# The Effect of the Neurotransmitter Dopamine, Lead Acetate, L-NAME, and Verapamil on the Metabolic Pathway in the Longitudinal Smooth Muscle

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**Abstract**

The purpose of this study was evaluating the effect of dopamine neurotransmitter in the constrictive response of Ileum longitudinal smooth muscle (ILSM) in White Leghorn chickens (Gallus gallus domesticus) in the relation of lead acetate, verapamil (as a calcium ion blocker).

This study also includes the role of epithelium and involvement of dopamine with and without nitric oxide synthesis inhibitor L-NAME (NOS blocker) that significantly increase the effect of ileum longitudinal smooth muscle (ILSM). While the application of lead acetate in different doses with dopamine has shown an increasing effect on the ileum longitudinal smooth muscle (ILSM) under "in vitro" conditions dependent on different concentration. Also, this research reflected the effect of verapamil as a blocker of the Ca2+ ionic channels that decreased the contraction of the ileum longitudinal smooth muscle (ILSM) in the application with L-NAME and lead acetate.

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## Introduction

Neurotoxicity describes neurophysiological changes caused by exposure to toxic agents, which may result in cognitive changes, memory disorders, and changes in mood or onset of psychiatric disturbances.[1,2,3] Common toxic agents include certain heavy metals, drugs, organophosphates, bacterial, and animal neurotoxins.[4] Chronic exposure may also result in prolonged and variable symptom presentations, including fatigue, decreased processing speed, fine and gross motor deficits, and generally decreased cognitive functioning.[5]

The presence of lead in the human body causes damage to the nervous system through several mechanisms. Direct effects on the nervous system may be classified as either morphological or pharmacological.[6] Morphological effects alter the development of the nervous system, particularly from the prenatal period through childhood. Such effects include disruption of key molecules during neuronal migration and differentiation; interference with synapse formation, mediated by a reduction in neuronal sialic acid production and premature differentiation of glial cells. [7, 8, 9]

Pharmacological effects result from the action of lead as a pharmacological agent in the CNS. Lead substitutes for calcium and, to a lesser extent, zinc and inappropriately triggers processes reliant on calmodulin.[10] Lead also interferes with neurotransmitter release, disrupting the function of GABAergic, dopaminergic, and cholinergic systems as well as inhibiting NMDA-ion channels during the neonatal period. [11, 12] *In vitro* studies have shown that lead activates protein kinase C in capillary cells and inhibits Na+/K+-ATPase in the cell membrane, interfering with energy metabolism.[13,14] Within the cell, lead appears to interfere with calcium release from the mitochondria, resulting in formation of reactive oxygen species, speeding mitochondrial self-destruction through formation of the permeability transition pore, and priming activation of programmed cell death processes.[14, 15]

Lead exposure has effects on

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neuropsychological functioning that vary across the lifespan. Prospective studies have found that prenatal exposure, as measured by lead levels in umbilical cord blood, predicted slower development in the sensorimotor and visuomotor domains, as measured by the Bayley Scales of Infant Development.[40] Numerous studies of children have shown that lead exposure reduces overall cognitive functioning in children, both cross-sectionally and longitudinally, but most such studies examine omnibus measures of intellectual functioning rather than domain-specific effects.[35] In adulthood, it is apparent that chronic exposure to lead is more harmful to cognition than acute exposures. In a sample of demographically diverse, primarily middle-aged US adults, bone lead levels predicted worse cognitive performance in several domains, whereas blood lead level did not. [35]

In this environment condition as a society, contaminating with heavy metals, especially lead, is still current due to a relatively large lead and zinc reserves. There is a lot of scientific evidence that by the time there were no preventive measures, lead poisoning was one of the most pronounced deaths.

Kosovo is among of the countries with the highest degree of pollution in Europe. Currently, the main sources of pollution are Kosovo's Power Plants. According to the present data, coal resources in Kosovo are estimated at about 10.5 milliard tones. Almost entirely, this coal is being used for producing of the electricity in Kosovo's power plants. Monitoring showed that only one of five units of Power Plant "Kosova A", within one hour releases 25 tonnes of dust and ash. Emission level is about 74 times above European standards.[1] Particular environmental problem represents ash landfill, not only for microlocality where it's located but even far away including Prishtina. Chemical gamma- spectroscopical analysis of dust and particles showed that it contains several toxic elements (As, Pb, etc.), and radionuclides (238U, 235U, 226Ra, etc.) which endanger plant, animal and human health in a vicinity of power plants.[31,32]

Currently, about 700,000 citizens inhale the mixture of toxic gases released from Power Plants "Kosova A and B". An indicator of this pollution is the level of public health in Kosovo, the number of deaths of newborn infants that is the highest in Europe. According to a report published by the UNDP (2002), the infant mortality rate in Kosovo is around 34-35 per 1000 live births.[34]

Based on scientific evidence, lead is multidrop that attacks the organism. Lead causes cognitive and neurophysiological deficiency, in addition, lead interferons with $Ca^{2+}$ skeletons and in the function of adenosine monophosphate cyclic AMP-c, inhibits synthesis, release, and mechanism of action of neurotransmitters such as dopamine, norepinephrine, dopamine as well as gamma acid system aminobutyric acid (ATSDR).

It is known that lead is a polypropylene protoplasmic poison, its effects appear above all at baseline, damage cell structures, first of all, mitochondria, and inactivate various intra-cellular enzymes.

Although no proper steps have been taken in our society against its poisoning, public health today is at risk. What will we do to alarm his danger and damage to the human body?

*Lead and accumulation in the nervous system*

Much of the body's dysfunctions occur because they absorb lead amounts, which have the ability to imitate $Ca^{2+}$ ion channels by preventing calcium in the cell.

The lead when it enters the body interferes with the normal functions of the cell and its physiological processes. Some of the basic physiological effects include; damage to the peripheral nervous system and central nervous system (SNP), then blood cells, vitamin D and calcium metabolism, toxicity to the reproductive system. The nervous system seems to be more sensitive in cases of lead poisoning.

Neurons are functional units of the nervous system and are specialized in transmitting signals from one brain location to another. The neurons' dendrites receive signals and transmit them to the rest of the neuron. The neurons' axon conveys this signal in the direction of its effector. Joint axons have a specialized system known as the synaptic terminal initially receiving signals from other cells using one of the "ambassadors" sent to the chemical neurotransmitter. The neuron will extract synaptic vesicle molecules from the synaptic molecule only when it dopamines depolarization on the synaptic terminal surface.

Lead, even in low concentrations, has the ability to increase the basal release of neurotransmitters from the presynaptic nerve

center. This can happen both in PNS and CNS. Lead micromolecular concentration may spontaneously cause dopamine release, acetylcholine and gamma-aminobutyric acid (GABA).

A neurotransmitter receptor has the responsibility for the transduction of nerve impulses, which reflect muscle contraction.

GABA is an amine-acid classed as a neurotransmitter with inhibitory effects. GABA, according to some studies, is thought to play an increasing role in hormone secretion. Almost the same condition is manifested in some of the brain processes as a response to movement control and emotions, which are reflected by the amount of dopamine and serotonine concentration.

*The effects of lead on smooth muscles*

Muscle experiments in branched pelvic plexus on the chicken 2 weeks lead-acid treatment in the country resulted in a drastic change in the 8-week period. Tonic airway muscle tone, submucosal gland secretion and blood circulation in the airway are regulated through the central nervous system and the neuronal circuit within the airways.

*Lead and Dopamine neurotransmitter*

As we know that lead is involved in the synthesis and release of neurotransmitters. Thus, lead through imitation of $Ca^{2+}$ role, activates the mechanism of release of synaptic vesicles with neurotransmitter, activates and facilitates the activity of protein kinase II through the use of $Ca^{2+}$ calmodulin pathway. As a result, the spontaneous release of neurotransmitters into the central nervous system (CNS) and peripheral nervous system (PNS) occurs.

Tetraethyllead contributes to the release of neurotransmitter in the package (quanto), so the synthesis and release of neurotransmitter are sensitive processes in organic lead neurotoxicity. On the other hand, in particular, lead nitrate and lead chloride can block the release of neurotransmitters from the preganglionic neurons. Thus, lead is implicated in the transmission of dopaminergic, cholinergic and catecholaminergic and Serotonergic neurons processes.

In experimental animals, it has been shown that lead alternates with a number of neurotransmitter systems such as dopamine, norepinephrine, serotonine and gamma-aminobutyric acid systems (ATSDR).

Numerous studies find that lead distorts the catecholamine system, respectively lead poisoning along with decreasing the secretion of acetylcholine results in a decrease in norepinephrine on the one hand and dopamine increase on the other. Thus, the bullet shows the role of an agonist with known substances such as dopamine. Other studies report a stimulation of catecholaminergic function to lead-exposed people.

The administration of lead acetate (50, 100 and 200 mg/kg) does not potentiate blockade induced by dopamine receptor antagonists. Lead can inhibit neuronal conductivity that uses neurotransmitters dopamine, noradrenaline, serotonin and acetylcholine without selectivity.

*The effect of neurotransmitters on smooth muscles*

The origin of neurotransmitters containing nerve fibers that help digestion in the chicken tract has been investigated with the use of colchicine and immunohistochemistry. Neurotensin-immunoreactive of nerve fibers is found in muscle layers of soft gut and duodenum.

The density of their distribution was very high in the gullet and its culture (maximum value means:1315 / $mm^2$ in the sectoral zone in the nutritional elements of the muscular mucous membrane) and to progressively decrease the duodenum.

The number of immunoreactive neurotensins in plexus muscle cells in the throat (28.3 ± 2.7 / ganglion), but lower in gastric, ventricular and proven trick ulcer plexus.

These chicken scores indicate that most of the immune-active intestinal neurotensin, with internal origin in the upper plexus digestive tract and are mainly distributed in mild muscle cells.

*Dopamine as neurotransmitter*

Studies in animals and humans have shown that dopamine affects the growth of gastrointestinal muscle tone and stimulate their motor activity. Dopamine is a neurotransmitter that is able to cause the condition to feel good. Its deficit causes malnutrition, schizophrenia, parkinsonism, etc.

Dopamine has been found to be beneficial in treating the gastric motor and regulating the strain of the middle and lower esophagus of the esophagus and the treatment of the inability of the ring muscle. Metoclopramide and domperidone, unlike betanecol, affect the growth of gastric acid secretion. While betanecol directly stimulates the muscular mucosa of the

gastrointestinal muscle, metoclopramide is supposed to act indirectly, hence an increase in acetylcholine. Domperidone antagonists of norepinephrine and dopamine-induced relaxation of the isolated muscle. There is evidence for experiments of dopamine receptors in the esophagus, stomach, domperidone is supposed to affect the mobility of the muscles of the pigs, at least in this species, by blocking the 1-adrenoreceptor. Nerve messages from one neuron to the other are sent as electrical impulses along the axon. They are sent from the axon ends to another nerve cell.

Serotonergic neurons have project actions that affect different brain functions, so dopamine affects many different behaviors; sleep, dispositional affective behavior, etc.

## Materials and methods

*Tissue preparation*

Research model in these studies was the White Leghorn chickens (Gallus gallus domesticus) respectively duodenal ileum, based on the reasons: the relatively easy way the provision of appropriate homogeneous groups (genetic basis, age, sex, treatment, etc.). Relatively low cost of keeping chickens in adaptation and growth conditions an easy opportunity for sacrifice and intense metabolism. An integral part of this research was conducted at the Faculty of Medicine, respectively in the Biomedicine Laboratory, Institute University Clinical Centre of Kosovo · Institute of Pathophysiology.

In this case experiments were conducted in conditions "*in vitro*" in isolated organs of ileum White Leghorn chickens (Gallus gallus domesticus) were taken from the farm and kept-in the rehabilitation of the Faculty of Medicine until the time of the experiment, with adequate treatment (concentrated chicken food and ad-libidium water), until the time of sacrifice. Chickens aged 2-3 months were sacrificed by decapitation.

Then a 6 cm long segment of ileum was prepared, and the first cut of 3 cm was made from the ileocaecal junction, and the second one of 6 cm long was made above (about 9 cm from the ileocaecal junction). Truncated segment then was placed in the Krebs Hensley (KH) dip composed of (in gr / l) NaCl 6.9; KCl 0:35; $CaCl_2$ 0:28; $MgSO_4$ 0145; glucose 2.1; $KH_2PO_4$ 0:16 and $NaHCO_3$ 2:09; (pH = 7.4).

Dopamine preparation at the bottom should be linked to the fixed non-moving part, while the upper part should be tied to the transducer (FORT 10 Transducer, World Precision Instruments). Signals of the resisting force should be measured by four-channel register (Watanabe 6600, Germany).

*Preparation of long-acting ileum muscle*

After a 40-60 minute incubation (until baseline muscle tonic stabilization), the same preparation will be provoked in a series of solutions of different reagents (Dopamine in concentrations determined by the nature of the substance - the neurotransmitter in this case).

After dopamine treatment, it will again be expected at least 30-40 min. until stabilization-balancing the basal tone of smooth muscle before starting the second treatment, and so on, every day before the start of dopamine experiment.

The contents of the bath were continually airy with air streams from the mix gas flask: 95% $O_2$ and 5% $CO_2$.

*Experimental procedure*

A cumulative dose-response was analyzed by adding concentrations of neurotransmitter were based on preliminary studies where we found that the minimal dose to induce the detectable response of the tissue. To study the role of dopamine, additional longitudinal rings (n=10) were used dopamine with $Ca^{2+}$ in the ileum longitudinal smooth muscle (ILSM) response, the muscle preparation was treated with a series of dopamine doses (in the concentration of dopamine ($10^{-10}$ x $100^{-10}$-1 x 100 µl / l). The preparation was treated with cumulative doses, then washed and stabilized for 30 min. For analyze the constrictive effect of dopamine with LN~nitro arginine methyl ester (L-NAME) in the ileum longitudinal smooth muscle (ILSM) response, the L-NAME in the aqueous bath was added in dose series (0.037M) then treated with the dopamine's series. Also, we analyzed the role of $Ca^{2+}$ channel effect on these responses, after provoking muscle control with the dopamine concentration range (at concentrations $10^{-10}$ x 100-$10^{-1}$ x 100 µl / l) and incubation in medium treated with verapamil (as blocker of $Ca^{2+}$ channels) in the concentration of 0.02 -0.021 mM/ l. Also the different concentrations of lead acetate on dopamine the muscle product was treated with different values

of dopamine (in the concentration of $10^{-10}$ x 100-$10^{-1}$ x 100 µl / l). Before repeated the experiments the basal tonus of the preparation without physical interference will be balanced (until its tonus stabilizes for 10-15 min) at the initial frequency.

*Statistics*

Data are calculated as the arithmetic mean ± SEM. For statistical analysis, two-way ANOVA with repeated measurements was used. While $p < 0.05$ was considered significant results.

## Results

Neurotoxicity involves fundamental, essential and physiological changes induced by toxic exposure. Some of the most commonly known toxic agents include heavy metals and it is known that acute and chronic exposure to heavy metals (Pb) can affect fundamental neurophysiological and neuropsychological deficits, depending on the level and duration of exposure. Current neuropsychological studies show that exposure to Pb can result in intelligence, memory, memory dynamics, comprehension and reading deficits, perceptual abilities, motor skills and, at a lower probability, even to executive skills. As a result of this, in people affected or intoxicated, feelings of anxiety, depression, and phobia can be manifested, while the dynamics of developing of this symptoms depend on the rehabilitation and the level of toxic exposure. There is also evidence of increased antisocial behavior in people exposed to toxic lead action.

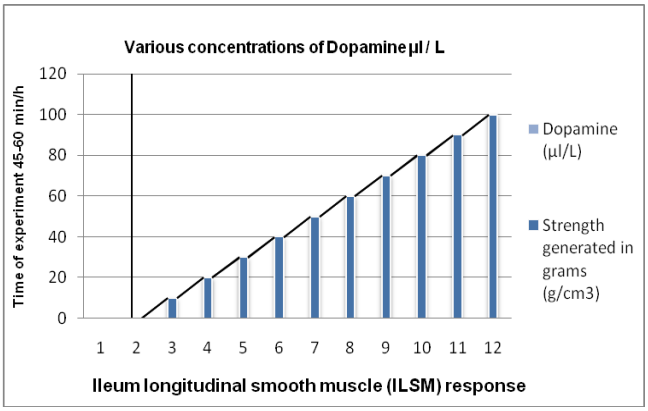


**Figure 1.** The average response value of the Ileum longitudinal smooth muscle (ILSM) (strength generated per gram) to different concentrations of dopamine ($10^{-10}$ x 100-$10^{-1}$ x 100 µl / l).

Inadequate rehabilitation treatment evidenced that early detection and accurate assessment are important treatment measures, especially early prevention can help eliminate some post-toxic effects. The use of neuropsychological and neurophysiological profiles of persons after toxic exposure to Pb can assist in the accurate clinical detection, prevention, evaluation for rehabilitation and the development of adequate treatment policies. Differences in response to the Ileum longitudinal smooth muscle (ILSM) of different dopamine levels are apparent from the initial concentration, but the significant effect from our experiments was found at concentrations of $10^{-5}$ x 100 - $10^{-4}$ x 100 µl / l (Figure 1).

The high contraction effect was expressed in the concentration of dopamine ($10^{-10}$ x 100-$10^{-1}$ x 100 µl / l), while the lowest effect was in these concentrations ($10^{-8}$ x 100 µl / l). So in other experiments with dopamine, there is an oblique ratio between the dose of concentration and the shrinking effect on Ileum longitudinal smooth muscle (ILSM). By reducing the dose of the substance (in this case dopamine), the constrictive force increases in the longitudinal Ileum smooth muscle (ILSM) of course, to a certain limit when it comes to the effect of substance saturation, and no longer reacts regardless of the substance dose used.

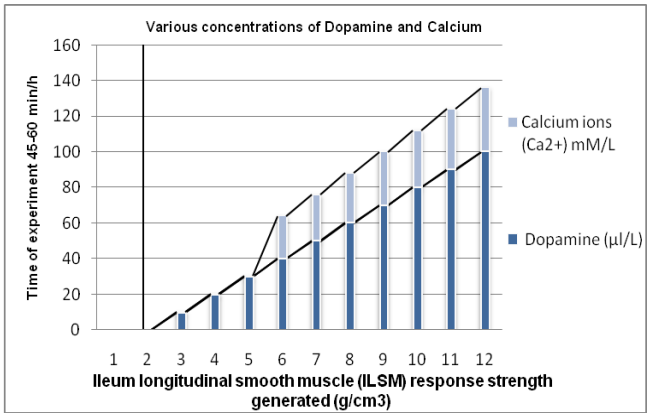


**Figure 2.** The extracellular Ca2+ influence on modulating the Ileum longitudinal smooth muscle (ILSM) contraction (strength generated in gram) to dopamine (concentrations 10 -8 x 100 to 10-1 x 100 µl / l).

*The influence of Calcium ($Ca^{2+}$) effect with dopamine in constrictive response to Ileum longitudinal smooth muscle (ILSM).*

The comparative aspect of the dopamine shrinking effect on Ileum longitudinal smooth muscle (ILSM) as well as the synergic action of dopamine and calcium (as effective contraction modulator) is given in (Figure 2).

It seems that calcium, when it interacts with dopamine, increase the Ileum longitudinal smooth muscle (ILSM), ranging from the dopamine concentration $10^{-6}$ x 100 µl / l up to dopamine the maximum concentration effect at concentrations $10^{-3}$ x 100 µl / l of dopamine.

*The effect of blocking (with verapamil) of $Ca^{2+}$ channels and dopamine in response to the Ileum longitudinal smooth muscle (ILSM) chickens*

The effect of blocking $Ca^{2+}$ channels in response to the Ileum longitudinal smooth muscle (ILSM) of dopamine-treated chickens has been studied in the ileum segments obtained from 10-15 chickens. Verapamil (in concentrations 0.02 mM/ l -0.021 mM/ l) was used in the experiment as ion blocker of calcium channel.

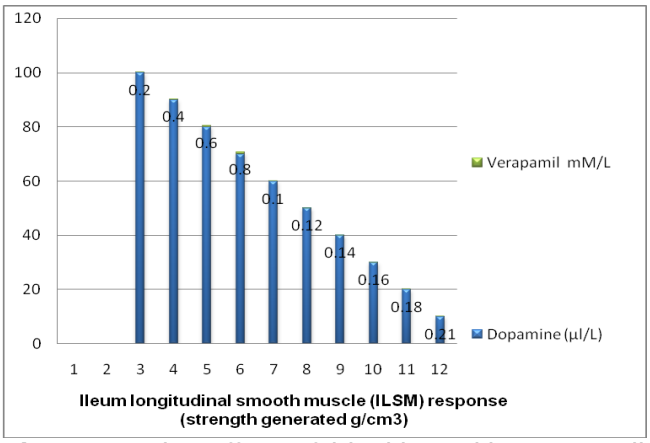


**Figure 3.** The effect of blocking with verapamil (0.02 mM/l - 0.021 mM/l) in response to the Ileum longitudinal smooth muscle (ILSM) (strength generated in grams) to different dopamine concentrations ($10^{-8}$ x 100-$10^{-1}$ x 100 µl / l).

The blocking of ionic calcium channel with verapamil has an effect and result in a reduction in the amplitude response of this Ileum longitudinal smooth muscle (ILSM) to dopamine concentrations of $10^{-8}$ x 100-$10^{-1}$ x 100 µl / l compared to the control (Figure 3). This does not result in complete blockade of muscle response, which also proves the role of $Ca^{2+}$ intracellular response in muscle response.

But as is know that are significant differences in the response of the Ileum longitudinal smooth muscle (ILSM) from dopamine when the muscle tissue is incubated with and without verapamil (Figure 3).

*The effect of nitric oxide ( NOS) blockade with synthesis nitric oxide synthase inhibitor L-NAME (N omega-nitro-L-arginine methyl ester) in response to the Ileum longitudinal smooth muscle (ILSM) with dopamine*

The role of NO in the mechanism of response to Ileum longitudinal smooth muscle (ILSM) provoked with dopamine was done through the study of the effect of the NOS blocker (L-NAME), which catalyzes the synthesis of NO. As NOS blocker is using L-NAME. In the preliminary experiments we tested the effect of various concentrations of L-NAME, during this experiment we found that five-minute incubation at 0.037 M / l concentration gives optimal effect on muscle modulating response.

The experiment was carried out in such a way that the influence of dopamine in the preparation without and previously treated with L-NAME was recorded so that the same muscle preparation after incubation at Krebs-Hesley's digestion was first treated with the selected concentrations of dopamine. The obtained results show that in the case where L-NAME was present in the medium, the amplitude of the muscle responses was higher compared to the cases when the medium was missing (Figure 4).

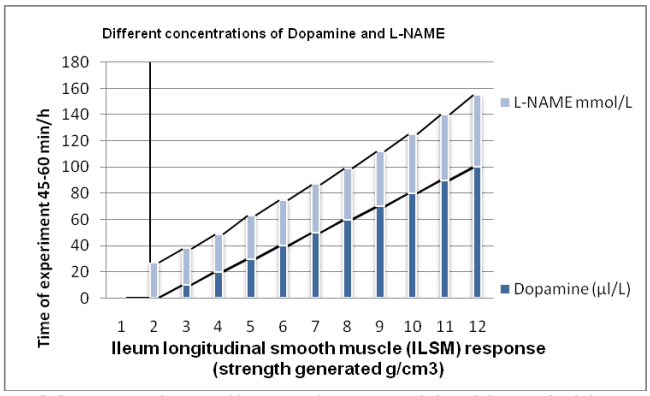


**Table 4.** The effect of NOS blocking (with L-NAME 0.037M / L) in response to the Ileum longitudinal smooth muscle (ILSM) (strength generated in grams) to different concentrations of dopamine ($10^{-8}$ x100 µl / l-$10^{-2}$ x 100 µl / l).

*The response of the longitudinal smooth muscle Ileum (ILSM) to dopamine and lead acetate (lead acetate ).*

After comparing the constrictive effect of lead acetate in different doses, although it was confirmed that dopamine has the highest constrictive effect at all its concentrations which we have used in our experiments,compared to lead acetate .

The response frequency of this Ileum longitudinal smooth muscle (ILSM) of the chicken to the lead series of lead acetate resulted in contractual response to confirm concentration. In this experimental series, the sections of the ileum taken from a series of 15 chickens treated with the series of cumulative doses were tested, starting with the lowest concentrations of doses at higher concentrations. During this study, the ileum segment was treated with the cumulative doses of dopamine. Then the same muscle preparation was treated with a dose of lead acetate (Pb acetate ) at one of the concentrations (3.06 x $10^{-2}$ -0.307 M/l) and the cumulative dose of dopamine as before.

When a specific concentration of lead acetate is present in the medium, the response (especially at initial doses) of the Ileum longitudinal smooth muscle (ILSM) will provoked with cumulative dopamine doses ($10^{-10}$ x100 µl / l-$10^{-1}$ x 100 µl / l) compared to the case where no lead acetate is present in the medium and sometimes (at certain doses) the opposite occurs (Figure 5).

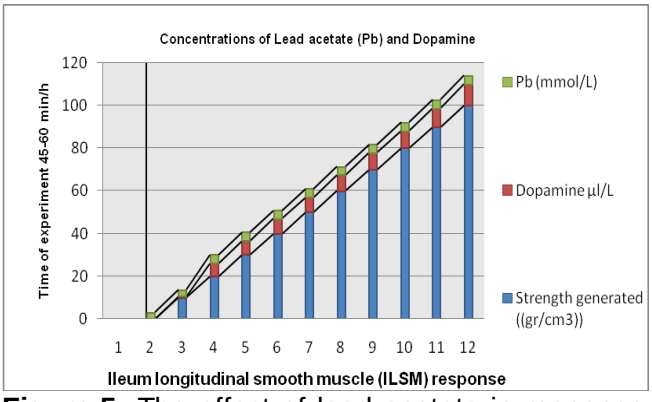


**Figure 5.** The effect of lead acetate in response to the Ileum longitudinal smooth muscle (ILSM) (strength generated in grams) to various concentrations of dopamine ($10^{-8}$ x100 µl / l-$10^{-2}$ x 100 µl / l).

## Discussion

From this experiments results were obtained which showed that; dopamine, and lead acetate (Pb acetate ) cause the contraction of this Ileum longitudinal smooth muscle (ILSM) of the chicken under "in vitro" conditions and this response is dependent on their concentration.

Also our results showed the effect of the blocking action of verapamil on various concentrations of lead acetate, dopamine, and the stimulus effect of muscle contraction in the presence of $Ca^{2+}$ ions at different concentrations of all these constituent substances. It is known that lead binds sulfhydryl groups (SH) of proteins that are a constitutive component of muscarinic receptors, increasing the sensitivity to agonists of these receptors.[32] Also it has been shown that lead interacts with $Ca^{2+}$ and protein kinase C initiating intracellular mechanisms of contraction.[33] In contrast to our findings, have shown that incubation of rat to dopamine preparations in lead reduced the constrictive responses to dopamine at lower concentrations but not at higher concentrations.[18] Below they found that this heavy toxic metal causes smooth muscle shrinkage of the same system and other systems, such as the digestive system (mammary ileum), the blood vessels, the aorta and some other vessels. The results of other authors indicate that lead exposure in low concentrations also causes hipertension and dopamineeal limb muscles.[34, 35] Lead also exhibits a constrictive effect on the transverse muscles.[37]

Studies show that the mechanism of action of lead in muscle contraction is direct and is dopamine after it enters interaction with PKC. The protein C protein enzyme is found in the cells of all tissue types, including smooth muscles.[38] The main path of initiation of PKC contraction is through the phosphorylation of light myosin strands (formation of AT-linked myocardial bridging bridges), while their dephosphorylation causes muscle relaxation.

It has been established that PKC and some isoforms there of, such as AMPc-dependent and $Ca^{2+}$ -calmodulin (CAM kinase II) dependent proteins in "in vitro" conditions, may cause phosphorylation of light myosin catheters.[39] Given the fact that smooth muscle contraction in general resembles PKC activation, this may be related to the fact that the shrinking effect of ML from lead is accomplished through stimulation of PKC.[40] Although lead biochemical mechanisms of lead toxicity are not fully clarified, however, lead in some biological systems interacts with

calcium-dependent cellular processes. Studies have shown that lead, for cytosol entry, uses $Ca^{2+}$ type L channels. [40] In this research, the use of lead L-type $Ca^{2+}$ channels was found in the case of blocking these channels with verapamil. Thus, although the amplitude of the contraction response of this Ileum longitudinal smooth muscle (ILSM) decreases to lead acetate, however, it does not extinct completely. This opens the possibility of the existence of other "alternative" lead-in cytosolic pathways, such as T-type channels, which are not blocked by verapamil, although this type of channels is less represented in ML cells, PKC is too much (i.e., PKC), but it does not have the same effect on the cytosolic ion more sensitive to lead than $Ca^{2+}$, so that picomolar concentrations of lead are equivalent to the micromolar concentrations of $Ca^{2+}$**.** The contraction of ML, apart from other factors, is also dependent on the concentration of $Ca^{2+}$ ions, which actively initiate the process of muscle contraction. $Ca^{2+}$ cytosolic origin may be from the extracellular environment and the intracellular calcium reserves in the sarcoplasmic reticulum. Causation of $Ca^{2+}$ extracellular cytosol in $Ca^{2+}$ is mediated by $Ca^{2+}$ ions, dependent on the voltage (electrical potential) and those that are activated by receptor mediation.[40]

The results of our experiments show that extraction of $Ca^{2+}$ extracellular ions is important for the generation of lead-provoked muscle contraction. This contraction is partially dependent on the inflow of $Ca^{2+}$ ions from outside within the muscle cell. Our results demonstrate this assertion that the contraction of this Ileum longitudinal smooth muscle (ILSM) is also generated in the case of provocation of the muscle in the medium without $Ca^{2+}$ similar to the $Ca^{2+}$ channel blockade. In this case, the muscle contraction process is initiated by the release of $Ca^{2+}$ from its intracellular reservoirs in the sarcoplasmic reticulum. This release of $Ca^{2+}$ lead is accomplished by activation of phospholipase C and production of IP3.[40] Our results are in accordance with other authors whose research found that heavy metals $Pb^{2+}$ and $Hg^{2+}$ can induce active strain directly in the rabbit aorta ML by acting on the processes which are supported on extracellular $Ca^{2+}$.Various authors have concluded that the lead effect on increasing muscle sensitivity to certain agonist substances, noradrenalin, caracol, has significantly resulted in the case when tissue is incubated in Krebs Hensley (KH) digestion with large amounts of $Ca^{2+}$ compared with that when tissue is incubated in $Ca^{2+}$ free digestion.This proves that the mechanism of lead action in the muscular contraction process is also related to $Ca^{2+}$.

Some authors of high lead toxicity and other heavy metals explain in the context of high affinity with which these metals effectively replace $Ca^{2+}$ in the celodulus. [37]

In the Ileum longitudinal smooth muscle (ILSM) contraction process of lead acetate, there is also the endothelium from which NO is released, synthesized under the action of NOS, who's the research of the relaxing molecule was the goal of this research. From the experiments performed during this study, in the case of NOS inhibition with L-NAME, the response of this Ileum longitudinal smooth muscle (ILSM) of the chicken to lead acetate resulted in enhanced tension of contraction and elevation of the response amplitude muscle. This is because of the prevalence of constrictive mechanisms to those relaxing in the Ileum longitudinal smooth muscle (ILSM) cells, namely the production of smaller amounts of NO. From this it is clear that small amounts of NO increase the constrictive effect of lead acetate. Enzyme Inhibitory Activity (NOS) responsible for NO synthesis is the key explanation about the mechanisms and effects of lead action in this Ileum longitudinal smooth muscle (ILSM).

These findings coincide with the results of other authors, who also found that lead inhibits NO release through NOS inhibition and as a result manifested lead hypertension.[38] In our study, the interaction of single-dose lead acetate and cumulative doses of dopamine, with the exception of some initial doses, did not result in constrictive response strain of this Ileum longitudinal smooth muscle (ILSM) of chicken to dopamine compared to the case where lead acetate was lacking in the medium.

Despite this found that lead causes anon crossing muscle anxiety to agonist substances (carbahol, noradrenalin, etc.) in rats.[37] Lead also affords convex response to dopamine in smooth rheumatoid muscle at rats. Mechanisms dependent on extracellular $Ca^{2+}$ are implicated in this mechanism.

NO "compromises" the activity of the creatine kinase system in the myocardium (heart muscle), for example: injecting the heart's NO inhibits the CK-cardiac by 65%, which is expressed as a

decrease in muscle contraction. The same NO influence is not excluded from the other muscles.The constrictive effect of lead is also found in the smooth muscle of the digestive tract, respectively in the mammary ileum. Found that lead (Pb2+) protokens (highlights) the contraction caused by acetylcholine in normal medium or with calcium supplementation ($Ca^{2+}$).[39]

The results show the role of epithelial relaxation in the smooth muscle relaxation who experimenting with the bovine dopamine, noted that the constrictive effect of neurotransmitter is greater in the non-epithelial tract compared to the intake the dopamine. This answer clarified with the release of substances that have an adverse effect on the bronchoconstriction. By measuring the release of NO and PGE2 (prostaglandins 2) in epithelium, Sadeghi found that with the addition of histamine construct, and dopamine in dopamineeal bovine muscle, dog.[38]

On the other hand, suggest that organic lead affects has not only the synaptic transmission which is carried out with dopamine neurotransmitter, but also in the synthesis and release of acetylcholine.[39]

In the opposite sense, Hosh's give other data, which noted that lead three-ethylene inhibits the release of neurotransmitter from the brain. This suggests that acetylcholine transmission, the synthesis of acetylcholine and its release is susceptible to organic lead neurotoxicity.[40]

The results of our research that relate to synergic constrictive systolic lead amplification in smooth muscles treated with acetylcholine neurotransmitter are consistent with other authors' data that test their constrictive effect when acting together with any of the neurotransmitters mentioned in different research models. Thus, in the anokokcygeus muscle isolated from lead treated rats found their supernatant in norepinephrine, dopamine and 5-hydroxytryptamine.[37]

Lead neurotoxicity and its consequences on behavior were followed by which concluded that the first disorders in people's behavior and animal models are related to changes in cholinergic and dopaminergic neurotransmission in the CNS (like serotonine, dopamine, norepinephrine and acetylcholinesterase activity).[40]

There are data that the workers who have been exposed to lead (if this heavy metal concentration 41-80 µg / dl) show cognitive and neurophysiological deficits.

Some research has shown that with the application of lead or lead nitrate chloride in "in situ" or "in vitro" conditions in peripheral tissues, the release of neutrotransmitter from the preganglionic neurons is blocked. From this it results that lead is implicated in cholinergic and catecholaminergic transmission.

From this scientific data it appears that despite the existence of different thoughts on lead action in muscle contraction mechanisms, there is a common consensus among researchers on the implication of lead in both systems, both cholinergic and catecholaminergic.[41, 42]

This may lead to the conclusion that lead had a synergistic effect with calcium and dopamine in the smooth muscle contraction of the ileum. Based on results of this research we show that dopamine constructs a constrictive response of this longitudinal smooth muscle (ILSM) influenced by the concentration of $10^{-8}$ x 100 -$10^{-4}$ x 100 µl / l, while dopamine with $Ca^{2+}$ action is caused by the highest constrictive response of this longitudinal smooth muscle (ILSM) influenced by the concentrations of dopamine $10^{-8}$ x 100-$10^{-2}$ x 100 µl / compared with the constrictive response of this Ileum longitudinal smooth muscle (ILSM) without $Ca^{2+}$.

Dopamine with verapamil ($Ca^{2+}$ channel blocker) to the medium reduces the constrictive effect in concentration $10^{-4}$ x 100 to $10^{-1}$ x 100 µl / l compared to the high constrictive response of this Ileum longitudinal smooth muscle (ILSM) without verapamil. On the other hand dopamine with L-NAME - (NOS blocker) treated with 0.037 M concentration significantly increases the constrictive effect of Ileum longitudinal smooth muscle (ILSM) in dopamine from the concentration $11^{-8}$ x 100 - $10^{-4}$ x 100 µl /l. And, lead acetate with dopamine causes a high constrictive response of the longitudinal smooth muscle (ILSM) influenced by the concentration of (3.06 x $10^{-2}$ -0.307 M/l).

Also, the presence of verapamil ($Ca^{2+}$ channel blocker) in the medium decreases the constrictive effect of lead acetate of the Ileum longitudinal smooth muscle (ILSM) in concentrations (0.02 mM/ l - 0.021 mM/l).

## Conclusions

Results from this research show that dopamine has constrictive effects on the longitudinal smooth muscle of ileum (ILSM). Also, resulted between the effect of ILSM and the concentration of neurotransmitter exist an indirect division. Dopamine with L-NAME - (NOS blocker) and lead acetate significantly increases the constrictive response of ILSM. Also, the presence of verapamil (as $Ca^{2+}$ inhibitor) in the medium lower the constrictive effect of lead acetate and other neurotransmitters. Dopamine with the action of $Ca^{2+}$ ions causes the highest constrictive response of the Ileum longitudinal smooth muscle compared with the constrictive response of this Ileum longitudinal smooth muscle (ILSM) without being influenced by $Ca^{2+}$. Verapamil as a $Ca^{2+}$ channel blocker in the medium reduces the dopamine constrictive effect in the Ileum longitudinal smooth muscle (ILSM) depends on the concentration. These findings confirm that the White Leghorn chicken birds can be used as an experimental model to reflect the effect of neurotransmitters and the role of NO and extracellular calcium in the pathway physiology and constrictive mechanism of Ileum longitudinal smooth muscle (ILSM).

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## Declaration of Interest

The authors report no conflict of interest.

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