3^-$ ingested in diet should be considered and cannot be ignored. Thus, one of the index of NO production is the $NO_2^-$. SOD

also appears to be important in the prevention of other neurodegenerative disorder such as Alzheimer`s, Parkinson`s and Huntigton`s diseases . SODs are metalloenzymes that catalyze the dismutation of super oxide anion to molecular oxygen and hydrogen peroxide and thus form a crucial part of the cellular antioxidant defense mechanism. There are three types of SODs have been characteristized according to their metal content copper, zinc (Cu,Zn) , manganese (Mn) , and iron (Fe). SOD is widely distributed in both plants and animals. SODs occur in high concentration in brain, liver, heart, erythrocytes and kidney.In human there are three forms of SOD ;cytosolic Cu/Zn-SOD , mitochondrial Mn-SOD, and extracellular SOD. Extracellular SOD is found in the enterstitial spaces of tissues and also in extacellular, accounting for the majority of the SOD activity in plasma, lymph, and synovial fluid.The decreased SOD activity in CDDP treated group is insufficient to scavenge the superoxide anion produced during the normal metabolic process . As a result of decreasing SOD activity , the level of nitric oxide increased because nitric oxide compete the SOD for superoxide anion resulting in formation of peroxynitrite which considered powerful oxidizing and nitrating agent, peroxynitrite causes change in protein structure and function, lipid peroxidation, chemical change in DNA and reduction in cellular defenses. These competition present only in the case of decreasing SOD activity or in SOD mutation, and this explain why the level of nitric oxide increase in case of SOD activity decrease . So nitric oxide play important role in kidneys injury.

The decrease in SOD activity after cisplatin administration might be due to the loss of copper and zinc in the kidneys . Zn is a trace element essential for living organisms. More than ٣٠٠ enzymes require Zn for their activity. It also plays an important role in the DNA replication, transcription and protein synthesis, influencing cell division

and differentiation. It has been noted that, Zn has a relationship with many enzymes in the body and can prevent cell damage through activation of the antioxidant system. Zinc is an essential component of the oxidant defense system and functions, at many levels Zn deficiency increases lipid peroxidation in various rates which are essential for the enzyme activity, ***(Ozdemirand and Inanc, 2005; Santos et al., 2008).***

The activity of GSHPx is also found to be decreased after cisplatin administration, this decrease may be due to the ability of the kidney to scavenge toxic hydrogen peroxide and lipid peroxides, and subsequence a significant decrease in the lipid peroxides level, ***(Tsuji et al., 2009).***

Also our results are in agreement with, ***Gao et al.,(2006)*** who investigated the changes of serum erythropoietin (Epo) during cisplatin – inducing anemia in rats. Anemia was induced with single intravenous injection of CDDP ( ٨ mg/kg body weight). Serum Epo, hemoglobin, blood urea nitrogen (BUN) concentration, and reticulocyte (Ret) counts were measured on ٧ and ٢١days after administration of the anticancer drugs. The changes of renal tissue were examined by light microscope. A single injection of CDDP decreased Ret counts and Hb concentration and increased BUN. Serum Epo was decreased on٧ days but was increased on ٢١ days after CDDP treatment; however, these results suggest that, in CDDP-induced anemia, the concentration of serum Epo level was low in relation to the level of anemia, and CDDP-induced nephrotoxicity might be the main cause of changes of serum Epo.

Also ***Canaparo et al., (2000),*** studied that, platinum chemotherapy has been shown to have potent antineoplastic activity against various tumours, especially testicular, bladder, ovarian, head and neck cancers. This activity is accompanied

by side-effects of nephrotoxicity and cumulative myelosuppression, the latter frequently presenting as severe anaemia. Cisplatin and carboplatin nephrotoxicity might lower erythropoietin (Epo) secretion and, by this mechanism, contribute to the anaemia that follows therapy with this chemotherapeutic agent.

Flavonoids and antioxidants have been reported to exhibit a wide range of biological effect and may play a dietary role in protection against chronic diseases, The present study was designed to evaluate the potential role of natural dietary antioxidant, such as resveratrol which present in black grape and capsaicin which present in hot red pepper, against cisplatin- induced changes in renal function, renal cortex status in experiment animals as rats. Patients with cancer take antioxidant supplements to enhance the benefits of treatment. Antioxidants may also reduce certain types of toxicity associated with chemotherapy. Antioxidants, such as vitamin E and vitamin C administration, was reported to protect against CDDP-induced renal toxicity in animal studies.In the present study,black grape and hot red pepper used as a whole after drying ,so it contain different ingredients which play important role as antioxidants example : in the hot red pepper as capsaicin and in black grape as resveratrol, polyphenols called ellagitannin and acutimissin A (which blocks the action of an important enzyme which is essential to the development of cancerous cells , also black grape contain vitamin C, ***(Nermien, 2010).***

Resveratrol is present in grape, is one of the most exciting studies, suggested that it can prevent oxidative stress damage caused by free radicals. Also others suggest that, resveratrol can inhibit the growth of liver,breast,and lung cancer, by inhibiting receptors on cells called the Aryl Hydrocarbon Receptors (AHR), ***(Tadolini et al., 2000).***

Also ***Bertelli et al.,(2002),*** concluded that , black grape is a food with high antioxidant power,it has been established that dried black grape may strengthen the antioxidant potential of the body and thus reduce some oxidant stress-induced health problems.

***Olas et al.,( 2005)***,investigated that resveratrol (trans-٣,٤',٥-trihydroxystilbene), a polyphenolic compound found in grapes and wine, has been shown to have anti-inflammatory, anti-oxidant, anti-tumor and anti-platelet activities. Using different methods, they showed that resveratrol reduces oxidative stress induced by cisplatin and selenium-cisplatin conjugate ([NH(٣)] (٢)Pt(SeO(٣)), Se-Pt) in human blood platelets, lymphocytes and plasma. Resveratrol decreased the production of ٨-epi-prostaglandin F(٢) (a biomarker of lipid peroxidation) in control blood platelets and platelets treated with platinum compounds (١٠ microg/ml), and markedly reduced activities of different anti-oxidative enzymes (glutathione peroxidase, superoxide dismutase and catalase) in these cells. A combined action of resveratrol and Se-Pt evoked a significant decrease of DNA damage (measured by comet assay) in lymphocytes compared with cells treated with Se-Pt only. Resveratrol also caused a distinct reduction of total anti-oxidant level in plasma after incubation with platinum compounds. Therefore, anti-oxidative activity of resveratrol may diminish oxidative stress and damage to cellular biomolecules (lipids, proteins and DNA) induced by platinum compounds.

Resveratrol has many antioxidant effects , preventing fibrosis, activating the phosphorylation of PKC, secreting transthyretin to prevent a beta aggregation , protecting dopam-inergic neurons, activating sirtuin family of NAD-dependent histone deacetylases, ***(Bastianetto et al., 2007).***