Review Article

Circulatory disorders induced by ochratoxin A

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ABSTRACT

Ochratoxin A (OA) or (OTA) is a mycotoxin produced by certain *Penecillium* (mainly *P. verrucosum*) and *Aspergillus* (mainly *A. occracies)* species of storage fungi. This toxin is a secondary fungal metabolites which has been detected in a variety of animal chows, human food and in up to 80% of human blood samples of several Western countries. Its main target is the kidney which is the causing agent of Danish porcine nephropathy and increases the incidence of renal carcinomas and adenomas in rats. OA produces many other adverse effects on animals and human since it is known as teratogen, mutagen and immunosuppressive agent. Several mechanisms of OA toxicity have been proposed by several investigators. The present manuscript introduces the circulatory disturbances as well as several cases of cardiac disorders which have been recorded following the acute administration of ochratoxin A to adult rats. A dose-dependent relationship of ochratoxin A has been established. OA decreased the heart rate of adult rats after 30 min from the IP injection. Hypotension as well as a significant change in mean arterial blood pressure and decrease in the conduction time of the heart cycle were established also following ochratoxicosis. Other electrophysiological changes included significant inotropic effect and increase of ventricle repolarization voltage have been reported after OA treatment. The abnormalities of heart function include abnormal sinus rhythms, arising of ectopic beats and atrioventricular block. A drop and change of pulse pressure of the arterial blood pressure have been recorded simultaneously with multiple cases of arterial premature contractions (APC) and sinus arrhythmia (SA). The cardiotoxic mechanism of OA has been established by both *in vivo* and *in vitro* study on the rat and frog hearts. Sympathetic and parasympathetic antagonisms as well as calcium channels blocker have been employed. It is suggested that OA exerted its cardiotoxic effects mainly through increased influx of extracellular calcium ions, which postulates a possible direct effect of OA on the myocardium cell membranes integrity. Morover, OA can exert a parasympathetic stimulation to the heart in both the *in vivo* and *in-vitro* study. Ochratoxin A seriously affects the circulating blood and the circulating body fluid which are currently defined as the internal environment that facilitate the metabolic processes and homeostasis. Microcytic hypochromic anemia, reduced mean corpuscular volume and decrease of other haematological measurements were reported in different species of animals toxificated by OA. Ochratoxin A not only produced amelioration in the pH of intersititial fluid which provides the exchange medium for substances moving between cells and capillary plasma, but also within the cell membrane on the intracellular fluid, which serve as the liquid environment for chemical reactions necessary to cellular survival.

KEYWORDS: ECG, blood pressure, Autonomic nervous system, Body fluids, calcium channels, rat, frog.

INTRODUCTION

Historical notes: Human and animal exposure to ochratoxins: Kidney is the target organ of ochratoxicosis in several species of animals as well as in human (Marquardt & Frohlish 1992). OA contaminated diet leads to nephropathy in pigs and to Balkan endemic nephropathy in human. The poineering work of (Elling & Moller 1973) with pigs demonstrated that OA was the major causal determinant of nephropathy disease. The renal lesions associated with that disease include degeneration of the proximal tubules, interstitial fibrosis, and hyalinization of the glomeruli (Krogh 1978). Cases of porcine nephropathy have been observed also in other European countries, including Sweden, Norway, Finland, Germany, Hungary, Poland, Yogoslavia, and Great Britain (Rutqvist *et al.*1978). Burns & Dwivedi (1986) reported that the target organ of OA for poultry seems to be the kidney, although other systems such as the liver, gastointestinal tract, lymphoid organs, skeletal

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system, hematopiotic tissues, and the reproductive organs can be affected. Moreover, a specific effect of OA is the reduction of growth to be a potent teratogen and immunosuppressive in mice, rats, hamsters, and chickens (Fukui *et al.*1987). Ochratoxicosis has rarely been reported in ruminants, presumably because of the ability of ruminal microorganisms to hydrolyse OA to the nontoxic alpha form (Sreemannarayana *et al.*1988). Plestina *et al.*(1990) studied the human exposure to OA in several areas of Yogoslavia. They observed a common causal relationship between the porcine nephropathy and the Balkan (endemic) nephropathy disease, they showed also a correlation between the highest frequency of OA contaminated food in specific area in Yogaslavia and the incidence of the Balkan nephropathy disease in the same area.

In Saudi Arabia, (Abdel-Gawad & Zohri 1993) recorded ochratoxin A and other mycotoxins like aflatoxins and citrinin contaminated nut seeds for human consumption. In Sweden, OA was found in 14% of the cow's milk samples (10-40 ng/ml) and in 58% of the human milk samples (10-40 ng /ml) (Breitholtz *et al.*1993). Maaroufi *et al* (1996) reported that Tunisia appears to be a second hot spot of kidney disease due to the prevalane of OA in human blood and tissues.

Ochratoxicosis in Egypt: Today toxins from *Aspergillus occracies* (ochratoxin) and *Aspergillus flavus* (aflatoxin) and several other mycotoxins received a great attention in Egypt. Almost food and feed, particularly of plant products, may be infected by fungi and thence contaminated with these mycotoxins (Abdelhamid *et al.*1996 and Naguib *et al.* 1999). EL-Maraghy and Zohari (1988) isolated one hundered and thirty isolates of Aspergillus and Penicillia from 100 broad beans seed samples collected from different localities in Egypt. In Sinai, several species of mycotoxic fungi of barley grains have been identified (Abdel-Hafez & Maghraby 1987). Zohri & Abdel-Gawad (1993) carried out a survay of mycoflora and mycotoxins of some dried fruits in Egypt. (Naguib *et al.*1999) screened *Aspergillus ochraceus* in green (50 samples) and coffee roosted beans (25 samples) for the presence of OA. The samples were randomly collected from different governorates and were inoculated individually on several Agar extracts, Their results showed that ochratoxin A was determined in samples under investigation using the AOAC method.

Chemistry of ochratoxins: Several forms of ochratoxins each of varying toxicity have been isolated from *Aspergillus* and *Penicillium* fungal genera (Frisvad & Samson 1991). Ochratoxin A is the most toxic one and it is a chlorine -containing diyhdro-methylisocoumarin compound with L-β-phenylalanine linked through the 7-carboxy group by an amide bound. (Marquardt & Frohlich 1992). Several analogs of OA have been prepared by substitution of phenylalanine with other amino acids (Creppy *et al.* 1983). The tyrosine, valine, serine and alanine analogs are most toxic. The methionine, tryptophan, and glutamic acid analogs have intermediate toxic effects, while, the glutamate and proline analogs have low toxicity. Hadidane *et al.*(1991) have reported that the serine, hydroxyproline, and lysine analogs occur also naturally.

Toxicity of ochratoxins A: Ochratoxin A is acutely toxic to several species of animals, including chicks, hens, ducklings, turkeys, sheep, swine (Dwivedi & Burns 1986, Kuiper-Goodman & Scott 1989, Marquardt *et al.*1990). The acute toxicity of the ochratoxins, as assessed by the LD_{50} values, varies considerably from species to species and within a species is influenced by diet, age, sex, route of administration, genetic differences and the form of ochratoxin (Marquardt & Frohlish 1992). Significant increases in the concentration of creatinine and urea in the pigs blood were observed after the highest exposure to OA for 4-6 wk (Marquardt *et al.*1990). Several studies introduced the mechanism of ochratoxicosis on the kidney. Jung & Endou (1989) suggested that OA might enter the plasma membrane in S_2 and S_3 through the organic anion transport pathway and inhibit the mitochondrial oxidative phosphorylation. On the other hand, (Glahn *et al.*1989) suggested that OA may cause an osmotic diuresis in pullets by inhibiting the tubular reabsorption of electrolytes. The acute application of OA affects the postproximal part of the nephron, while the subchronic

application of OA results in a dramatic decrease of the transport maximum of p-aminohippuric acid (PAH), and reduced glomerular filtration rate, (Gekle & Silbernagl 1994). Moreover, there was a dose-response related increase in protein excretion consisting mainly of albumin and proteins with a similar or large molecular weight, indicating a glomerular proteinuria. The mechanism of ochratoxicosis of liver and hepatic cells was studied by (Khan *et al.* 1989). They suggested that OA disrupts microsomal calcium homeostasis by an impairment of the endoplasmic reticulum membrane and they suggested lipid peroxidation enhancement mechanism. Ochratoxin A induced alterations of some testicular enzymic activities after 4-8 wk in adult rats (Gharbi *et al.* 1993). These enzymes include alpha-amylase, alkaline phosphatase, and gamma-glutamyltransferase (GGT). The authors associated the increase of these testicular enzymes especially GGT with impairment of spermatogenesis and accumulation of premeiotic germinal cells induced by OA. They concluded that an earlier modification of the androgen status and testosterone level in testes can be induced by OA.

Carcinogenicity of OA has been established by several researches. OA induces DNA single-strand breaks as well as DNA adducts in many organs and produces mitotic and meiotic chromosomal abnormalities after the chronic application (Bose & Sinha 1994). Incidence of urinary tract tumors has been investigated in the endemic Balkan nephropathy disease in humans (Kuiper-Goodman & Scott 1989). Also, the incidence of renal tubular cell adenomas and carcinomas was observed in male rats much higher than that in female rats (Kuiper-Goodman & Scott 1989). On the other hand, the persisting of DNA adducts in kidney and their appearance and disappearance in liver and spleen rat after OA chronic application lead to the conclusion that DNA adducts are not likely quantitatively and qualitatively the same in several organs. This is due to difference of metabolism in these organs, leading to different ultimate carcinogens and may result also from differences in the efficiency of repair processes (Pfohl-Leszkowicz *et al.*1993).

Effect of ochratoxin A on the circulatory system: The circulatory system comprises its principal organs, the heart, blood and lymph vessels. Blood circulating in the vessels performs vitally important functions in the animal and human body. The function of the heart is to maintain a constant circulation of blood through the body. The heart acts as a pump and its action consists of a series of events known as the cardiac cycle (Wilson 1995). Studies concern the toxicity of ochratoxin A on the cardiac cycle as well as on cardiovascular physiology are considerably rare. The present review describes the cardiovascular lesions in rats induced by acute ochratoxicosis. In pharmacological experiments, the electrocardiogram has proved to be an invaluable method in detecting the actions of drugs on the heart especially with regard to possible cardiotoxic side effects (Cromwell *et al.*1999). Several cases of electrocardiographic abnormalities (arrhythmias) have been recorded by (Hussein 1996) following acute intraperitoneal administration of two doses of ochratoxin A. The changes were most obvious after the administration of the higher dose (1.7 mg/kg^{bw}). The abnormal sinus rhythms included cases of bradycardia, tachycardia and sinus arrhythmias. Bradycardia was recorded mostly after 30 min following the treatment of the two doses of OA and sustained until 2 hrs. On the other hand, tachycardia was observed later (after 2-3 hrs) from injection. The author concluded that OA can exert some effects on the rhythmicity of the sinoatrial node of the heart and thereby can disturb the automaticity of the heart. Moreover, the author considered the cases of premature and escape beats obtained after ochratoxin A application as a protective mechanism against the failure of SA node depolarization or the conduction block. Also, an atrioventricular block with first and second degree concomitant with cases of atrial and ectopic beats (Plate 1) were observed in the ECG recordings obtained following ochratoxicosis (Hussein 1996).

The cardiovascular alterations induced by several doses of ochratoxin A are described by (Hussein *et al.*1997). They reported that ochratoxin A not only exerted an arrhythmogenic effect on the ECG traces but also affected the arterial blood pressure waves since a change in

the pressure wave form with a remarkble drop in mean arterial blood pressure is obtained following the acute ochratoxicosis study (Plate 2). The decrease of arterial pressure as well as systolic, diastolic and mean blood pressure induced by ochratoxin A is a serious physiological alteration in the circulation since it is accompanied by a decrease in heart rate, cases of sinus arrhythmia and atrial premature contraction. It is known that low blood pressure leads to inadequate blood supply to the brain. It can lead to brief or more prolonged unconsciousness (fainting) possibly causing death, (Wilson 1995). Moreover, the authors proposed that OA strongly affects the performance of the myocardium as well as the cardiovascular system. they also suggested a direct or indirect effect that can be mediated on the circulatory system of the rat.

Plate 1

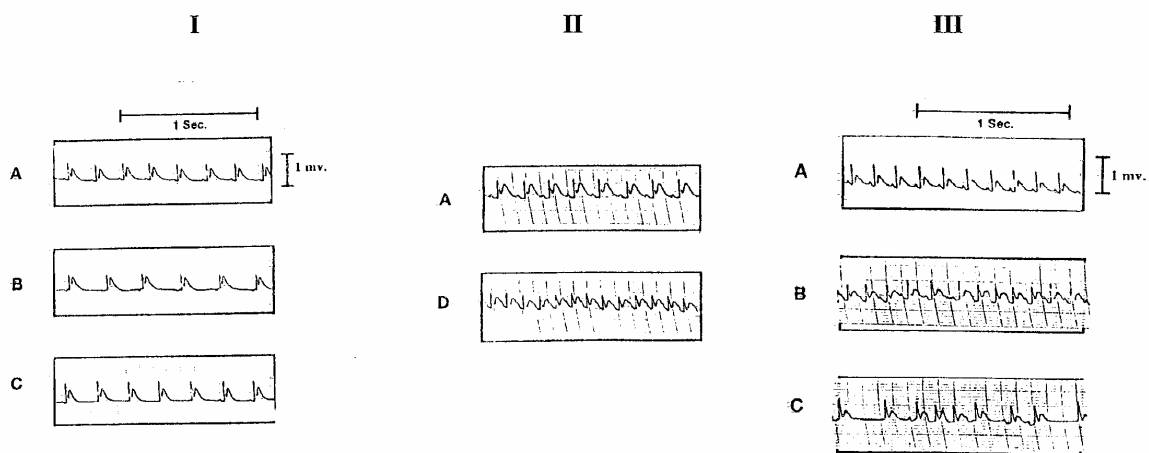Plate 1 (I): Abnormal sinus rhythm recorded after IP administration of OA.
        A: Control tracing.   B: Sinus bradycardia.   C:Sinus arrhythmia.
    (II): A: Control tracing.   D: sinus tachycardia.
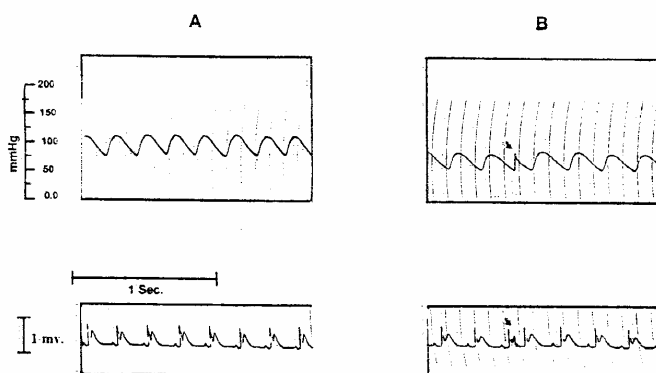   (III): A: Control tracing.   B:First degree block.   C: Second degree block.

Plate 2: Simultaneous recording of ECG and arterial blood pressure of rat demonstrate the toxicity of OA.   A: Control tracing.   B: Atrial extrasystole with pulse pressure change.

It is reported that OA enhances lipid peroxidation production and hydroxyl radicals generation (Aleo *et al.*1991). Hydroxyl radicals may affect the myocardium and the conductive system in different ways. (Kaneko *et al.*1993) indicated that oxygen free radicals may inhibit myofibrillar creatine kinase activity by modifying sulfhydryl groups in the enzyme protein. This reduction in creatine kinase activity may lead to a disturbance in the energy utilization in the heart and may contribute to cardiac dysfunction. The previous hypothesis supports the data obtained by (Hussein 1996). Since she suggested an arrythmogenic quality of OA on the myocardium and therby cardiac dysfunction.

Another hypothesis of (Wei *et al.*1985) described that OA inhibited the respiration of whole mitochondria by acting as a competitive inhibitor of carrier proteins located in the inner mitochondrial membrane supports the observations of the significant hypotension concomitant with a decrease of cardiac cycle obtained by (Hussein *et al.*1997). They concluded that an agent affecting an energy consuming process presumably could produce an inhibitory mechanism as well as a delay in the impulse conduction through the A-V bundle in the heart. The ability of ochratoxin A to increase the myocardial contactility has been reported also by (Hussein *et al.*1997). They proposed that the positive inotropic effect of OA may be due to calcium increase within the myocardium. This conclusion is in agreement with the report of (Khan *et al.*1989) that OA enhanced lipid peroxidation which was also accompanied by leakage of calcium-loaded microsomes leading to an influx of calcium to the cell causing changes in the metabolic activity within the cell.

Alteration in the repolarization of the ventricle after ochratoxicosis has been shown by (Hussein 1996; Hussein *et al.*1997). This alteration is represented by the increment of T-wave amplitudes of the rat ECG. Repolarization disorder of the ventricle is attributed mainly to a disturbance in ionic distribution especially the potassium ions around the myofibrils (Brown & Kozlowski 1997).

The possible cardiotoxic mechanism of OA was explored in the study of (Hussein 1998). The author introduced *in vivo* and *in vitro* study to manifest the mechanism of ochratoxin A in affecting the heart rate, conductivity, contractility and excitability of the rat and frog heart by the pretreatment or posttreatment by sympathetic, parasympathetic antagonists, as well as, calcium channels blocking agent (Plate 3). The results indicated that the calcium channels blocker verapamil hydrochloride 40 mg (2mg/kg [bw]) application could abolish the positive inotropic effect of OA on the myocardium, an effect which was not induced by the sympathetic blocking agent propranolol (1mg/kg[bw]).
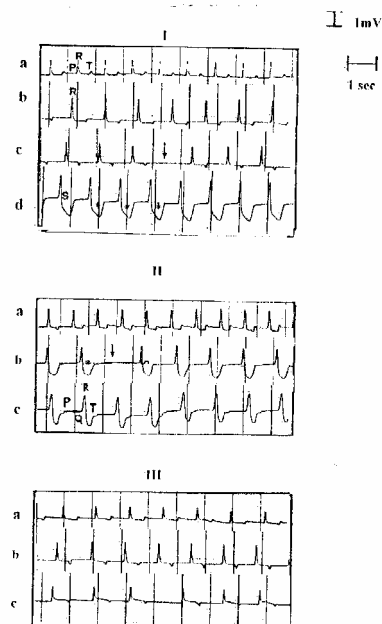

Plate 3: *In vitro* ECGs records of isolated frog hearts perfused with 5μg/ml of OA solution showing the effect of OA application (I) on the HR, P-R interval, R-wave amplitude, and S-T depression. (II), and (III) effect of atropine and verapamil 20 min after OA application.
A: Normal record.   b: OA application.   c: Atropine or verapamil application.

(↓) showing sinus arrythmia.   (*) showing S-T depression.

Moreover, atropine nearly regulated the heart rate and sinus arrhythmia but could not abolish the other electrocardiographic disorders induced by OA. The author's study suggested that OA exerted its cardiotoxic effects through the increased influx of extracellular calcium ions, which postulates a possible direct effect of OA on the myocardium cell membrane integrity. (Bailey *et al.*1989) reported the decrease of calcium, potassium, and inorganic phosphorus serum concentration is due to the biochemical lesions induced by OA in chickens'heart. Their results come in agreement with the conclusion of (Hussein 1998) on the heart of frog and rat.

Recently (Eder *et al.*2000) reported that ochratoxin A activates mitochondrial Na^+/H^+-exchange (NHE) by interfering with cellular Ca^{2+} homeostasis in the renal cells and subsequently change the cellular pH (pHc) homeostasis and mitochondrial function. The effects of OTA on cellular calcium homeostasis ($[Ca^{2+}]i$) and another cell parameters were also investigated by (Benesic *et al.*2000). They concluded that OTA may impair cellular Ca^{2+} and cAMP homeostasis already at low nanomolar concentrations, resulting in concentration-dependent $[Ca^{2+}]I$ oscillations. The above studies which discuss the role of OTA on calcium ions homeostasis agree with the conclusion of (Hussein 1998) study on the heart of rat and frog through the *in*-vivo and *in*-vitro experiments.

Toxicity of ochratoxin A on the internal environment and its relationship to systemic circulation. Currently, the internal environment is defined as the body fluids (blood, lymph, tissue and cerebrospinal fluid) that participate in the metabolic processes and homeostasis (Georgieva 1989). The blood circulating in the vessels provides one of the means of communication between the cells of different parts of the body and the external environment and it establishes specific defense mechanisms against toxins (Wilson 1995).
Several studies have reported the toxicity of ochratoxin A on the blood, hematopoietic system, immune system as well as on body fluid homoestasis. Short notes of these studies will be emphasize within this report to communicate several toxicities of OA with the circulatory disturbances induced by this toxin.

Toxicity of ochratoxin A on haematological, biochemical and blood minerals measurements: The bioavalability of ochratoxin A, which is the amount of OA reaching the systemic circulation, greatly affects the toxicity of OA. Another factor that affects OA toxicity is the high binding affinity of OA to plasma constituents (Uchiyama & Saito 1987). This not only facilitates the passive absorption of the nonionized form of OA from the digestive system (Kumagai 1988) but also retards OA elimination and limiting the transfer of OA from the bloodstream to the hepatic and renal cells and consequently contributes to the prolonged half-life of the toxin (Hagelberg *et al.*1989).

Plasma of several species of animals have proteins with different binding affinities for OA (Hagelberg *et al.*1989). This may be the reason of different susceptibility of haematological and biochemical measurements changes among different species of animals. In mammals, OA causes decrease of serum phosphorus, calcium, potassium, cholesterol and several haematologic values with an increase of uric acid and creatine concentration in the plasma of pigs (Harvey *et al* 1989). Other investigators supported the previous findings in rats (Mueller *et al.*1995; Nada *et al.*1996).

In poultry, there are microcytic hypochromic anaemia, reduction in mean corpuscular volume, reductions in serum levels of total protein, albumin, globulin, urea nitrogen, cholesterol, triglycerides, and potassium. An increase in uric acid and creatine levels as well as in the activities of serum phosphatase, cholinesterase and γ-glutamyltransferase have been reported by (Kubena *et al.*1989 and Sreemannarayana *et al.*1989). An increase of ion excretion (Na, K, Ca^2, P) by kidney toxificated by OA have been reported by (Glahn *et al.*1989). They suggested that OA may cause an osmotic diuresis by inhibiting the tubular reabsorption of electrolytes. Other researchers have shown that a decrease in renal phosphoenol-pyruvate carboxykinase (PEPCK) activity was a highly sensitive and specific indicator of OA in pigs, but not in rats (Krogh *et al* 1988). Recently (Li *et al.*1997; Schwerdt

et al.1999) studied the pharmacokinetics of ochratoxin A. They concluded that ochratoxin A has a long half-life and is very slowly cleared from the body. Moreover, its metabolies are cleared at much faster rate with much shorter half-lives. Another recent study (Gekle *et al*.2000) discussed the nephritogenic, carcinogenic, and teratogenic action of ochratoxin A. They reported that OA induces c-jun amino-terminal-kinase (JNK) activation and apoptosis in MDC7 cells at nanomolar concentrations.

Effect of ochratoxin A on circulating body fluids: Changes and amelioration in pH of extracellular fluid and intersititial fluid in mammalian body have been reported by several studies. Rodeheaver & Schinellmann (1993) obseved an extracellular acidosis which has been produced by ochratoxin A in rabbit renal proximal tubules suspensions *in- vitro*. They assumed lipid peroxidation mechanism which potentiates free radical production and decreases free radical detoxification. Gekle & Silbernagl (1993) described the ability of ochratoxin A to produce reduction of glomerular filtration rate (GFR) in rats. They attributed this reduction to a decrease of renal plasma flow (RPF) and increase of total renal vascular resistance (TVRF). Another study of (Gekle & Silbernagl 1996) working on cultured kidney cells concluded that the nanomolar concentrations of OTA could induce a blockade of anion conductance in the plasma membrane with subsequent disturbance of cellular acid-base homeostasis. Moreover, they reported that the renal hamodynamics and the secretory function of the proximal tubule are affected by OTA after prolonged but not after acute exposure. Recently, (Benesic *et al*.2000) reported that OTA interferes with hormonal Ca^{2+} signaling, which leads to alteration of cell proliferation. Also, the high concentrations of OTA lead to the reduction of cell viability which is not depend on Ca^{2+}.

In conclusion, it seems that there may be more than direct and indirect effects of OA. OA induces circulatory disturbances as well as several cases of cardiac disorders in adult rats. Diagram (1) summarizes the toxicity of OA on the circulatory system of rats. A dose-dependent relationship of ochratoxin A has been established. OA decreases the heart rate of adult rats. The abnormalities of heart function include abnormal sinus rhythms, arising of ectopic beats and atrioventricular block. Hypotension as well as a significant change in mean arterial blood pressure and decrease in the conduction time of the heart cycle are also established following ochratoxicosis. A drop and change of pulse pressure of the arterial blood pressure have been recorded simultaneously with multiple cases of arterial premature contractions (APC) and sinus arrhythmia (SA). Other electrophysiological changes include significant inotropism and increase of ventricle repolarization voltage have been reported after OA treatment. It is suggested that OA exerts its cardiotoxic effects mainly through increased influx of extracellular calcium ions, which postulates a possible direct effect of OA on the myocardium cell membranes integrity. Moreover, OA can exert a parasympathetic stimulation to the heart in both the *in-vivo* and *in-vitro* study. The ability of OA to increase intracellular calcium permiability is well documented.

Of the recent mechanisms expaining in the nephrotoxic effect of OA is the blocking of the plasma membrane anion conductance and inhibition of electrolytes reabsorption by the tubules. Moreover, deranges of the pH homoestasis in the intersititium of the renal papilla could lead to the impairment of urinary acidification and extracellular acidosis which potentiate the cell injury and death. OA toxicity has related to its ability to increase lipid peroxidation and glutathione disulfide formation and decrease glutathione peroxidase and glutathione reductase activities, a mechanism which is most likely to be related to free radicals production and lipid peroxidation enhancement.

Another mechanism of OA toxicity is its effect on mitochondrial ATP production. It was observed that OA inhibits the respiration of whole mitochondria by acting as a competitive inhibitor of carrier proteins located in the inner mitochondrial membrane. The decrease of cellular ATP content produced by OA is due to the ability of OA to enter the plasma membrane in S_2 and S_3 through the organic anion transport pathway and to inhibit mitochondrial oxidative phosphorylation.

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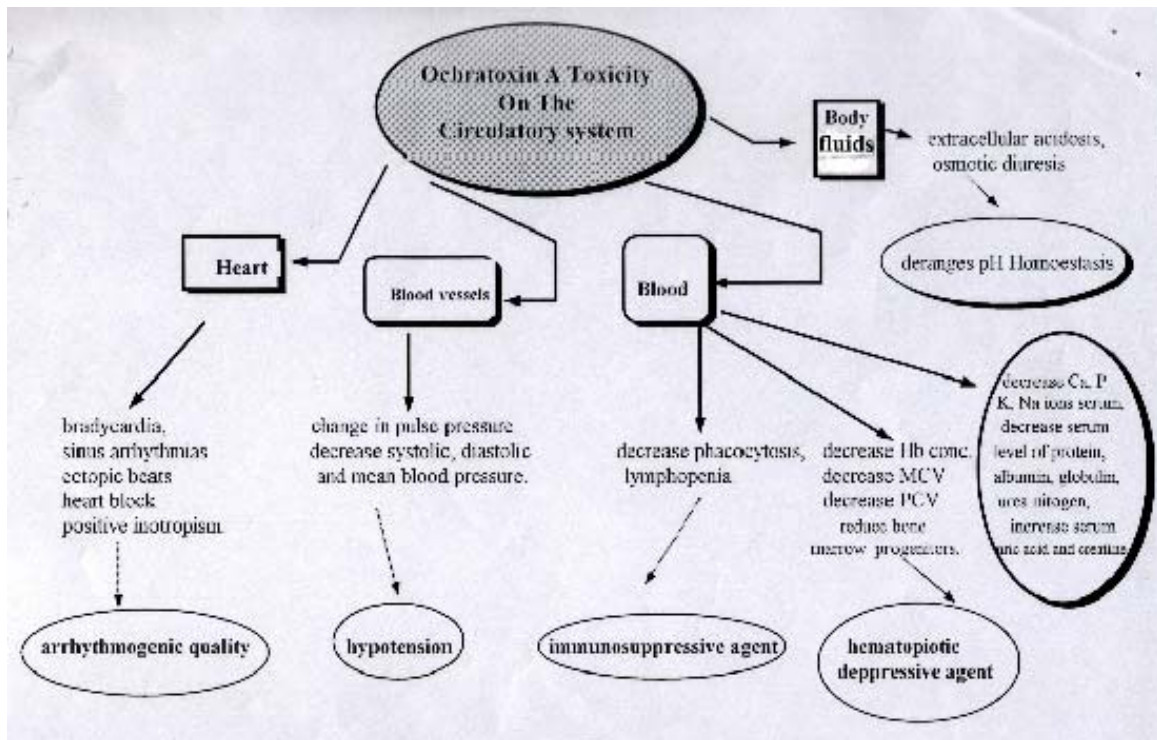
Diagram 1: shows the different possible mechanisms involving in the toxicity of OA in circulatory system of rat