Air Quality Monitoring in Egypt

&

A Practical Approach for Assessment of Environmental Pollutants

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Presentation Outlines

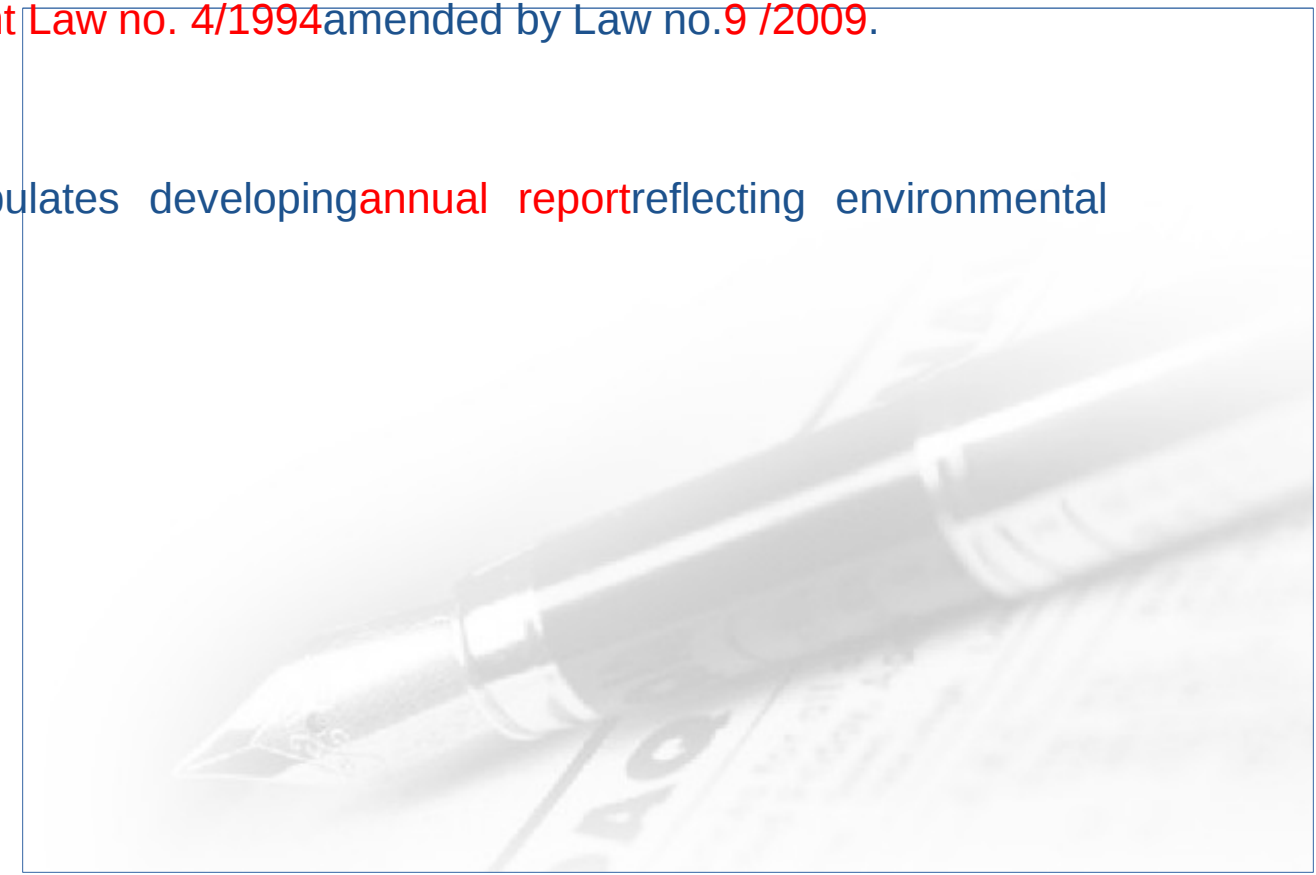
- Background
- Recent State of Environment Report (EEAA)
- Major ambient air pollutants in Egypt
- National Network for Monitoring Air Pollutants
- Some results of air quality monitoring indicators
- Monitoring Industrial Facilities Emissions
- Emissions from rice straw burning
- Health and economic impacts of air pollution
- A Practical Approach for Assessment of Environmental Pollutants
- Take- Home Message

Ambient airconservation is one of themajor challengesfacing Egyptian Government due to presence of multiple sources of pollution.

Egyptian Government taking all measurestopreserve environment, move towards green economy, support integrated environmental management, activate sustainable development policy, address impacts of climate change and mainstream environmental dimension within national policies

The presented data are captured from the recentState of Environment Reportwhich is issued for the consecutive years for the pursuant to article (5) of chapter II ofEnvironment Law no. 4/1994amended by Law no.9 /2009.

The Egyptian law stipulates developingannual reportreflecting environmental status in Egypt.



Ambient air pollutants

Ambient air pollutantsdivided intotwo major types; thesuspended inhaled particlesandgases.

The six major pollutantsindicate state of Egypt ambient air quality according to the guidelines of the US-EPA and WHO.

They includeprimary pollutantsresulting from human activities: Sulfur Dioxide, Nitrogen Dioxide, Carbon Monoxide, Ozone, Lead andinhaled ParticulateMatters).

Maximum permissible limits according to Environment Law no. 4/1994 amended by Law no. 9 /2009					
Pollutants	Area	Maximum concentration (microgram/m3)			
		1 hour	8 hour	24 hour	year
Sulfur Dioxide	Urban	300		125	50
	Industrial	350		150	60
Carbon Monoxide	Urban	30 mg	10 mg		
	Industrial				
Nitrogen Dioxide	Urban	300		150	60
	Industrial	300		150	80
Ozone	Urban	180	120		
	Industrial	180	120		
PM <10	Urban			150	70
	Industrial			150	70
PM <2.5	Urban			80	50
	Industrial			80	50
Lead	Urban				0.5
	Industrial				1

National Network for Monitoring Ambient Air Pollutants

The Ministry of State for Environmental Affairs(MSEA)established theNational Networkcomposed of87 stationsdistributed in different regions of the Country for monitoring and controlling air pollutants periodically and continuouslysince 1998and till now.

Additionally, thenetwork measuresmetrologicalfactors(wind speed and direction, temperature and relative humidity) affecting on the distribution and transmission of pollutants.

National Network for Monitoring Ambient Air Pollutants

Monitoring processof pollutants conducted by the following two different methods:

First Method:using automatic equipment for instantaneous and continuous monitoring for the whole day 24hr/7days to measure concentrations and calculate their average per hour.

Second Method: using semi- automatic sampling equipment's that analyze samples collected on filters by specialized chemical laboratories to determine particulate concentrations.

TheMinistry of State for Environmental Affairsupdate and replaceold monitoring devices, operate new communication technology , increase stations number ,re-locate monitoring sites to cover the whole country.

MonitoringAir QualityNetworkincludes87 Stations, distributed as follows:

19stations for Industrial areas

36stations for urban and residential areas

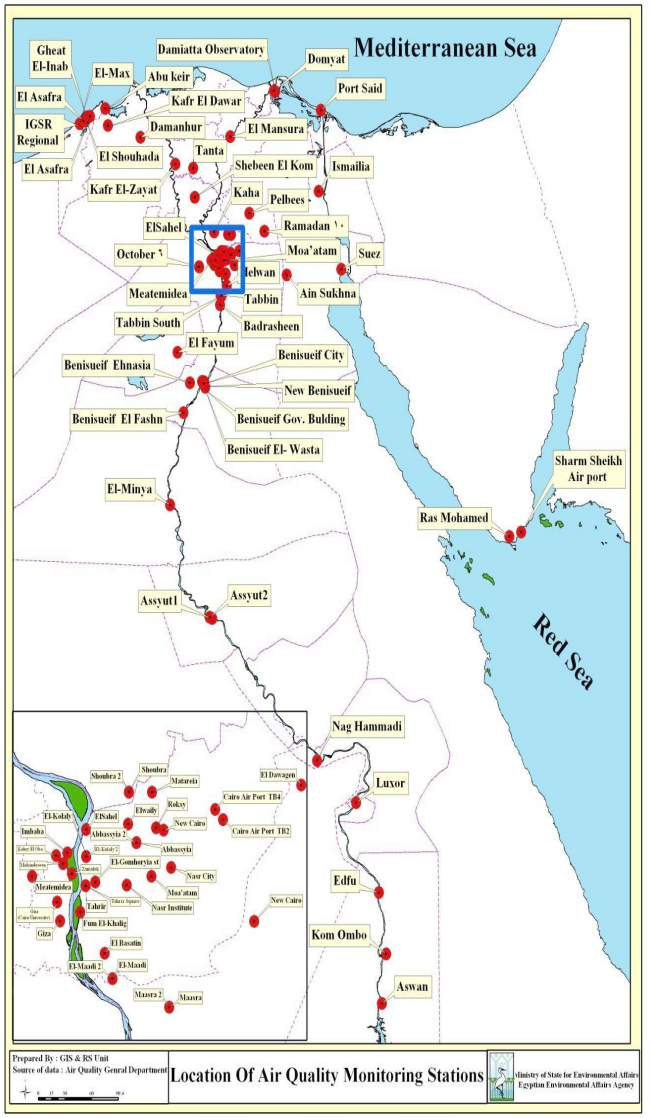
10stations for traffic dense areas

1station for remote areas

21stations for mixed areas

Geographical distribution of EEAA's National Network for Monitoring Ambient Air Pollutants

Site Type	Greater Cairo	Alexandria	Delta	Upper Egypt	Sinai and Canal Cities	Total
Industrial areas	8	3	4	3	1	19
Urban and Residential areas	14	4	8	9	1	36
Traffic dense areas	9			1		10
Remote areas					1	1
Mixed areas	16	1	2	2		21
Total	47	8	14	15	3	87

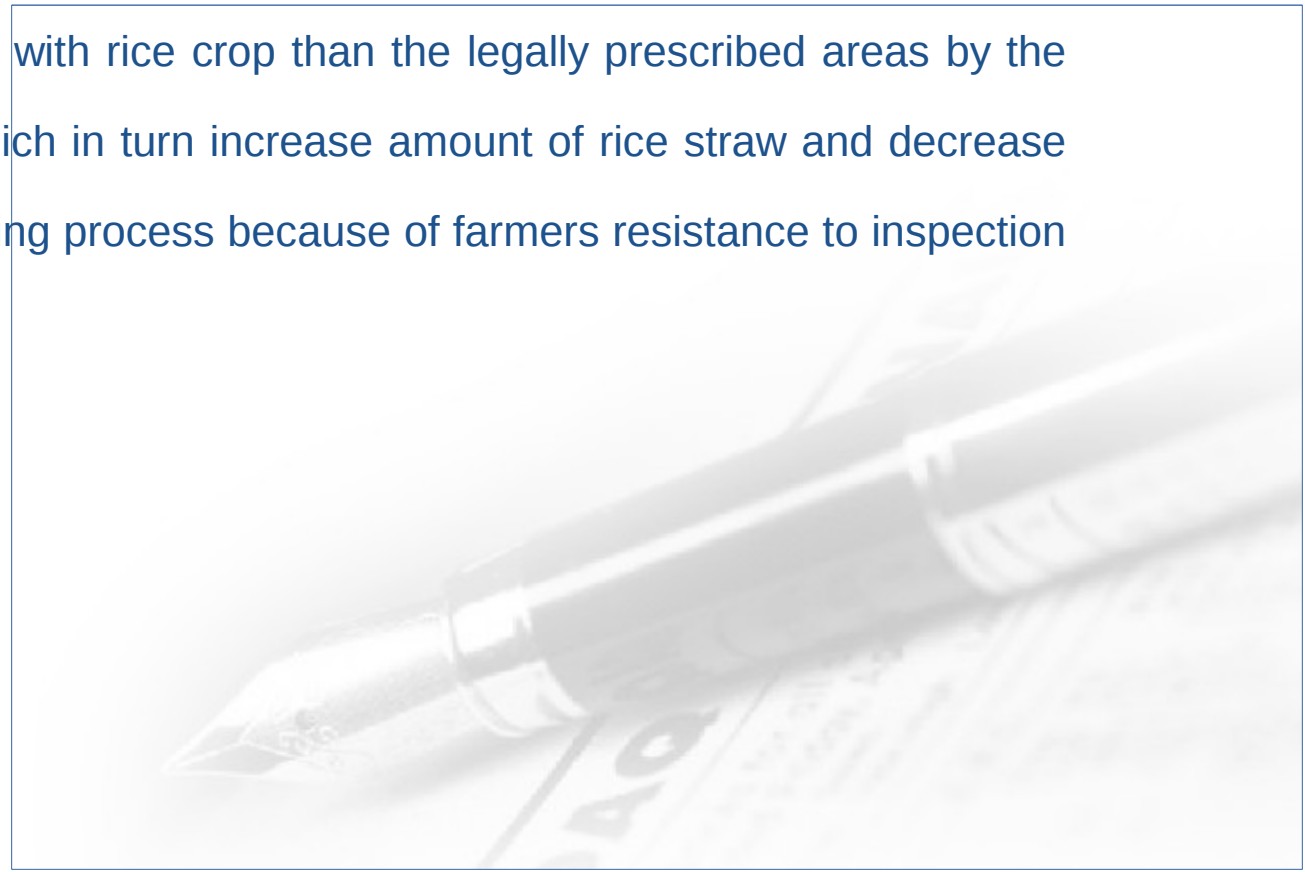


The most important results of ambient air quality monitoring indicators: Summary of the Environment state report 2013

- Results of theannual averagesconcentrations of carbon monoxide, sulfur dioxide, nitrogen dioxide, ozone and lead werewithin limits of the lawat urban and industrial areas.

- While results ofparticulate matter concentrations(PM2.5-PM10) exceeded permitted limits.
- ThePM10 concentrationsrecorded (172microgram/m3) at urban areas and (206microgram/m3) at industrial areas.i.eabout 146% and 195% exceed limits respectively.
- PM2.5the recorded concentration at greater Cairo was104microgram/m3 exceeding the permissible limit (50 microgram) at Urban areas.

- The excess concentration of PM (2.5-10) could be attributed to the reduction of the amount of recycled and compressed rice straw.
- This is due to the security conditions Egypt faced which resulted in farmers tend to cultivate larger areas with rice crop than the legally prescribed areas by the Ministry of Irrigation, which in turn increase amount of rice straw and decrease recycling and compressing process because of farmers resistance to inspection campaigns.



Air quality indicators

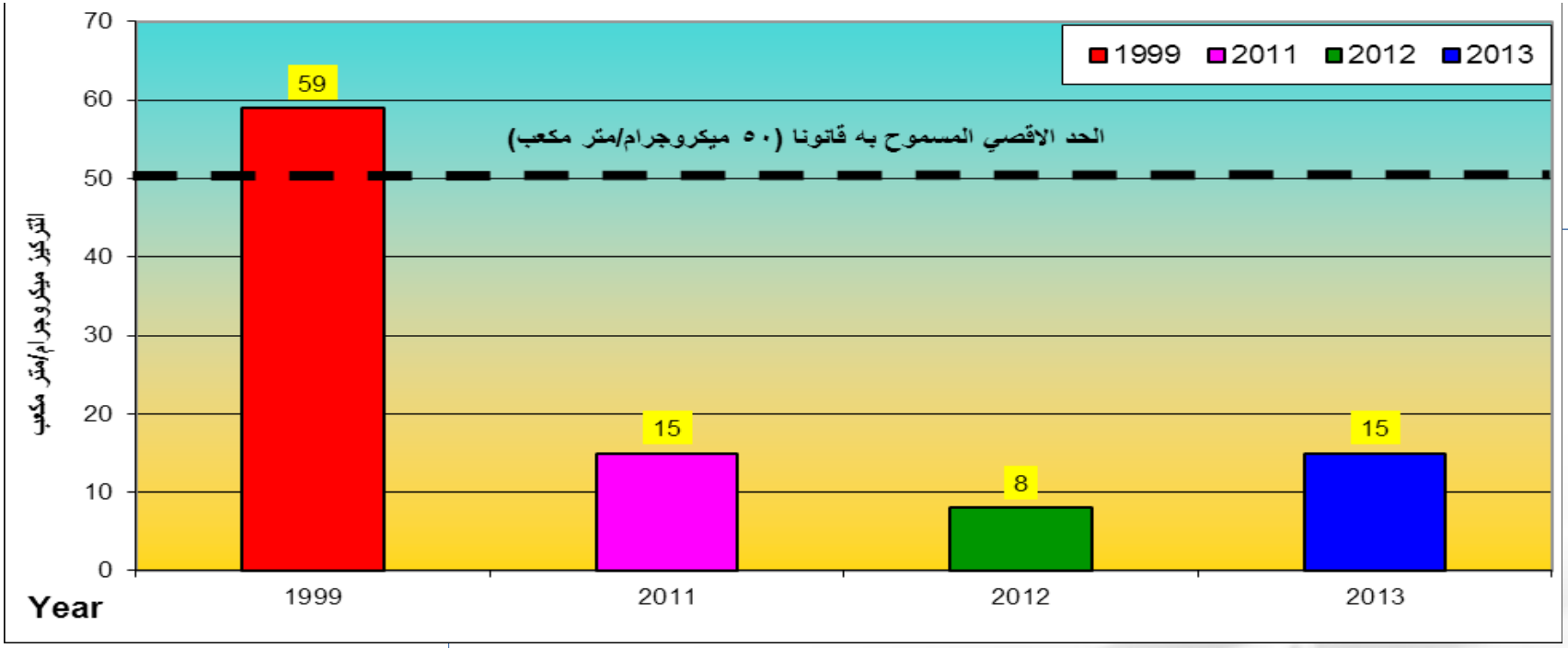
The National Network for air monitoring is considered primary reference for environmental air quality indicators and the base for studying variation during previous years.

These indicators and data are also used in preparing Integrated Environmental Assessment Reports, Sustainable Development Reports and State of the Environment Reports and following performance of policies to achieve the National Development Plan.

Sulfur Dioxide (SO2)

The permissible annual average limit in Annex No. 5 of the Executive Regulations of Law No. 4 / 1994 amended by Law 9 / 2009 is **60 µg / m3** for industrial areas and **50 µg / m3** for urban area.

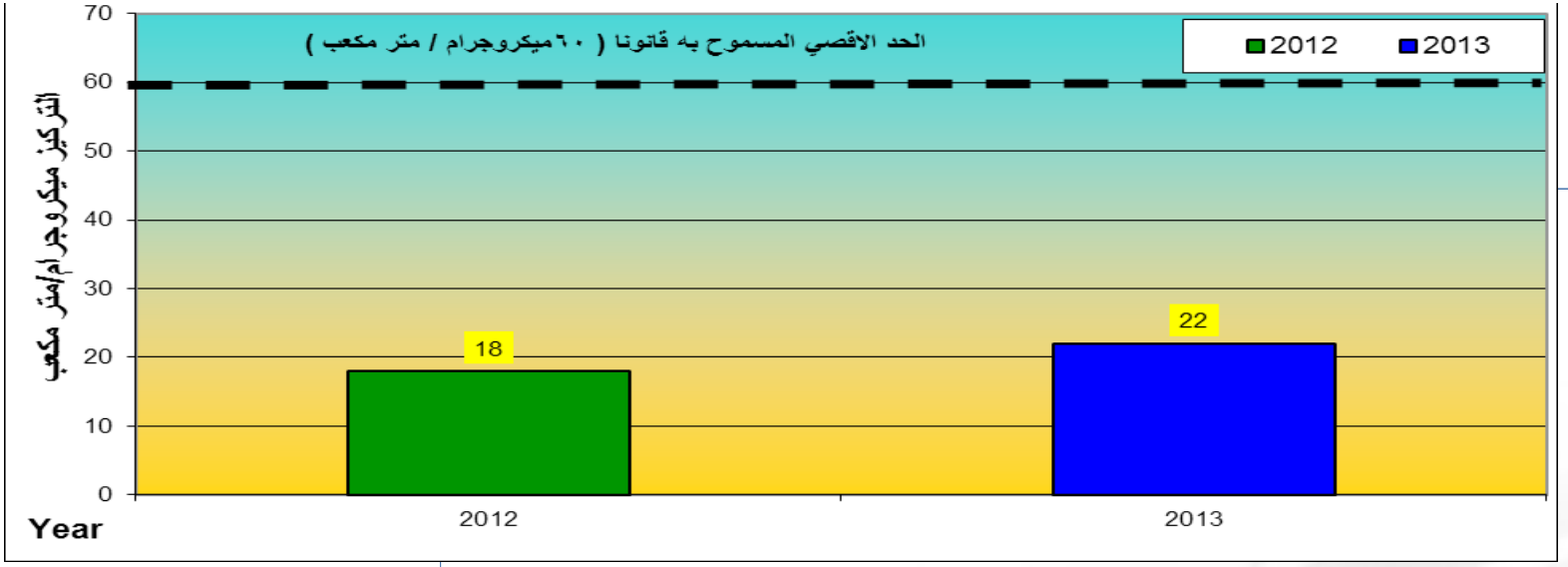
Annual average concentrations of SO2 at Urban/Residential areas



The 2013 annual average concentrations of SO2 all over the country at urban areas not exceeding permissible annual average limit in executive regulation law.

The improvement is clear during 2013 compared to the baseline year 1999. This can be attributed to the shift to operate factories and power plants by using natural gas in replacement of other types of fuel and adjusting environmental conditions of many facilities

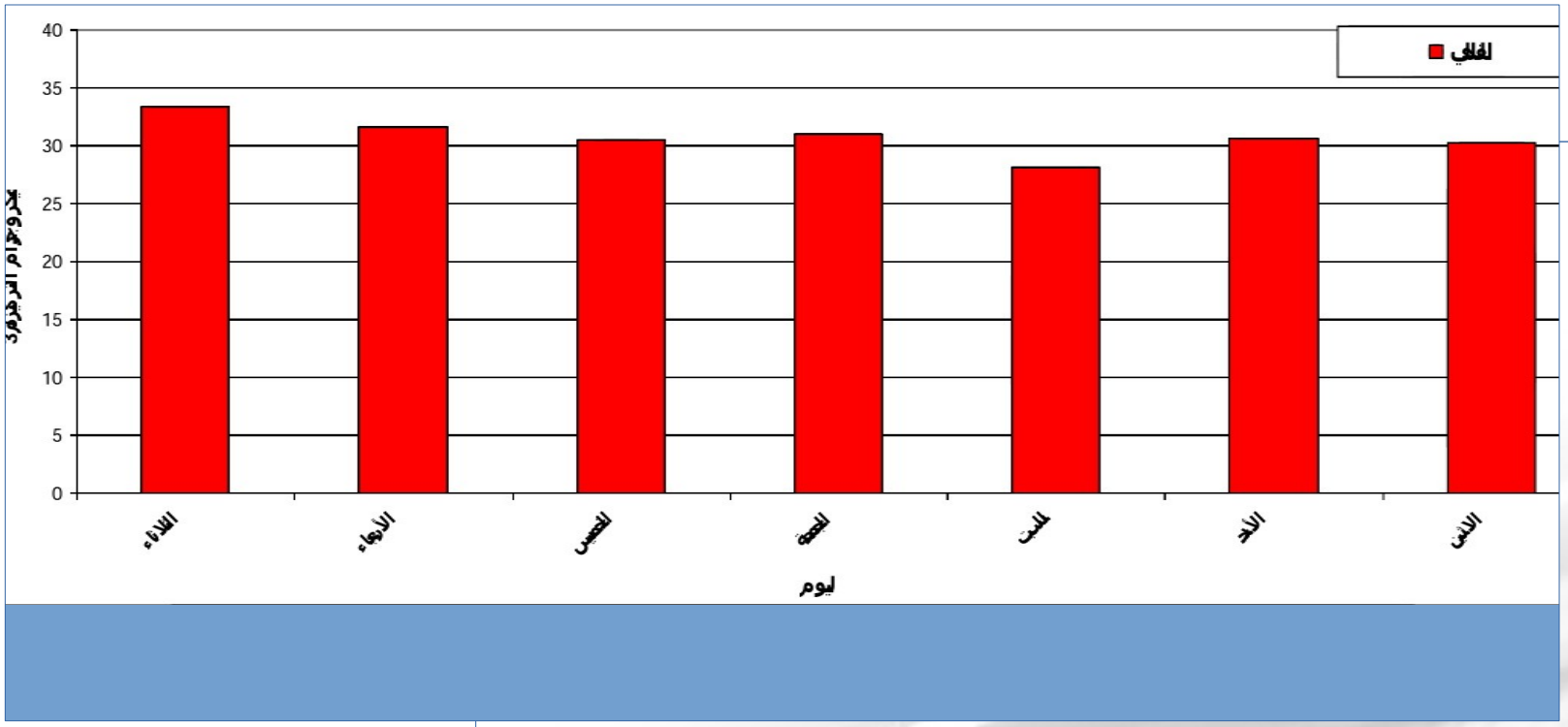
Annual average concentrations of SO2 at industrial areas



- The annual average concentrations of SO2 at industrial areas are within the permissible annual average limit in executive regulation law (60ug/m3.

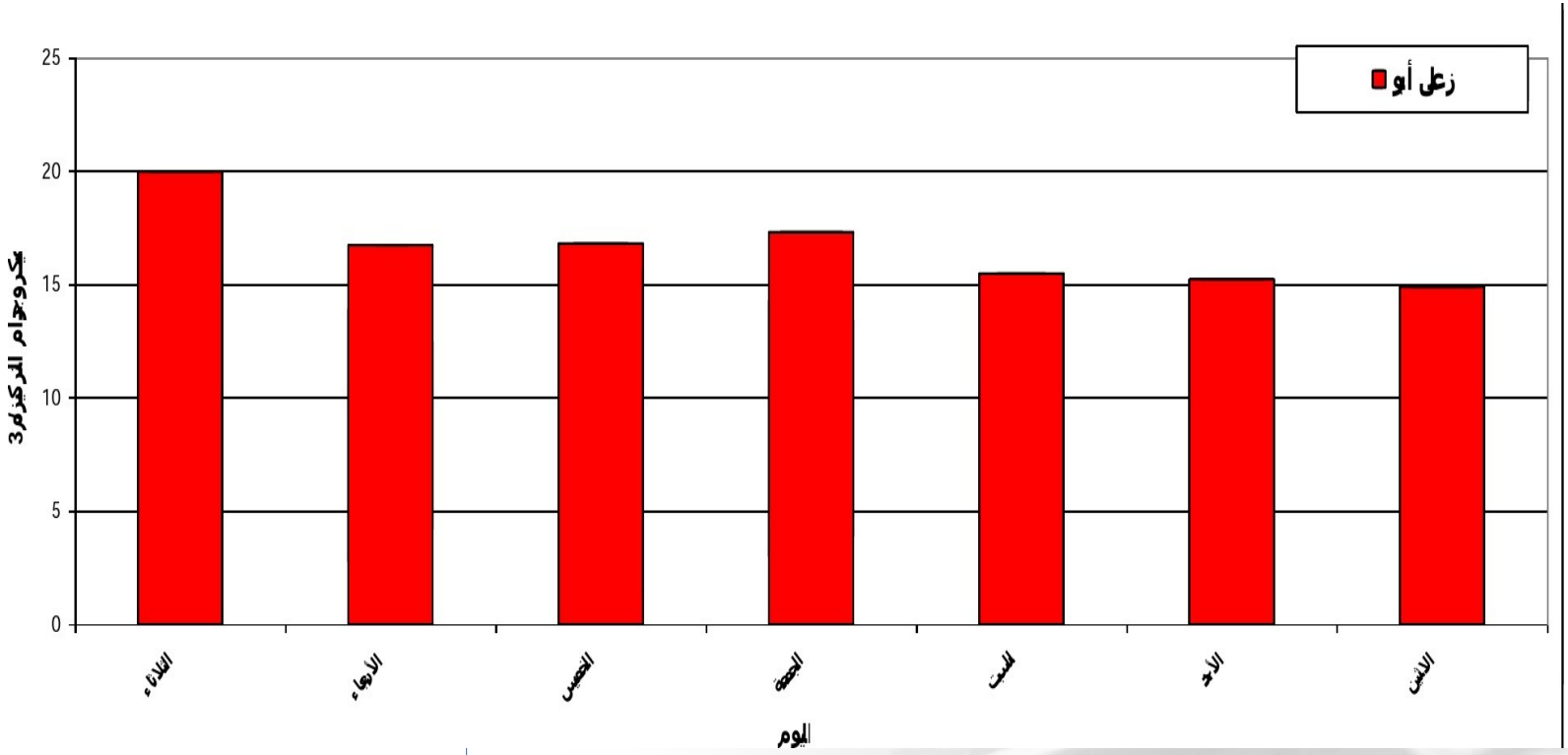
- There is slight increase in the limits compared to 2012

Dailyaverage concentrations of SO2during a weekatKolaly monitoringstation



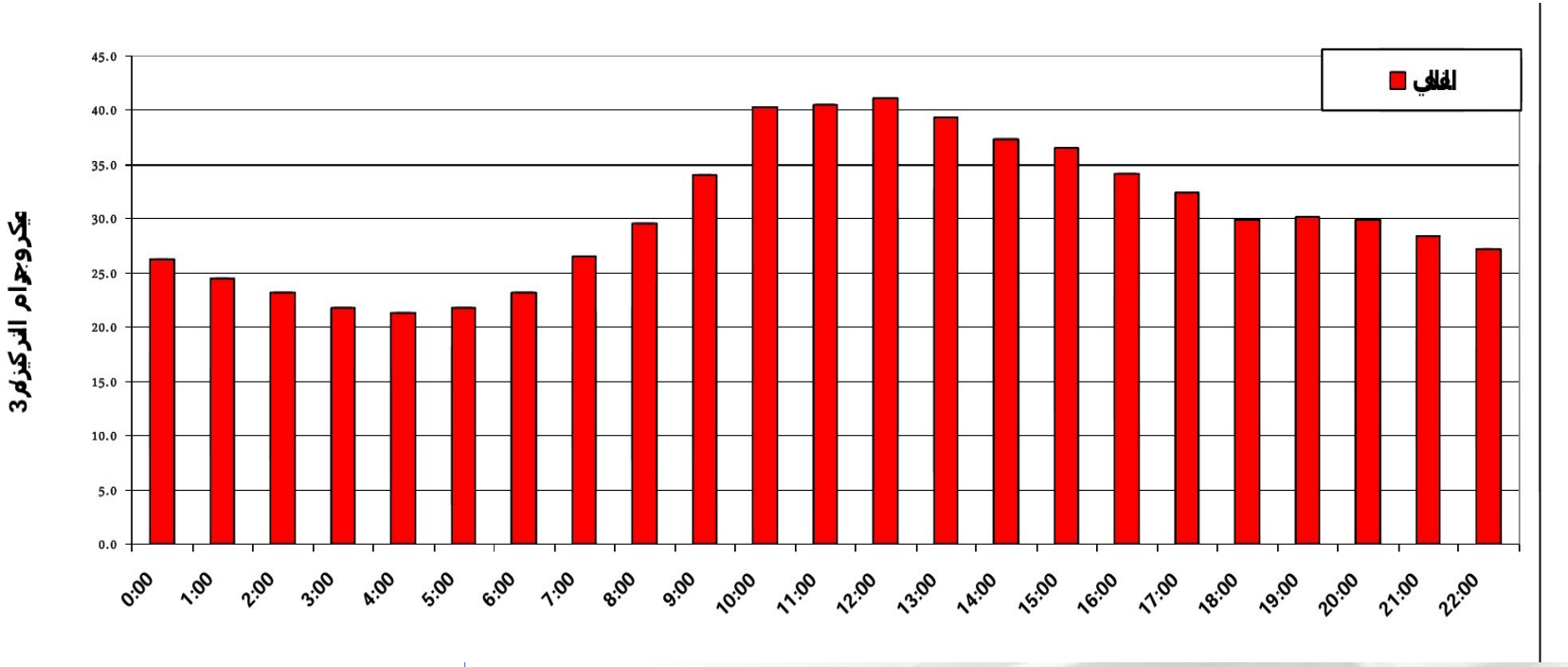
The graph shows therelations betweentheaverage concentrationandtrafficand the observedreductionat week end

Dailyaverage concentrations of SO2during a weekatabozabel industrialmonitoring station



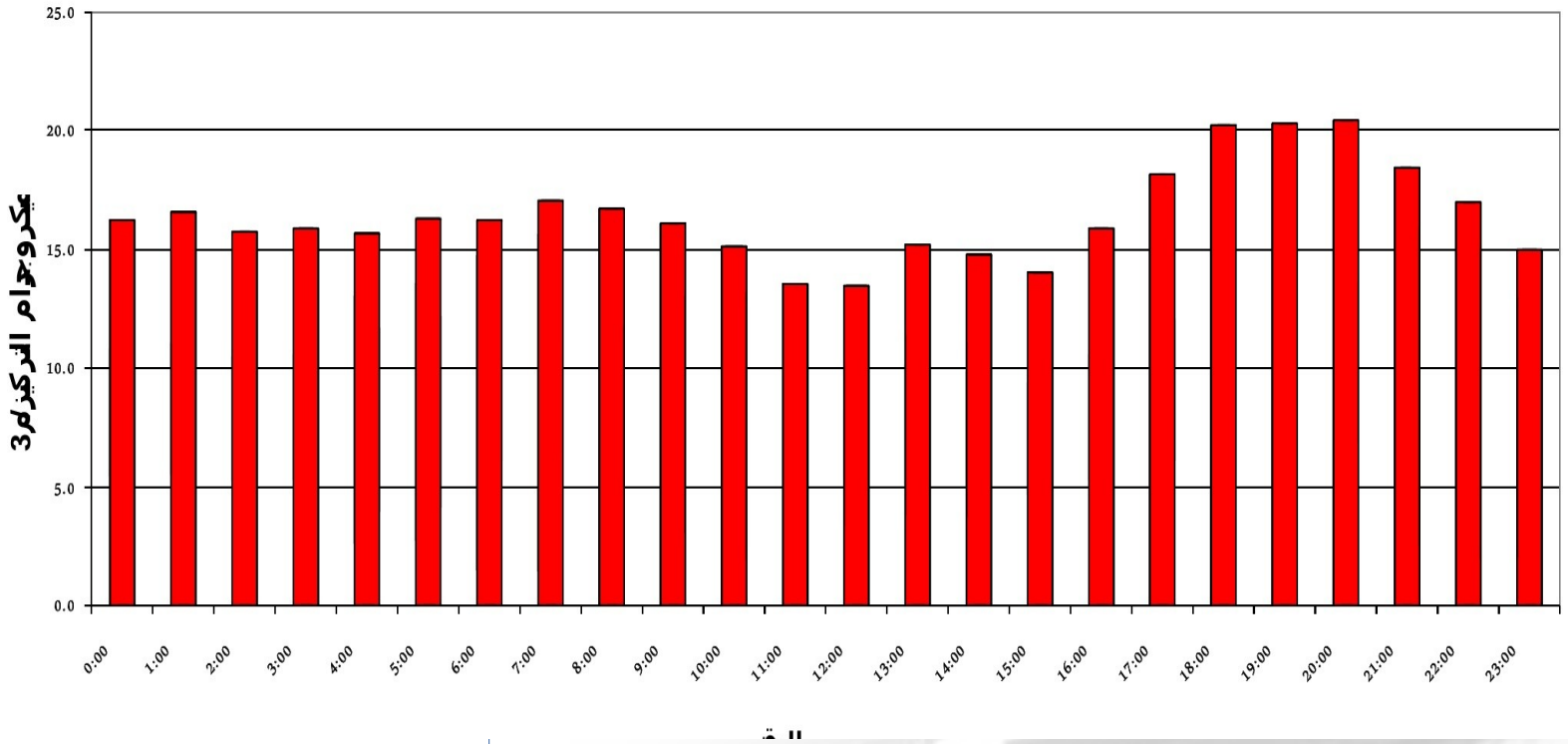
Shows the relations between theaverage concentrationandworking daysand the observed reductionat beginningof the week.

Average concentrations ofSO2 during a dayat urban area monitoring station



The most concentration increasesat human activity period from6 am-6 pm

Average concentrations of SO2during a dayatabozabalindustrial monitoring station

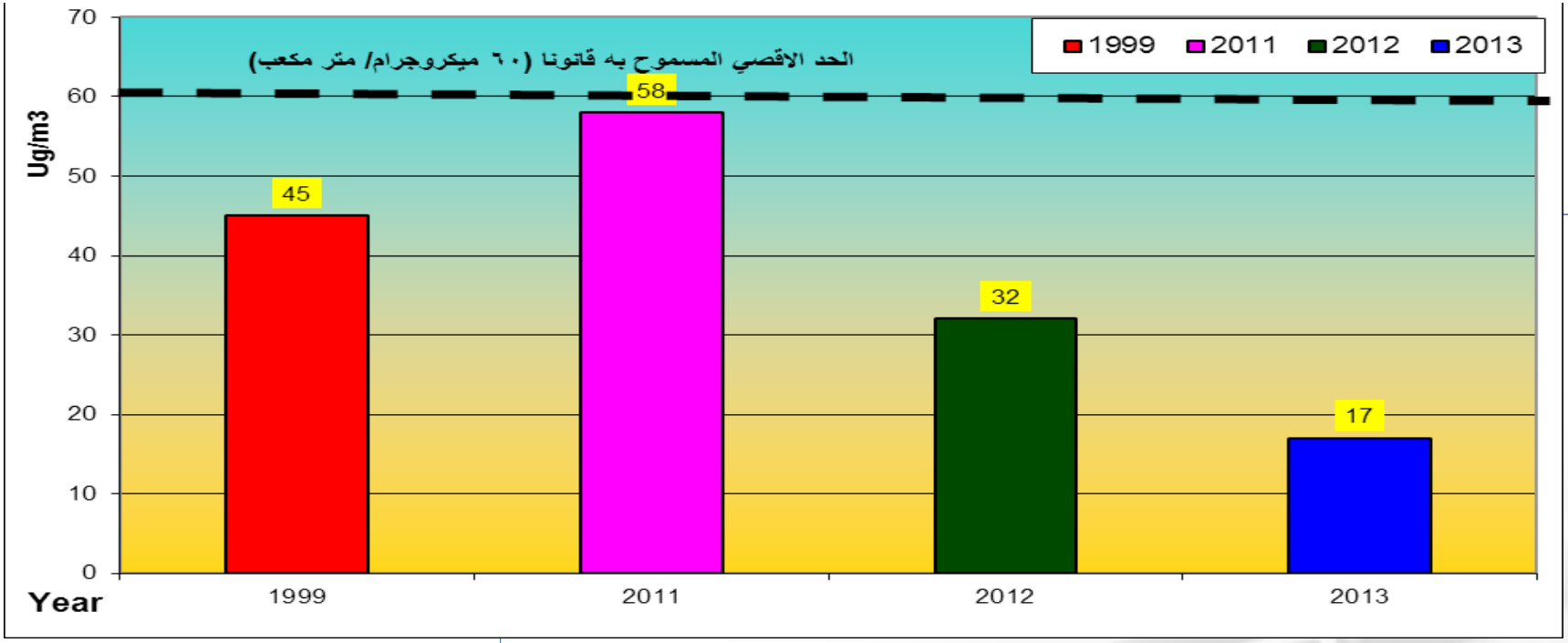


Thehighest concentrationrecorded atevening timeandearly morningtime

Nitrogen dioxide (NO2)

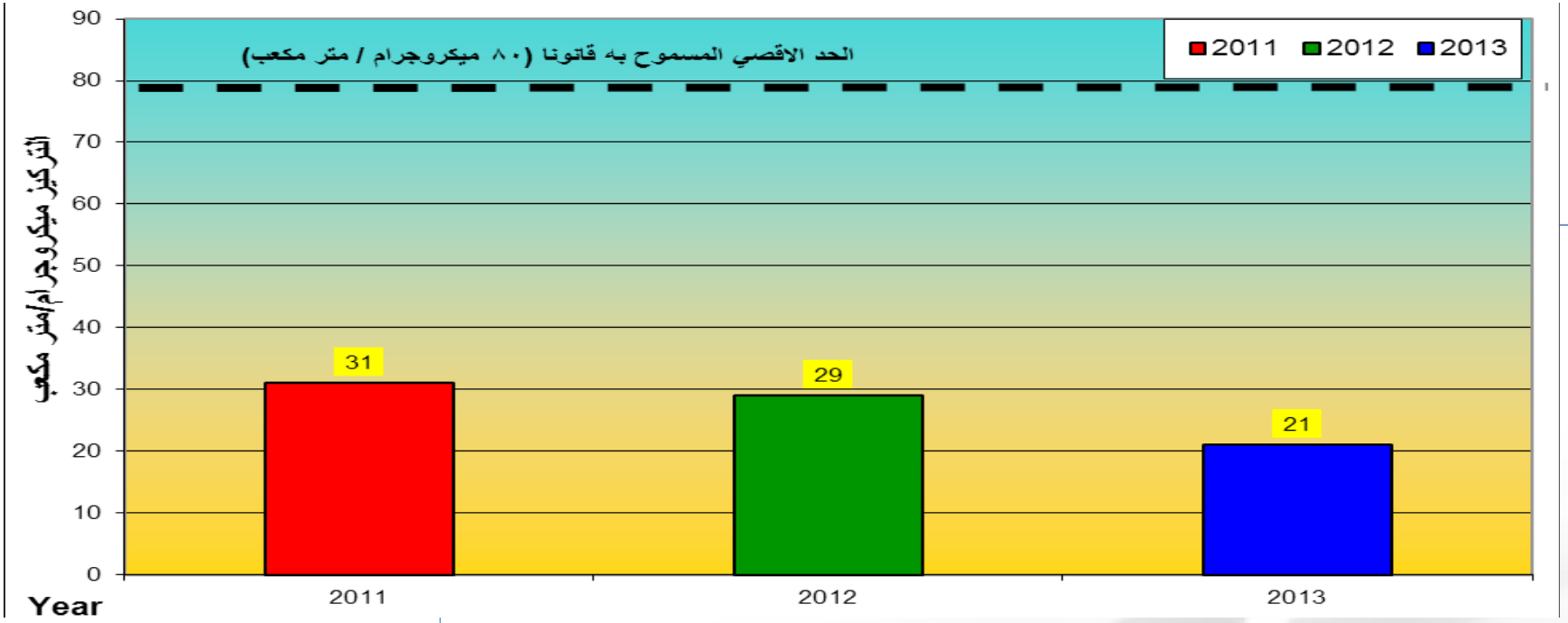
- Executive Regulation ofEnvironment Lawno. 4/1994 identify maximum annual average limit for itsurbanareas concentrations60µg/m3and80µg/m3forindustrialareas

Annual average NO2 concentration at urban area



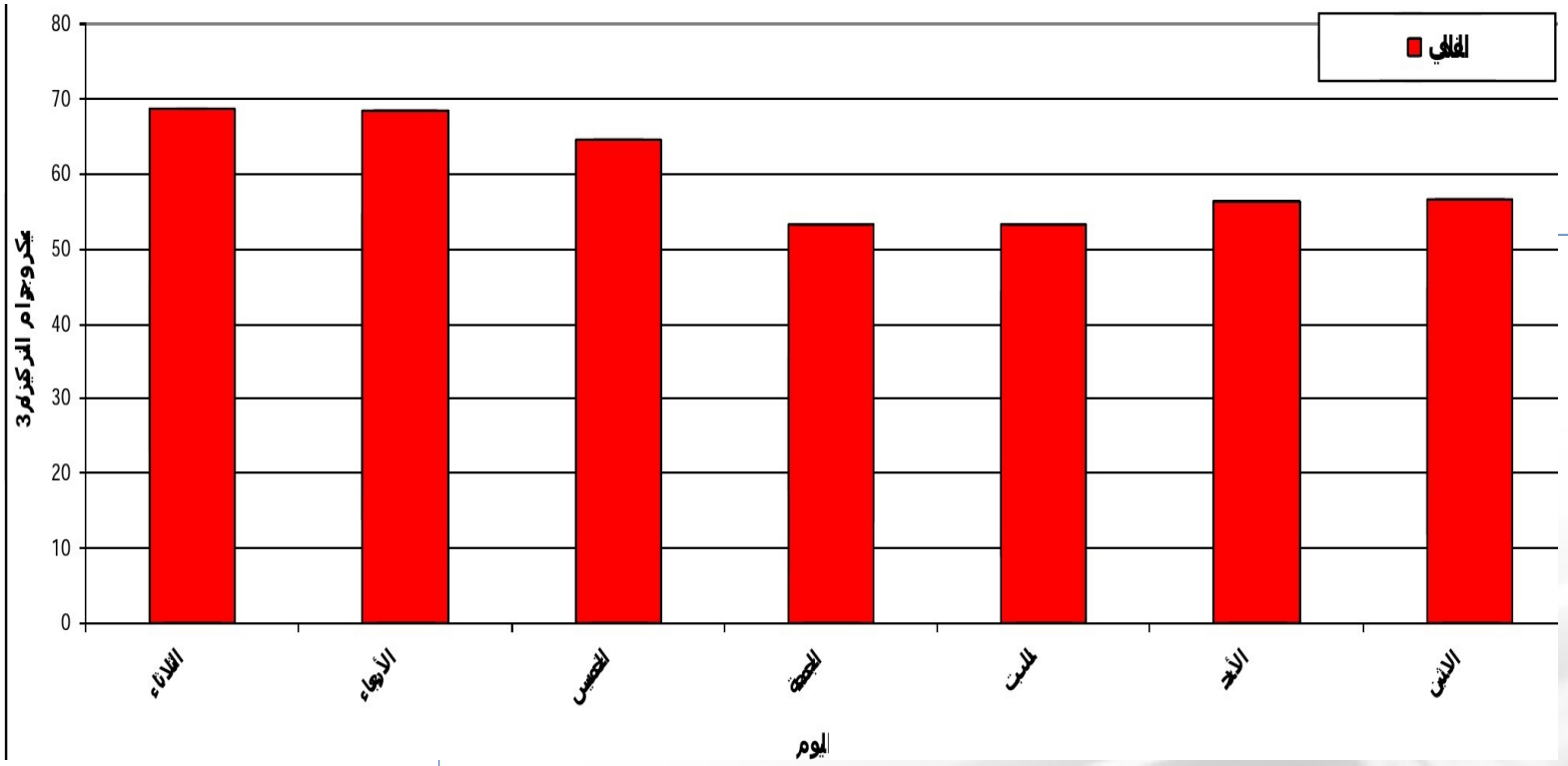
- Annual averageconcentration during 2013 recorded17µg/m3less than the average permissible concentrations(60µg/m3).
- Comparingannual average of 2013 with2012, adecreaseof about46% was recorded during 2013, in addition toa decreaseof about62% in comparison with1999(baseline year) 45µg/m3 .

Annual average NO2 concentration at industrial areas



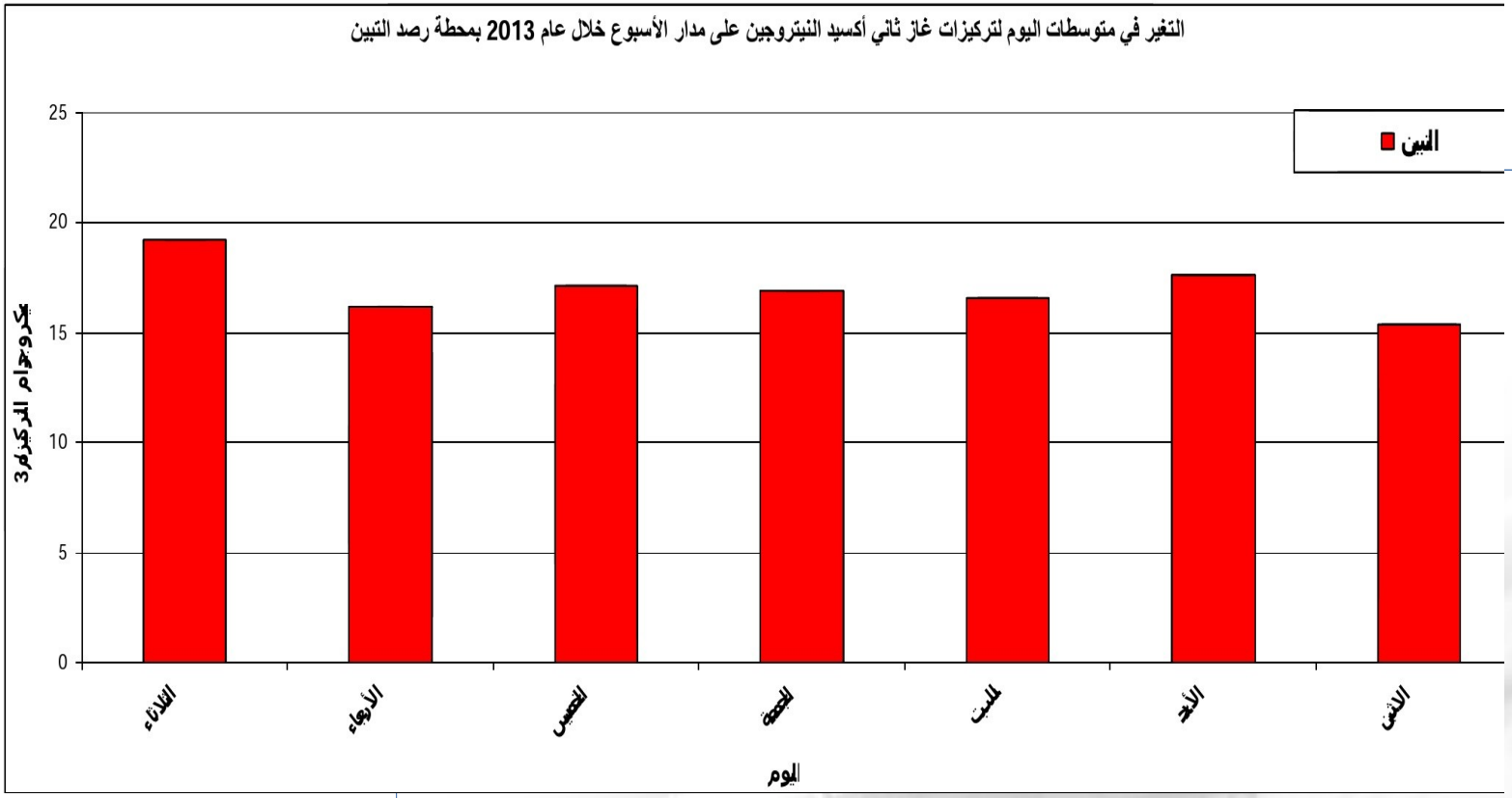
- Annual averageconcentration during 2013 recorded21µg/m3 less thanthe average permissible concentrations(80µg/m3).
- Comparing annualaverage of 2013 with2012, adecreaseof about28%was recorded during 2013, in addition toa decreaseof about32%in comparison with2011(31µg/m3)

Daily averageNO2concentrationduring a weekat urban areas

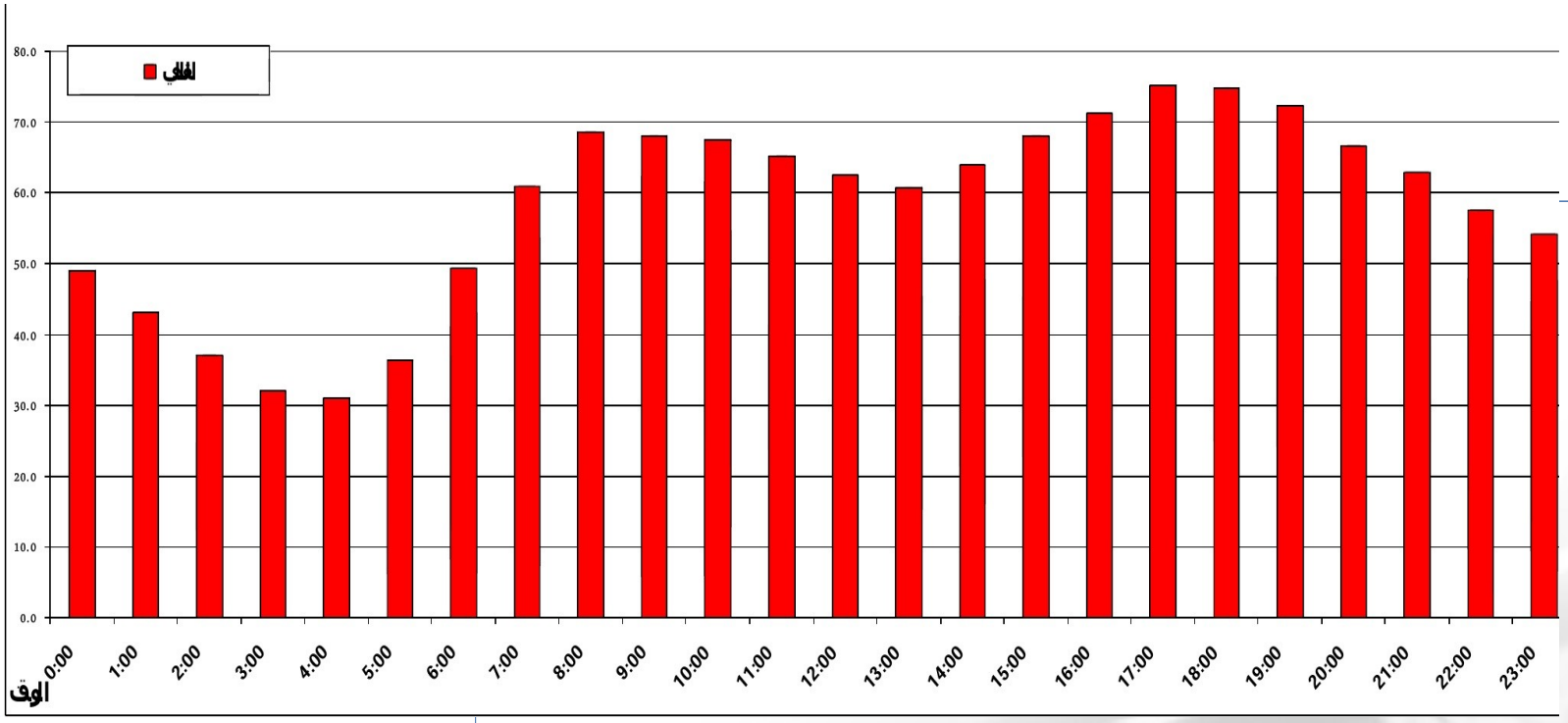


Thehighest decreaserecorded atweek endhowever, thehighest increaserecorded during the 5 working days.

Daily averageNO2concentrationduring a weekat industrial areas

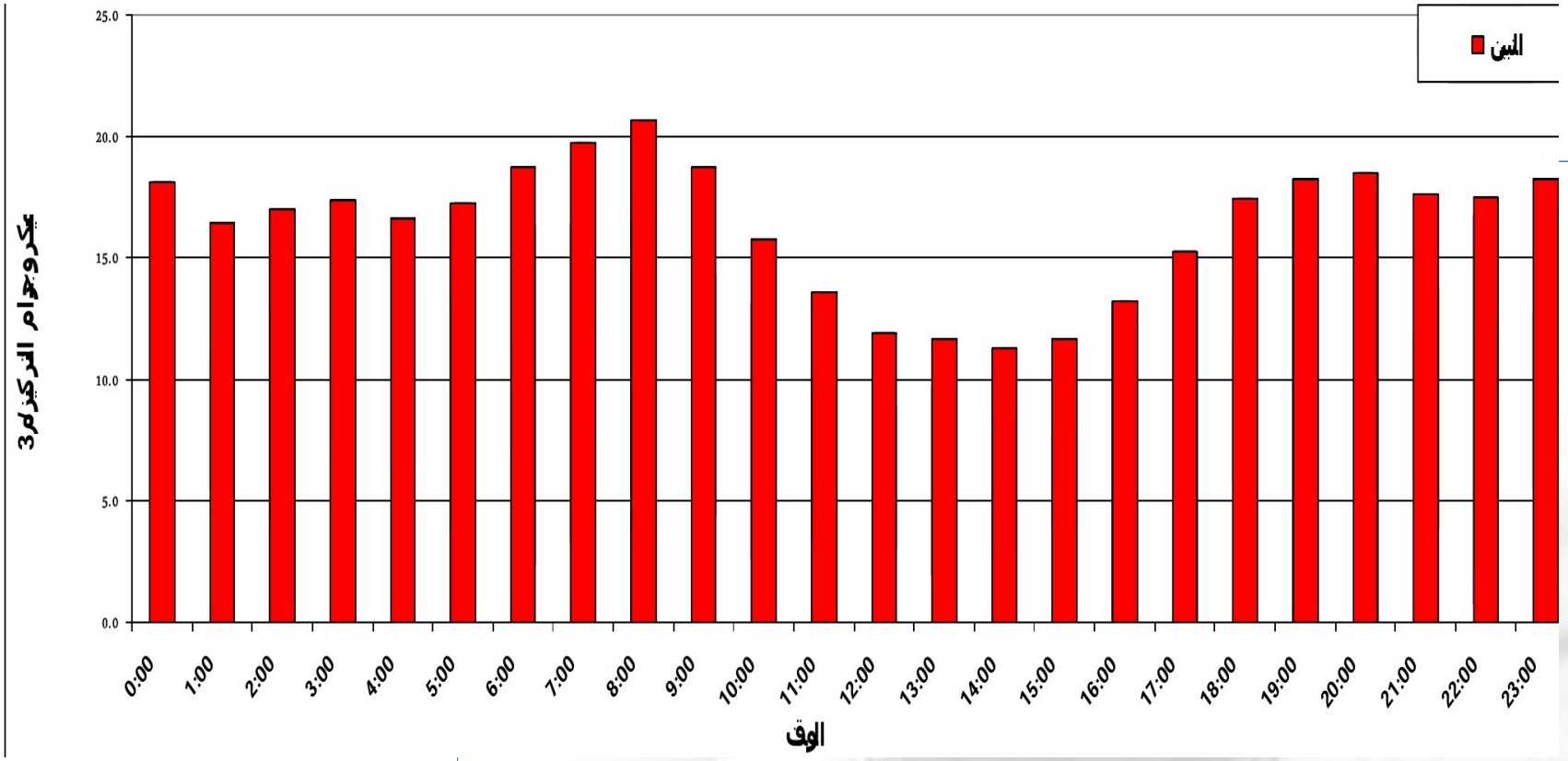


Average concentrations ofNO2during a dayat urban monitoring station.



The highest concentrationrecorded at time of increased the numberstraffic vehiclesduring a day.

Average concentrations of NO2 during a day at Tibin industrial monitoring station (have many industrial pollution sources)

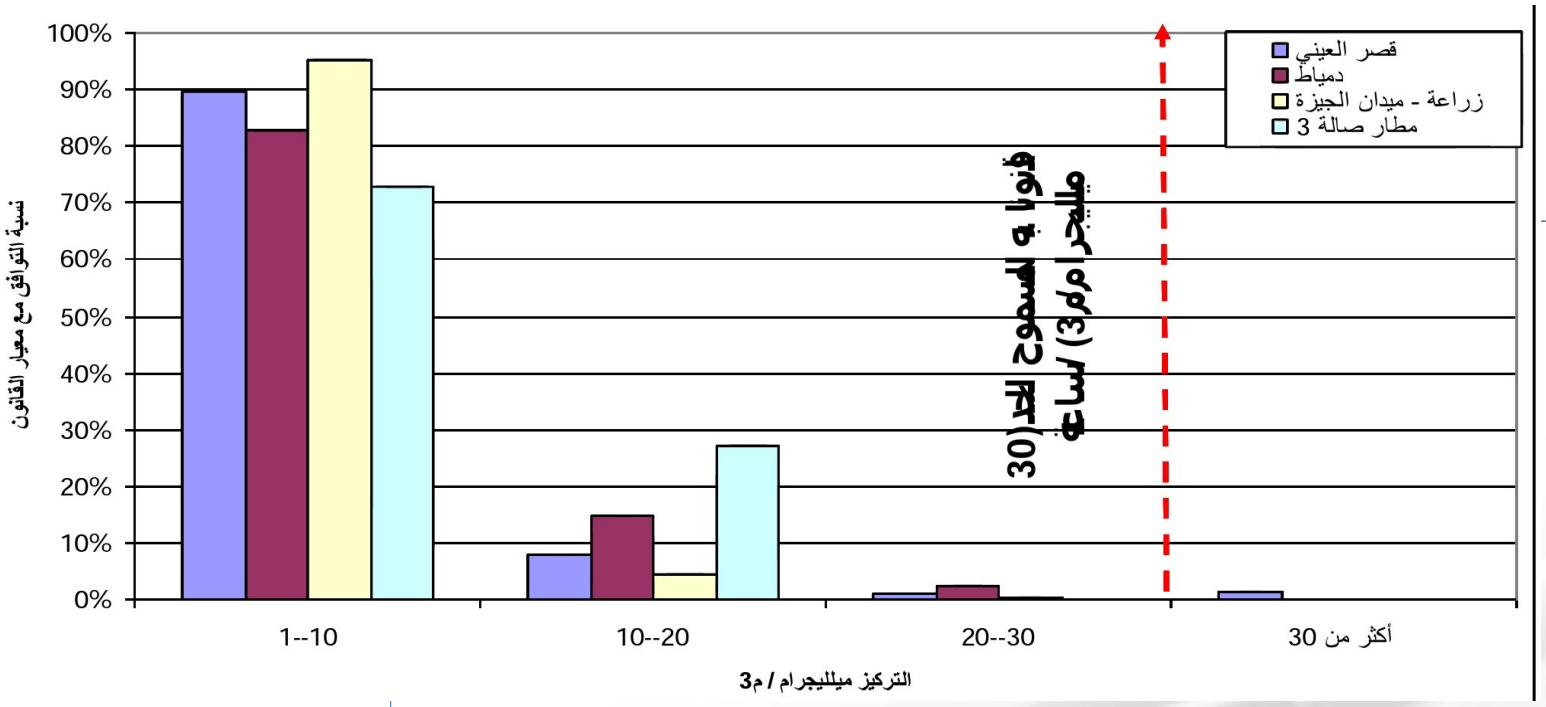


The highest concentration occurred at evening and early morning hours.

Carbon Monoxide "CO"

Themaximum allowableexposure to Carbon Monoxide gas forone houris30 mg /m3, and for8 hours 10 mg / m3, according to the Executive Regulations of the Environment Law.

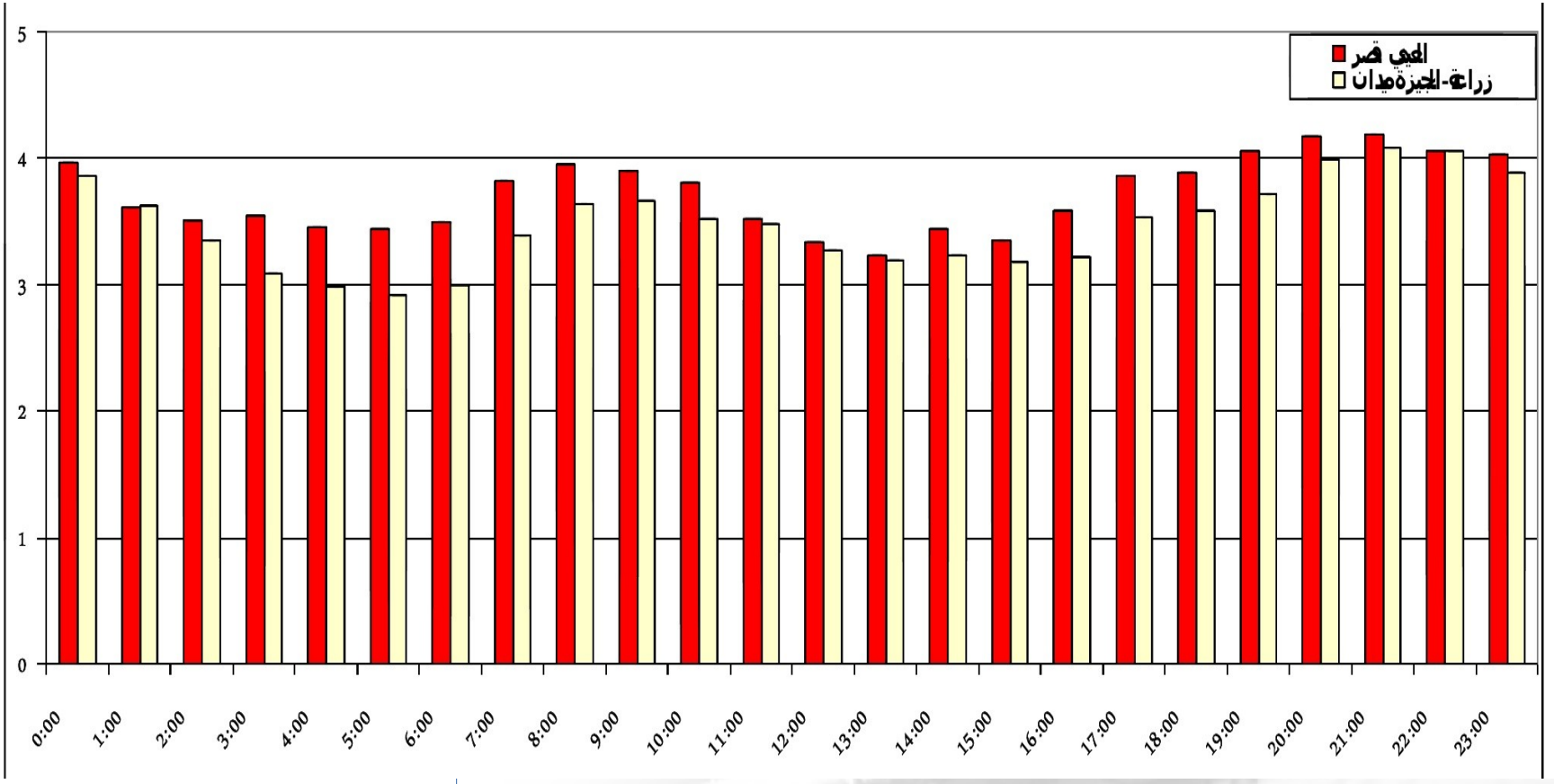
Averages concentrations of Carbon Monoxide gas per one hour:



Averagesconcentrationsper hourduring 2013 werewithinthe permitted limit(30mg/m3/hr).

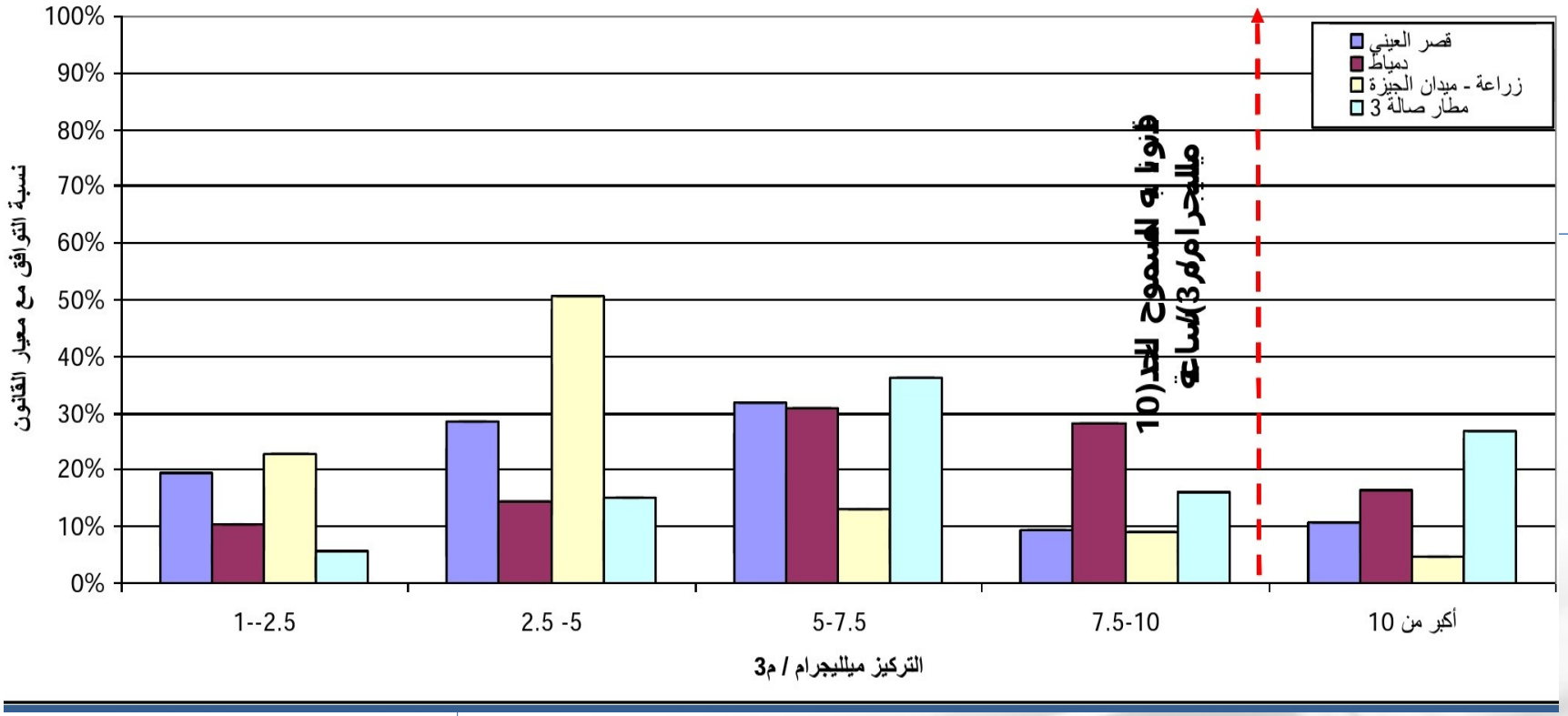
Bycomparing2013 averages with those of2012stabilityof the compatibility ratio will be noticed during 2013 with the maximum limits by 99 % on average hour.

Hourly average frequency distribution of Carbon Monoxide concentrations during 2013.



Thehighest concentrationforone hourwas recorded at time of activity andhigh trafficat Giza and Kasr Alainy monitoring stations.

Averages concentrations of Carbon Monoxide gas per 8 hour:



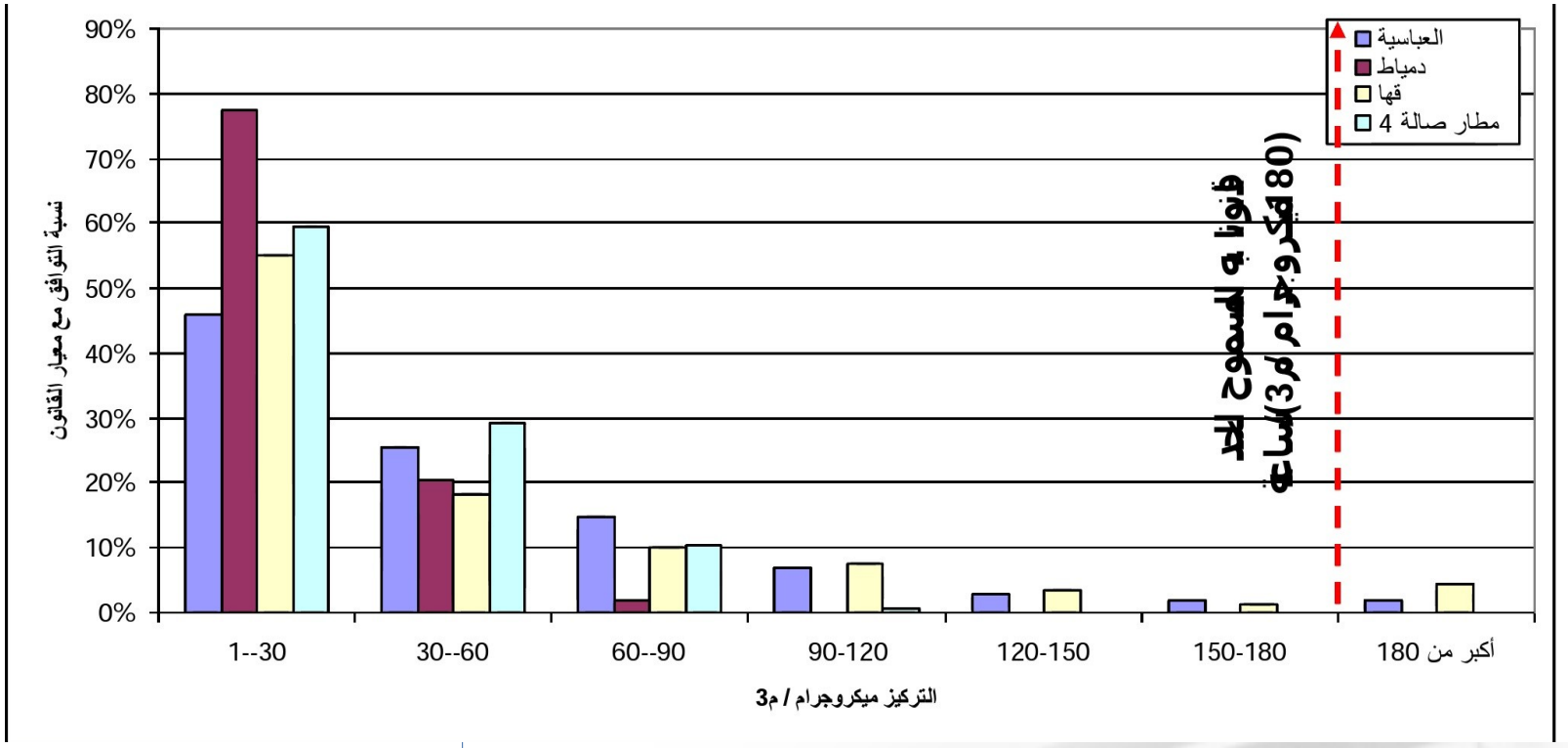
-Averagesconcentrations per8 hourduring 2013 werecompatiblewith the maximum permitted limit(10mg/m3/hr) by 85%.

- This could be attributed to increased traffic close to assessed stations with incompatibility of the vehicles with international standards.

Ozone "O3"

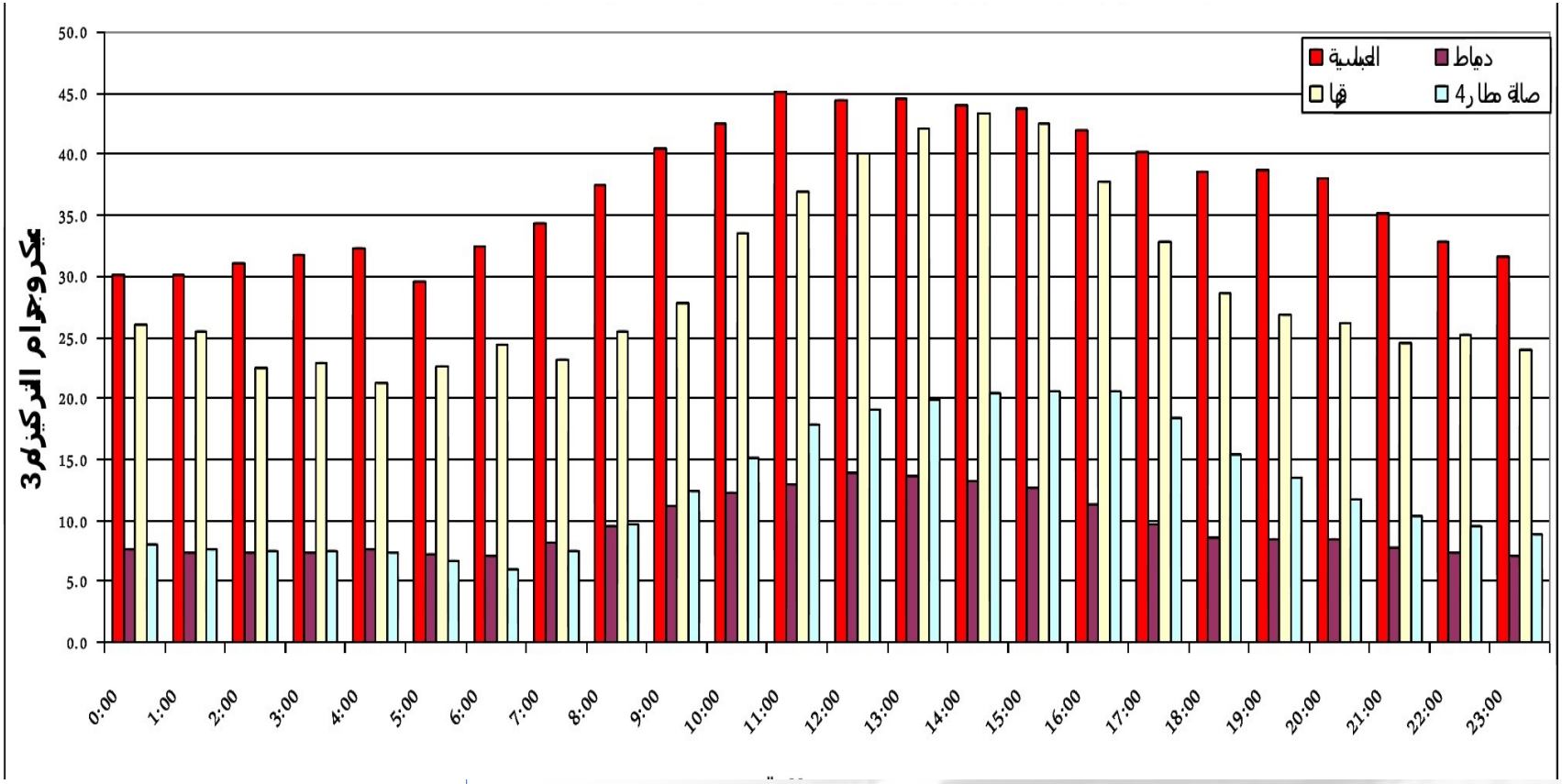
Executive Regulation of Environment Law stipulates that itsmaximum concentrationmust not exceed180 µg/m3 in one hour, while its limit during8 hoursmust not exceed120 µg/m3.

Averages concentrations of Ozone per one hour:



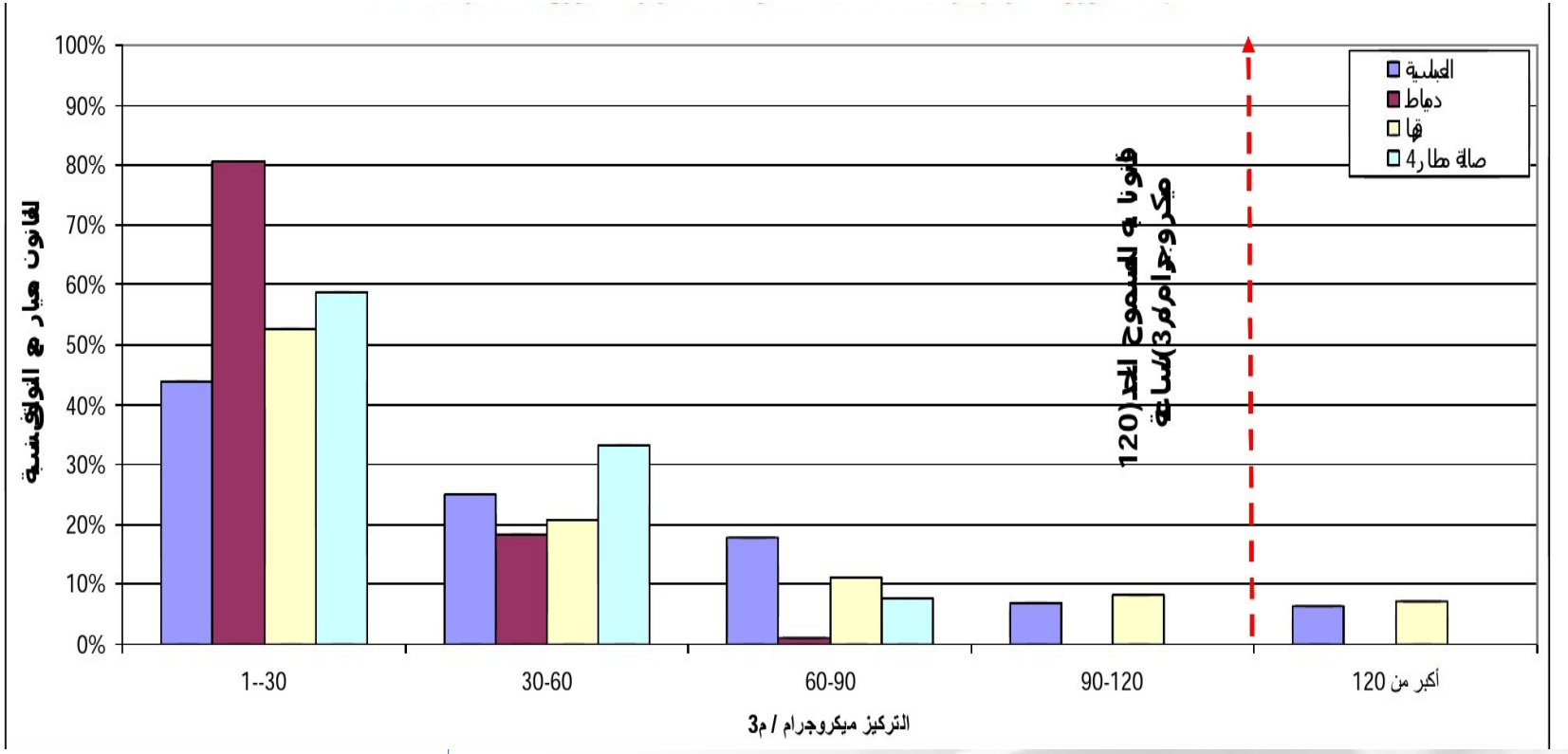
- The results of Ozonehourly averageconcentrations during 2013 werewithinthe maximum limits permitted by law andcompatibleby98%.
- Bycomparinghourly average concentrations to2012thecompatibility ratio recorded100%.

hourlyaverage relative frequency distribution of ozone concentrations



The results indicates thehighest concentrationsstartedafter sunshineand reached themaximum after noontime (sunshine time and increased temperature)

Averages concentrations of Ozone per 8 hour:

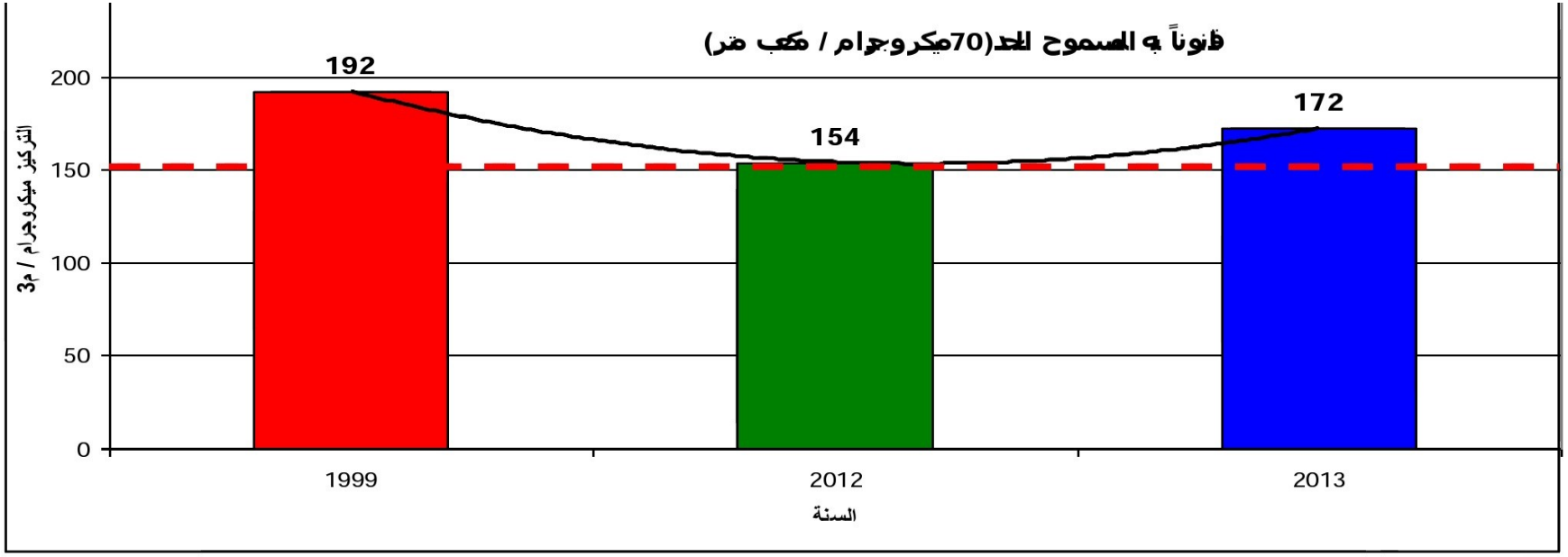


The results ofOzone 8 hour averageconcentrations during 2013 werewithin themaximum limits permitted by law(120 mg/m3/8hr) and compatible by 97%.

Inhaled Particulates Matters:

- Therates of Inhaledparticulate increased in Egypt because of the variety of pollutionsources,geographical natureand itslocationin the area of North Africa's desert belt characterized byscarcity of rainfall.
- Inhaled particulates considered one ofthe main indicatorsof increasedpollutionlevels in Egypt, especially in Greater Cairo and neighboring areas.
- During recent years theMinistry of State for EnvironmentalAffairs pays great interest toupdate monitoringdevices of all types of Inhaled Particulates Matters, particularly those less than 10µin diameter (PM10), and less than 2.5µ(PM2.5) which have negative impacts on human health.
- Annex No. 5 of the Executive Regulation of Law No. 4 / 1994 amended by Law 9 / 2009 stipulated that the allowed annual average for(PM10) is (70 µg/m3), (PM2.5) is (50 µg/m3)

Inhalable Particulates Matter PM10 at Urban and Residential areas



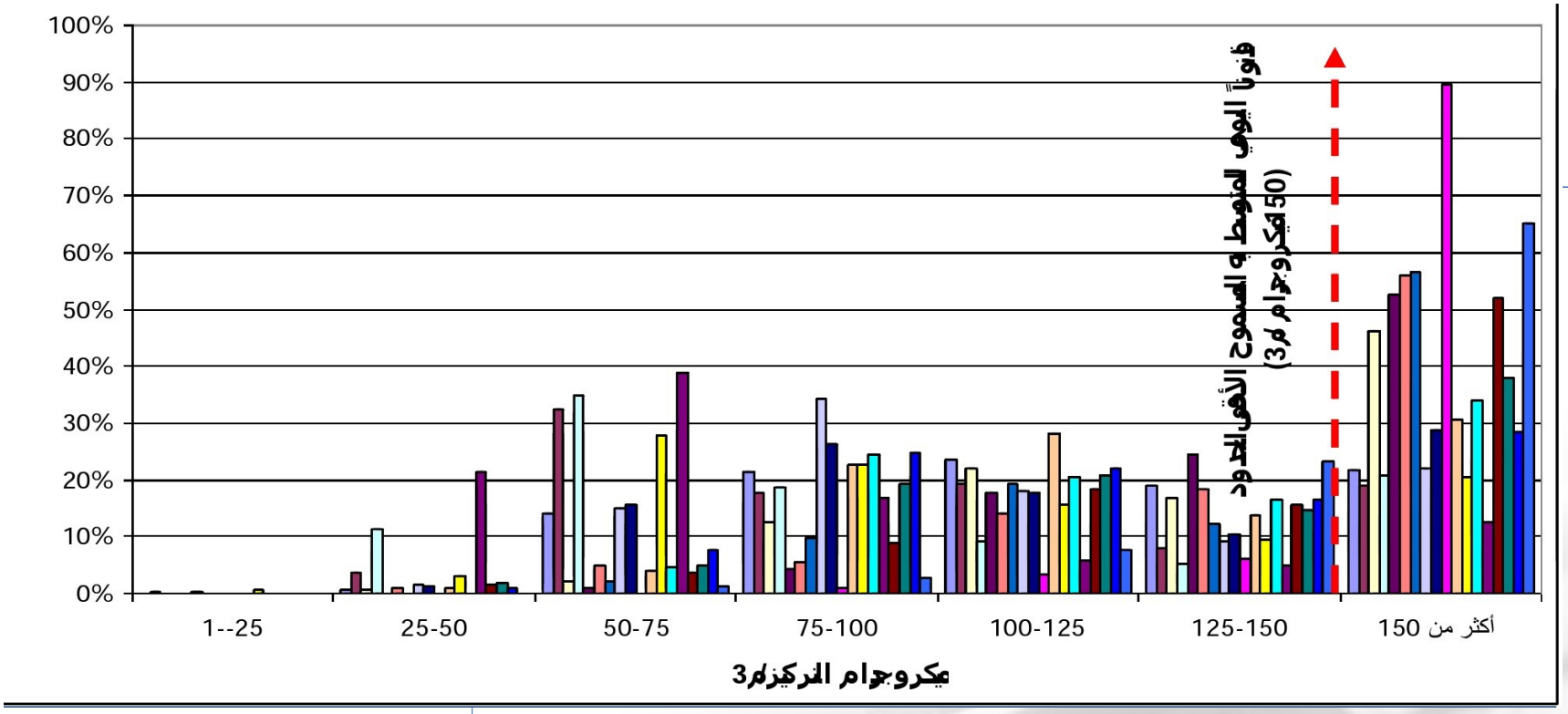
Obviousincreaseinannual averageduring 2013(172µg/m3) exceeding stipulated limits of EnvironmentLaw(70µg/m3) with about 146% ; thisindicatesincreasing sources of pollution as a result of burning municipal wastes and increasing rates of vehicles' emissions during 2013 .

Significantimprovementin the annual average concentrations during 2013 is noticeable .It recorded 172µg/m3compared to 192µg/m3 during 1999with improvement percentage of 11%

Inhalable Particulates MatterPM10atindustrial areas

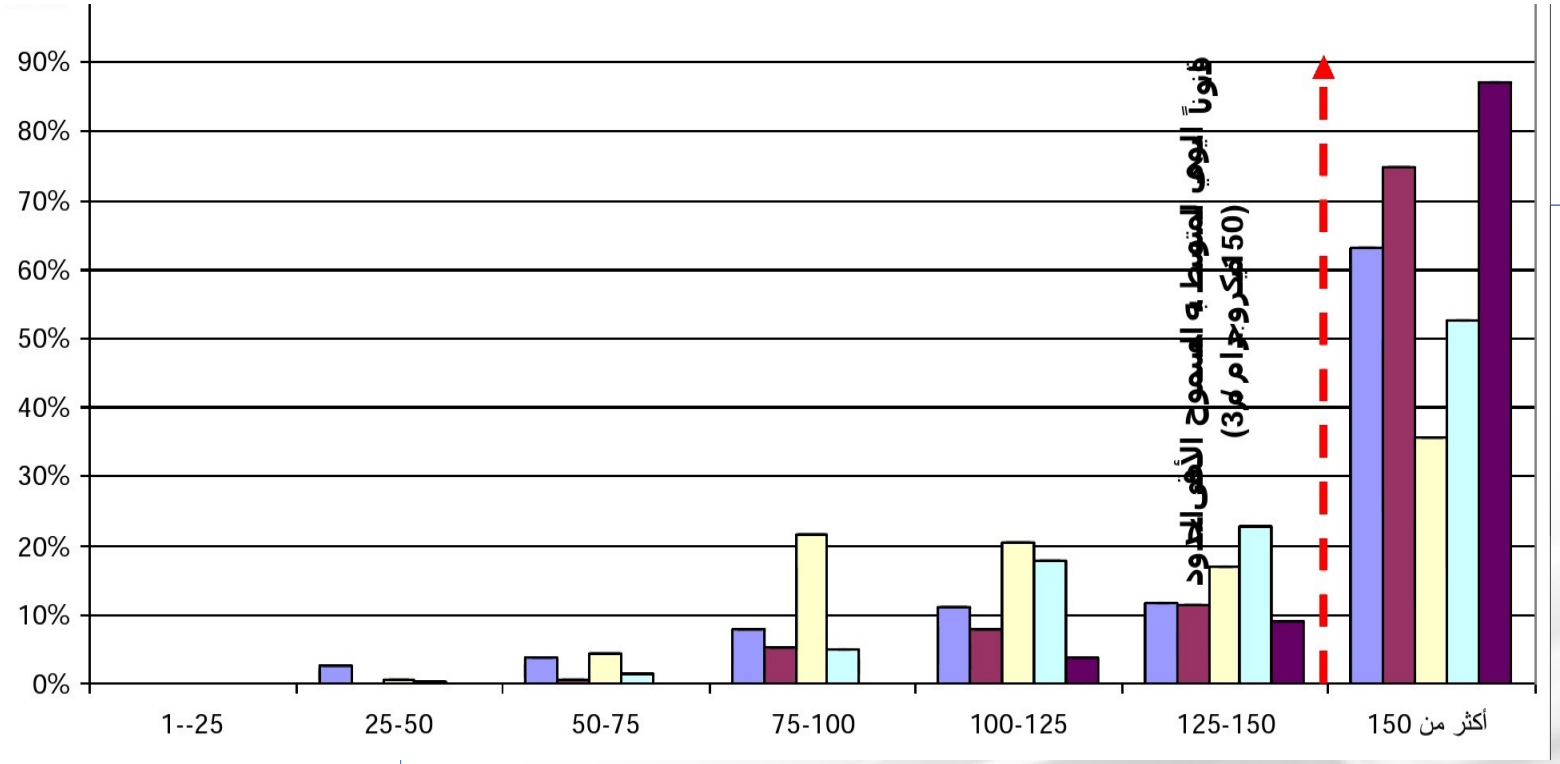
Significant increase in annual average during 2013 (206µg/m3)exceeding stipulated limits of Environment Law (70µg/m3) with about195%.

Average 24-hourconcentration at urban areas monitoring stations



Thecompatibilityrate reach39%of the permissible limit of daily averages(150µg/m3) stipulated in Annex No. 5 of the Executive Regulation of Law No.4 / 1994 amended by Law 9/2009

Average 24-hourconcentration at industrial areas monitoring stations



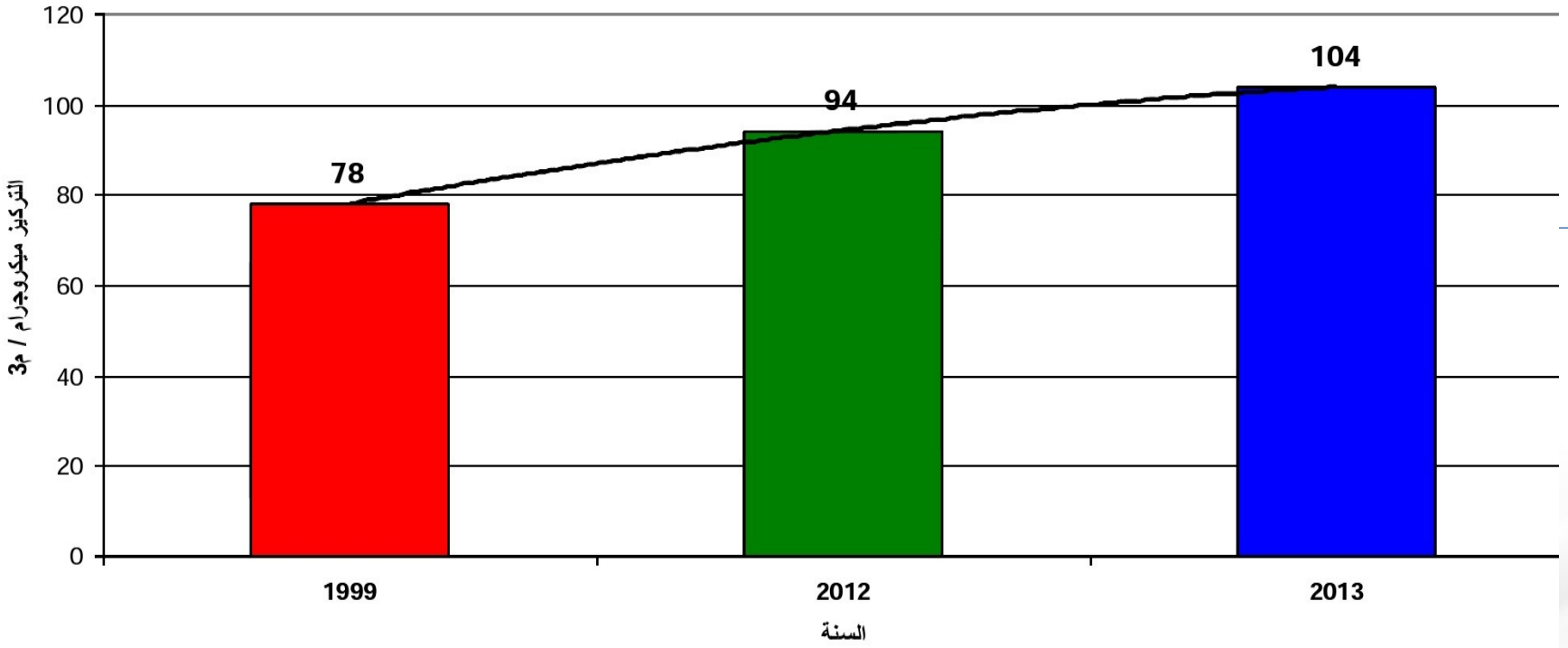
Thecompatibilityrate reach43%of the permissible limit of daily averages(150µg/m3) stipulated in Annex No. 5 of the Executive Regulation of Law No.4 / 1994 amended by Law 9/2009

Annual average concentrations of Particulate Matters during (2010-2013) compared to 1999 (baseline year) in Greater Cairo and Delta

Year Area	1999	2010	2011	2012	2013
Concentration microgram/m3					
Greater Cairo	234	126	135	157	172
Delta	150	138	140	161	167

- Significant decrease in the annual average of Greater Cairo from 1999 to 2013 with about 23%.
- Comparing the annual average of 2012 with 2013 noticeable increase in the annual average of 2013 with about 15% due to the lack of control over vehicles' exhausts and stopping inspection campaigns due to the security conditions experienced by the country.
- Obvious increase in the average concentrations of the Delta region from 1999 to 2013 by approximately 11%,.

Inhalable Particulates Matter PM2.5



- Comparingannual average of 2013(104 µg / m3) with the limits stipulated in the Executive Regulation of Law No.4 / 1994 amended by Law 9/2009(50 µg / m3) limitsobviously exceededcriteria by108%.
- Comparingannual average of2013(104 µg / m3) with the annual average of1999(baseline year)(78 µg / m3) relative increase with approximately 33% and 10% compared with 2012.

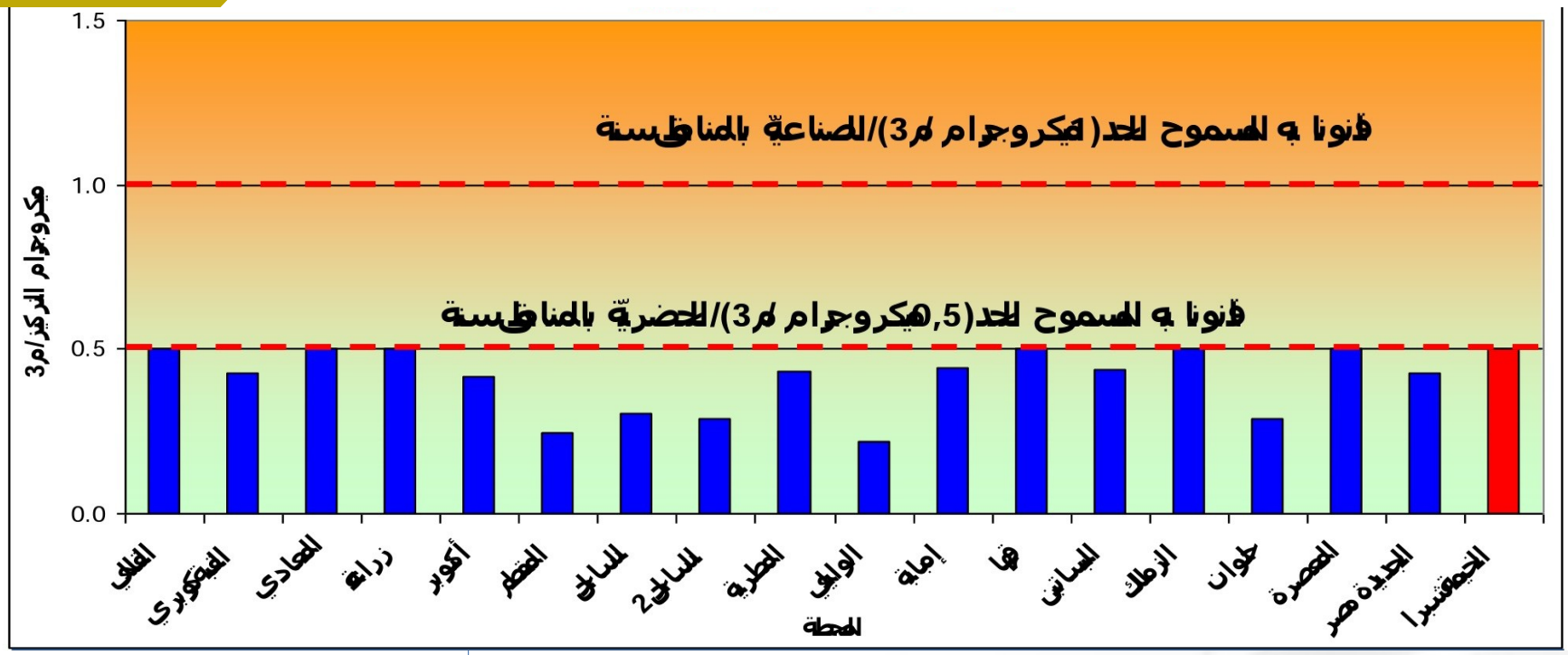
Lead

MSEA has focused on more updated methods of control to reduce proportion of lead in air:-

- Smelters are considered the first factor and the most important industrial source of lead emissions. MSEA adopted some policies, which resulted in:
  - Transferring smelters from populated areas.
  - Establish modern smelters to reduce lead emission.
  - Use lead-free gasoline in operating vehicles.

-All these efforts resulted in great reduction of lead concentrations due to those policies since 2000 and to date.

Lead



- During 2013,monitoringstarted with17monitoring stationat urbanarea in addition toone industrialmonitoring area (Shoubra Alkhaima).
- The averageannualconcentrations0.42 microgram/m3recordedfor urbanarea and0.5 microgram/m3forindustrial area.
- Thelimits are belowthe permissible limits stipulated in the Executive Regulation of Law No.4 / 1994 amended by Law 9/2009, Annex 5, 2012(0.50 µg / m3 for urban areas and 1 µg / m3 for industrial areas)

National Network for Monitoring Industrial Facilities Emissions

- Due to thesizeofmajor industries,multi sources of emissionsand their presence within residential areas particularly the old,Ministry of State for Environmental Affairsdevelopedsystemformonitoring industrial emissionsfrom stacks of industrial facilities
- The executive Environment Law No. 4/1994, amended by Law No. 9 /2009obligates"the companiesto establish continuous self- monitoring networksand provide EEAA their data".
- This network aimsto continuously and effectivelymonitorindustrial emissions to determine theirenvironmental compatibilityover 24 hours.

Current situation of the plants connected to the National Network for Monitoring Industrial Facilities Emissions

- First phase a Number of cement plants' stacks connected with the National Network increased as they reached to 110 sites for monitoring total Suspended Particulates' ~~emissions for 42 production lines in 20 Cement Companies.~~

- The first phase followed by establishment of connection for different companies dealing with fertilizer, and petrochemicals.

- The total connected companies became 30 with 127 self-monitoring sites.
- Monitored pollutants from cement companies increased to include all of the sulfur and nitrogen oxides through coordination with companies to conduct continuous self-monitoring for those pollutants.

Locations of monitoring Total Suspended Particulates emissions from stacks of companies connected with the National Network for Monitoring Industrial Facilities Emissions in Egypt

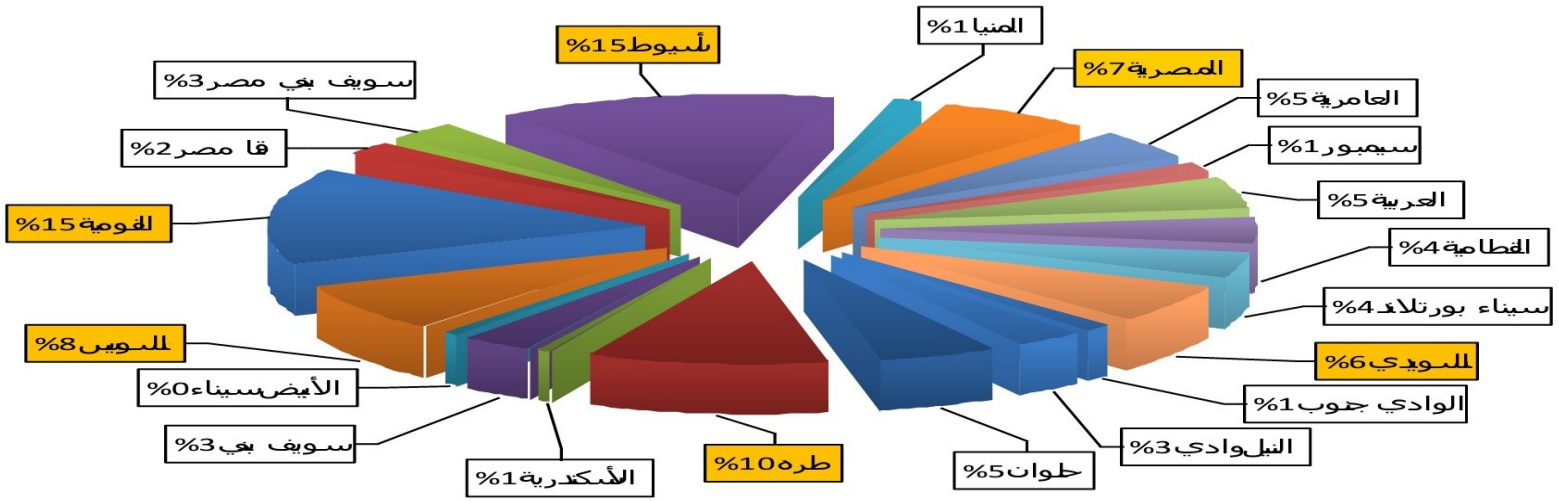
Serial	Company	No. Of sites	Pollutants indicator	Serial	Company	No. Of sites	Pollutants indicator
1	National cement company	11	PM	11	El Ameriya Cimpor cement company	1	PM
2	Portland Toura cement company	10	PM	12	Misr Quena cement company	2	PM
3	Helwan cement company	8	PM	13	Lafarge Cement company	5	PM NOx SO2
4	El katamyia cement company	3	PM	14	Sinai Portland cement company	4	PM
5	Suez cement company	4	SO2	15	Sinai White cement company	2	PM
6	Beni Suef cement company	8	PM	16	Misr Beni Suef cement company	9	PM
7	El-Ameriya cement company	4	PM	17	Alexandria Portland cement company	4	PM
8	Assuit cement company	11	PM	18	Arabia cement company	10	PM NOx SO2
9	El Menia cement company	2	PM	19	Elsewedy cement company	4	PM NOx SO2
10	Wadi ElNile cement company	4	PM	20	Ganoub Elwadi cement company	3	PM

Monitoring indicators at Cement Companies

Cementcompaniesconnected to the National Network for Monitoring Industrial Emissions areclassifiedaccording todatesof theirestablishmentand issuance of theEnvironment Lawas follows:

- **Old factories:**(Established prior issuance of the Executive Regulation of Environment Law -before 1995), the maximum allowed limits for these factories for PM is300 mg /m3.
- **New factories:**(Established after the issuance of the Executive Regulation of Environment Law –(after 1995-2005) the maximum allowed PM limits for these factories200 mg/m3..
- **Modern factories:**(Established after the amendment of the Executive Regulation of Environment Law – (after 2005-2011) the maximum allowed PM limits for these factories100 mg/m3.

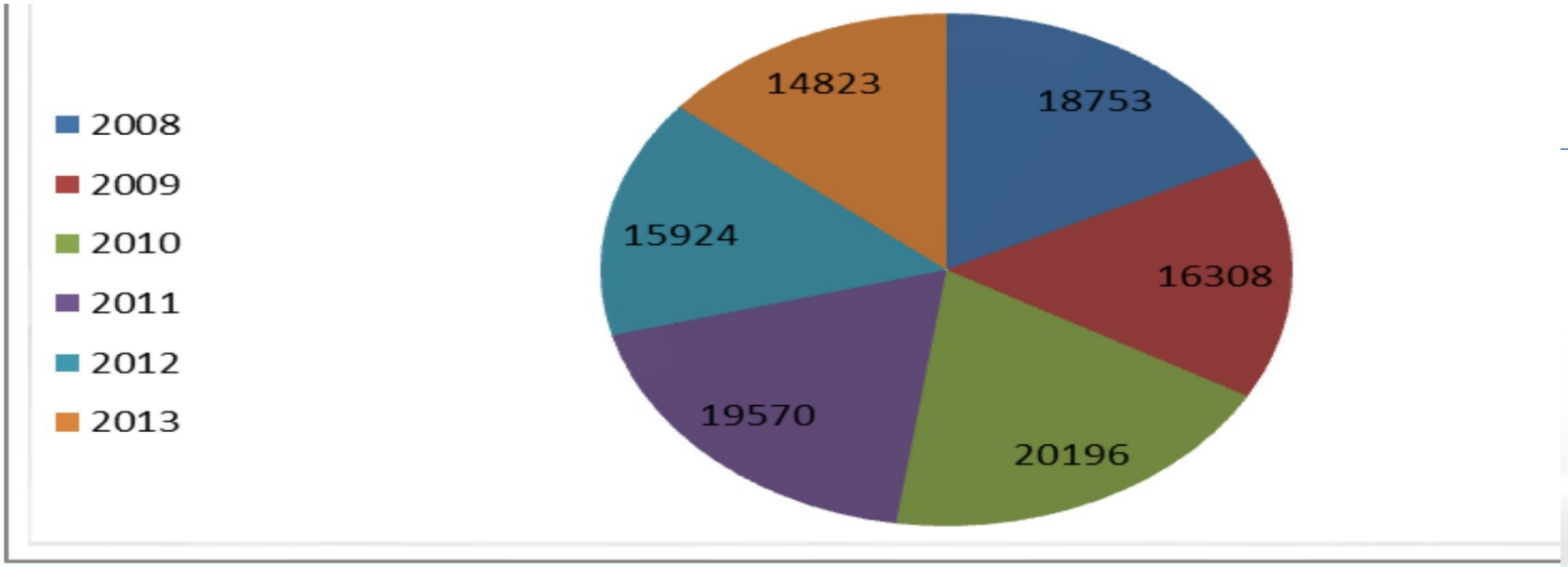
Environmental load of the total suspended particulates emissions emitted by cement stacks.



- Calculation pollution loadsof the totalsuspended particulatesemissions from factories connected with the National network for monitoring industrial emissions results clarified that:

-Five companiesare responsible about60%of the total environmental load of emissions (Torah – National Company - Suez - Assiut - Egypt).

Loads of Total Suspended Particulates at cement factories emissions



Loads of Total Suspended Particulates' emissions recordedobvious decrease during 2013compared to previous years.which isconsidered positive signdespite the increased number of self-monitoring and connected stacks to the National Network for Monitoring Industrial Emissions

Emissions from rice straw burning

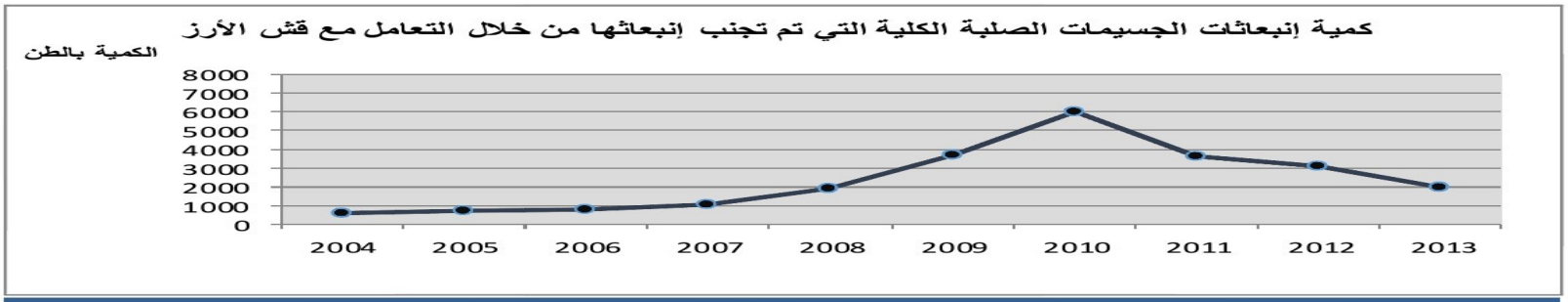
- MSEA's at 2008-2009conducted practical and scientific experiments todevelop the first Egyptian emission factor for rice strawin case of burning with conventional methods.

- This factor used in evaluating volume of emitted emissions.

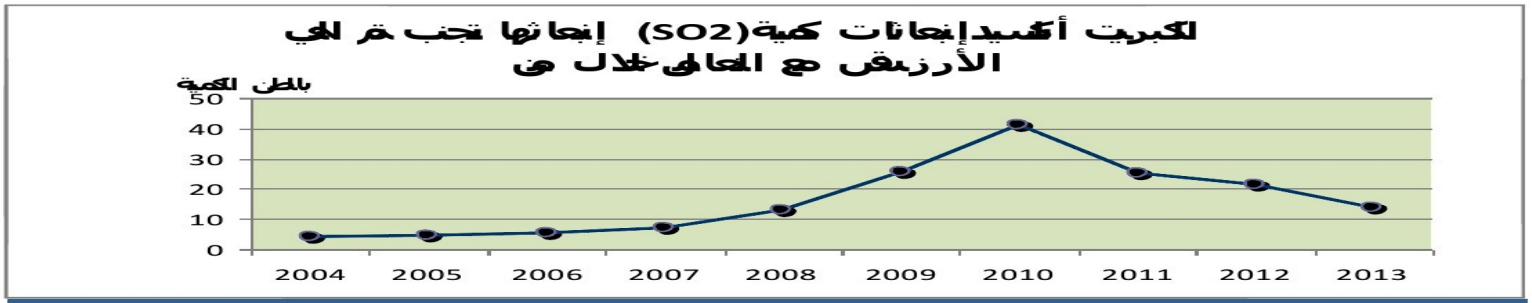
Emission factor of pollutants emitted during burning rice straw by conventional methods	
Pollutant	**Emission factor (Kg/ton)**
Total Suspended Particulates	10
Sulfur Dioxides	0.0685
Nitrogen Oxides	0.409

Total pollutants loads reduced as a result of rice straw treatment				
Year	Amount of treated rice straw (Ton)	Amount of decreased environmental load		
		Total Suspended Particulates T.S.P (Ton)	Sulfur Oxides SO_2 (Ton)	Nitrogen Oxides NO_2 (Ton)
2004	61500	615	4	25
2005	70500	705	5	29
2006	78500	785	5	32
2007	106000	1060	7	43
2008	191000	1910	13	78
2009	374000	3740	26	153
2010	600000	6000	41	245
2011	365274	3656	25	149
2012	312000	3120	21	128
2013	200000	2000	14	82

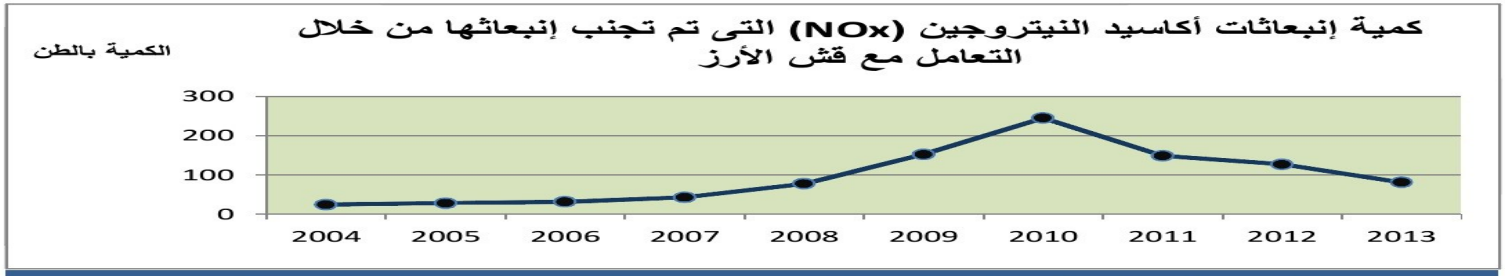
Amount in the environmental load of air pollutants resulting from open burning of rice straw



شكل (١-٤٣) كمية إنخفاض الحمل البيئى للجسيمات العالقة (TSP) بالطن نتيجة التعامل مع قش الأرز



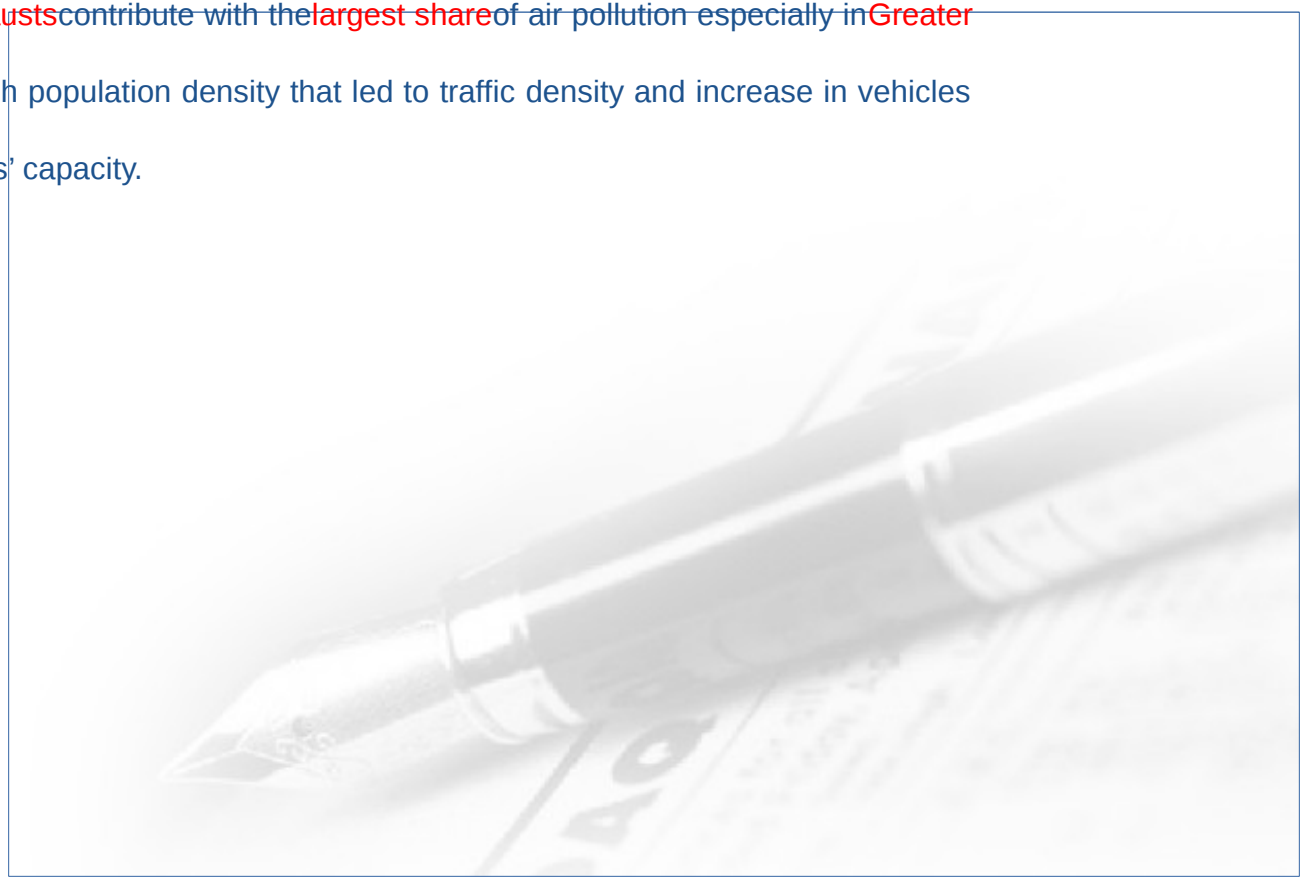
شكل (١-٤٤) كمية إنخفاض الحمل البيئى لثانى أكسيد الكبريت (SO2) بالطن نتيجة التعامل مع قش الأرز



شكل (١-٤٥) كمية إنخفاض الحمل البيئى لأكاسيد النيتروجين (NOx) بالطن نتيجة التعامل مع قش الأرز

Emissions from vehicles exhausts

- Vehicles' exhaustsrepresentone of the main sourcesthat directly affect ecosystem in general and air quality in particular.
- In Egypttransportation exhaustscontribute with thelargest shareof air pollution especially inGreater Cairowhich suffers from high population density that led to traffic density and increase in vehicles numbers compared to roads' capacity.



Implemented programs to reduce pollution from vehicles' exhausts:

- **Sustainable Transport Project**.
  - This project targets toimplement set of componentsto upgrade transportation system, improve air quality in Egypt
  - Establish modern buseslines work by natural gas and operated by private sector to connect new cities
  - Establish routes for pedestrians and bicyclesinFayoumandShebinElKomcities to encourage this environmentally friendly mean
  - Re-organize parking servicesdowntown Cairo and connect garages with advanced management and operation network .
- **Replacing old taxies project in Greater Cairo**
- **Inspection program of vehicle exhausts in traffic units**
- **Inspection program of vehicle exhausts in roads**
- **Inspection program of Public Transportation Authority Buses**
- **Program to use natural gas as fuel in transportation means Authority buses**
- **Smart card project and increase number of natural gas Supply stations**

Heath impacts of air pollution in Egypt

According to a study recently conducted by the Egyptian Environment Affairs Agency (EEAA),

Air pollution is responsible for the following:-

- An average of3400 deathseach year at Cairo
- About15000cases ofbronchitis
- About329,000cases ofrespiratoryinfection
- Large numberof cases ofasthmaeach year.

These figures are published in areview articleprepared byUNtitled (Air quality and Atmospheric pollution in the Arab region).

Economic impacts of air pollution in Egypt

- A recent study commissioned by the Egyptian Environment Affairs Agency (EEAA) calculated that the lower limit of the cost of air pollution in Cairo is in the range of US$ 1-2 billion per year or 3-6% of gross domestic product (GDP).
- Egyptian government has recognized that compressed natural gas will provide environmental, social and economic benefits.
- Improved air quality translates into a reduction of pollution-related health problems.
- Economic benefits are that at 0.45 pounds/cubic meter

A Practical Approach for Assessment of Environmental Pollutants

Environmental Pollutants

- The assessment of environmental pollutants in developing countries is one of serious challenges:Why?
  - Insufficient funds for assessment and research
  - Lack of advanced analytical equipment's
- Therefore,We developed a practical approach and system for pollutants assessment in which the results indicateapplicabilityandreliability

Isolated Rat Hepatocytes

as

In vitro Toxicology Model

for

Environmental Pollutants Assessment

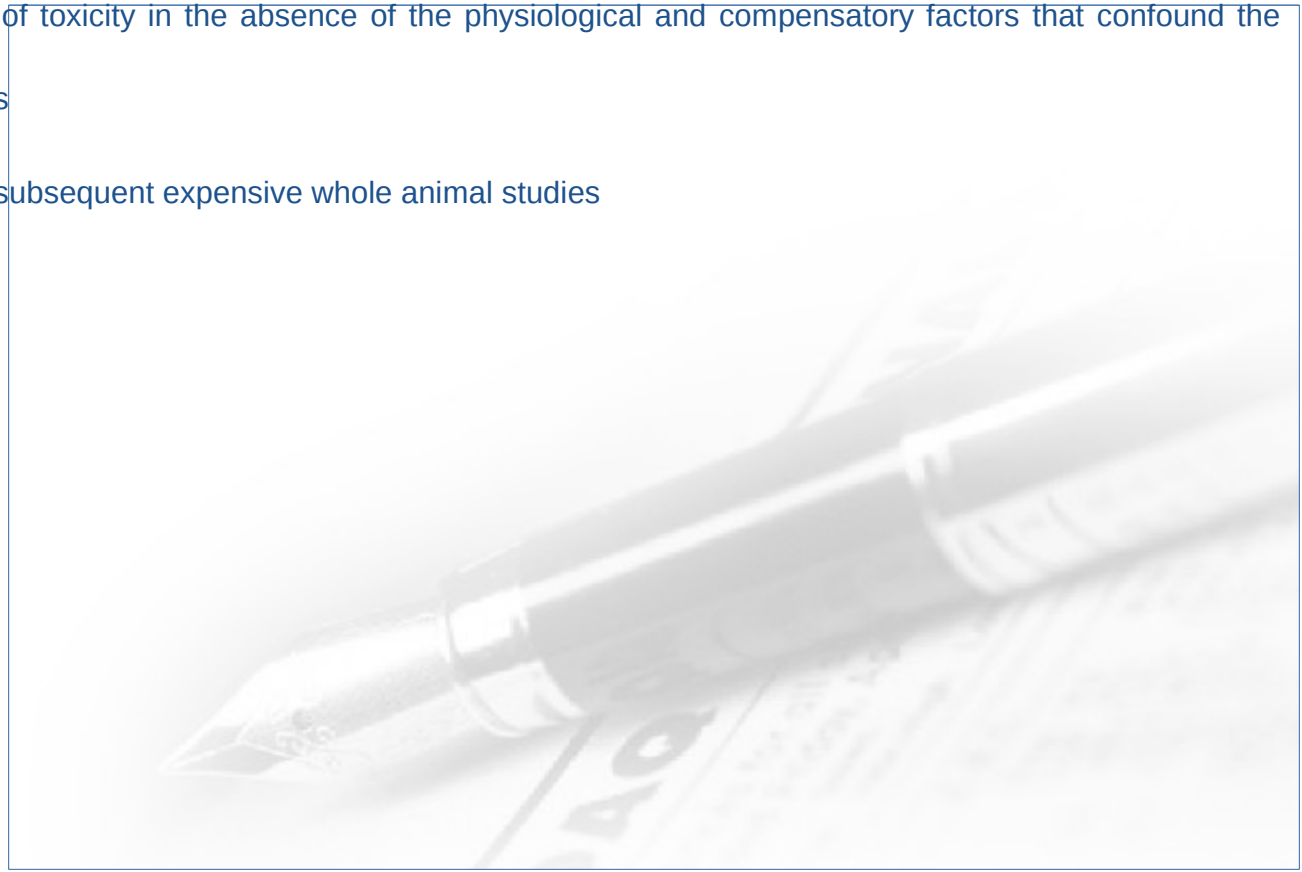
Outlines

- Background
- Research Techniques and Modifications
- Some Discoveries
- Future Prospective
- Take- Home Message

In Vitro Toxicity Testing

The development of ***in vitro* toxicity testing** is rapidly expanding in all developed countries; this is because:

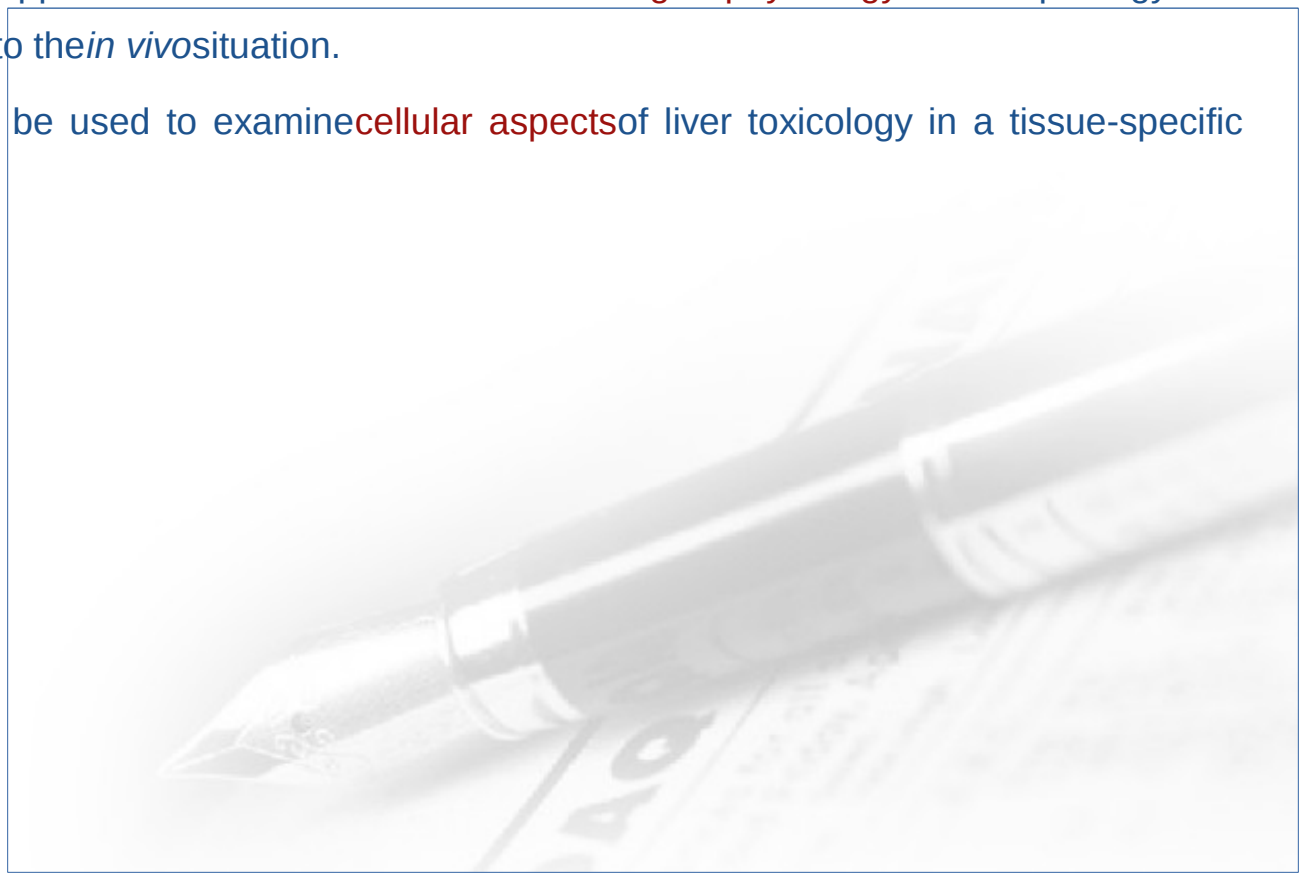
- The *in vitro* studies provide efficiency, rapidity and cost-effectiveness and allow greater control over the experiments.
- They mimic realistic conditions, thereby reducing the number of laboratory animals required for the testing.
- Identification of primary mechanisms of toxicity in the absence of the physiological and compensatory factors that confound the interpretation of whole animal studies
- Scope for improvements in design of subsequent expensive whole animal studies



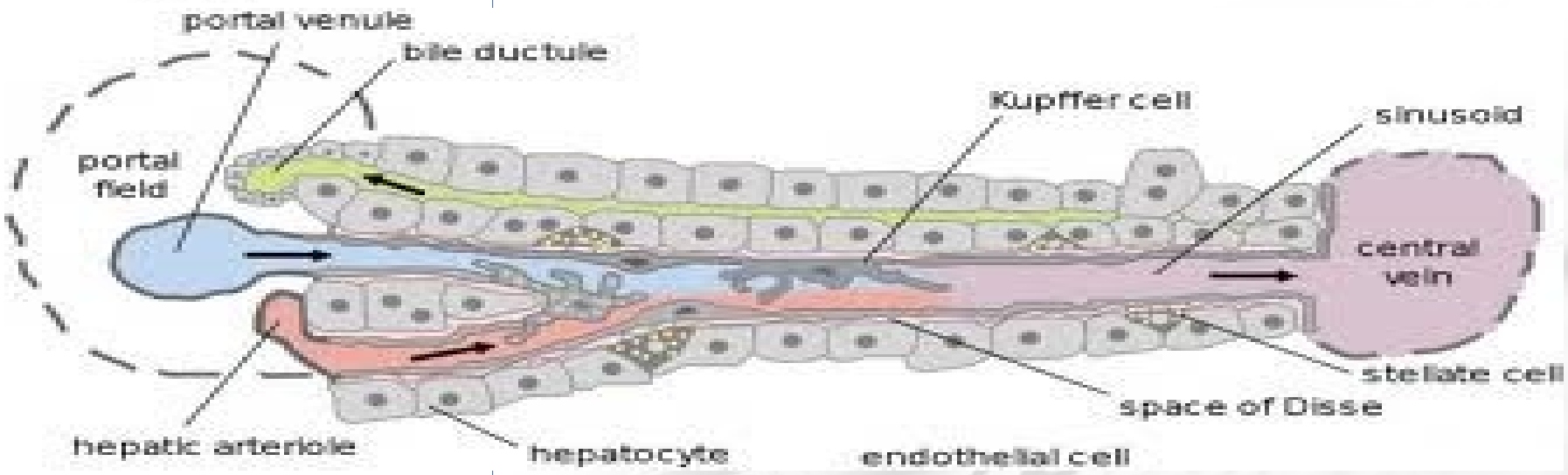
In Vitro Models of Hepatotoxicity

There are3 differentmajormodelsto study the hepatotoxic effects:

- **liver cell culture model:**is the most frequently used model that can be applied to examine effects of drugs/toxins onisolated hepatocytesat the cellular level.
- **Isolated organs:**display an approach towards the assessment oforgan physiologyand morphology and represent the closest model to the*in vivo*situation.
- Precision-cut**liver slices**can be used to examinecellular aspectsof liver toxicology in a tissue-specific background.



Why Isolated Hepatocytes?



Isolated Primary Hepatocytes

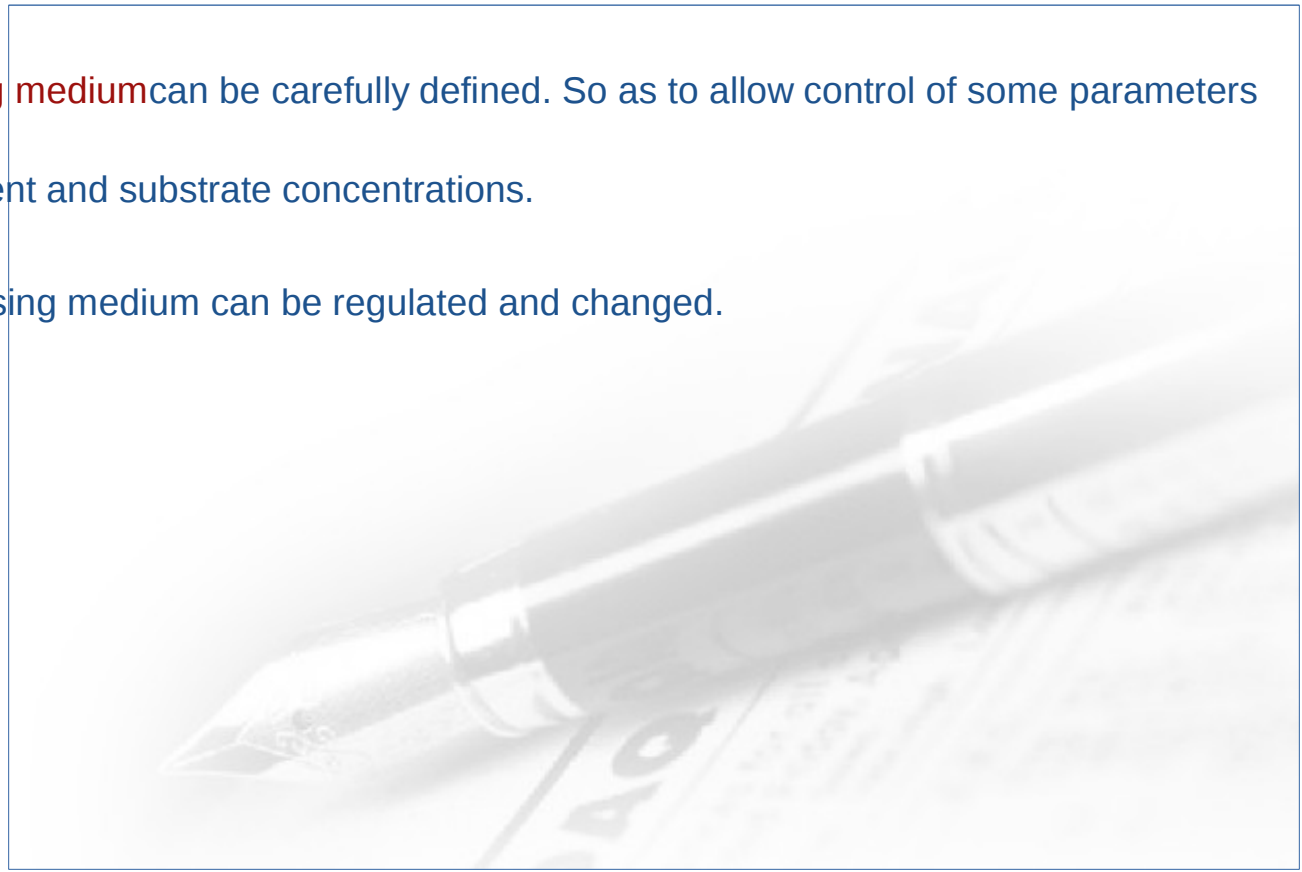
- Hepatocytes isolationand cultures have gained growing interest in toxicological and environmental researches.

- It is considered the system of choice for studying theenvironmental pollutantsbecause:

  - Most of these studies in laboratory animals entaillarge quantitiesof pollutants?
  - In addition,*in vivo*animal model do not clearly indicate themechanism of actionof the pollutants in liver.

Isolated hepatocytes offers the opportunity to study hepatic metabolisms in a system in which:

- Themetabolicand distributionalinfluencesof all extrahepatic tissues are removed.
- The cells remainmorphologically intactand normal uptake and metabolically functional.
- Oxygen, nutrients and substrates are delivered to the cells at normal required level.
- The composition of theperfusing mediumcan be carefully defined. So as to allow control of some parameters as pH, electrolyte content, nutrient and substrate concentrations.
- The rate of delivery of the perfusing medium can be regulated and changed.



- **Donor animals** may be subjected to pre-treatment. Thus, allowing metabolic studies in damaged as well as healthy tissue.
- Specific **metabolic inhibitors** can be added to the perfusate to permit the study of particular process.
- Isolated hepatocytes seem to retain many of the **essential properties** of the intact tissue including similar permeability characteristics. This has allowed studies on liver function, Cyt P450 linked **drug metabolism**, drug uptake, regulation of drug metabolism, and formation of drug metabolites.
- The isolated cell suspension permits precise **determination of the response** to the addition of substrates or hormones without the confusion of determining whether the parameter measured is a function of one or several of the distinct cell types present in the normal liver.

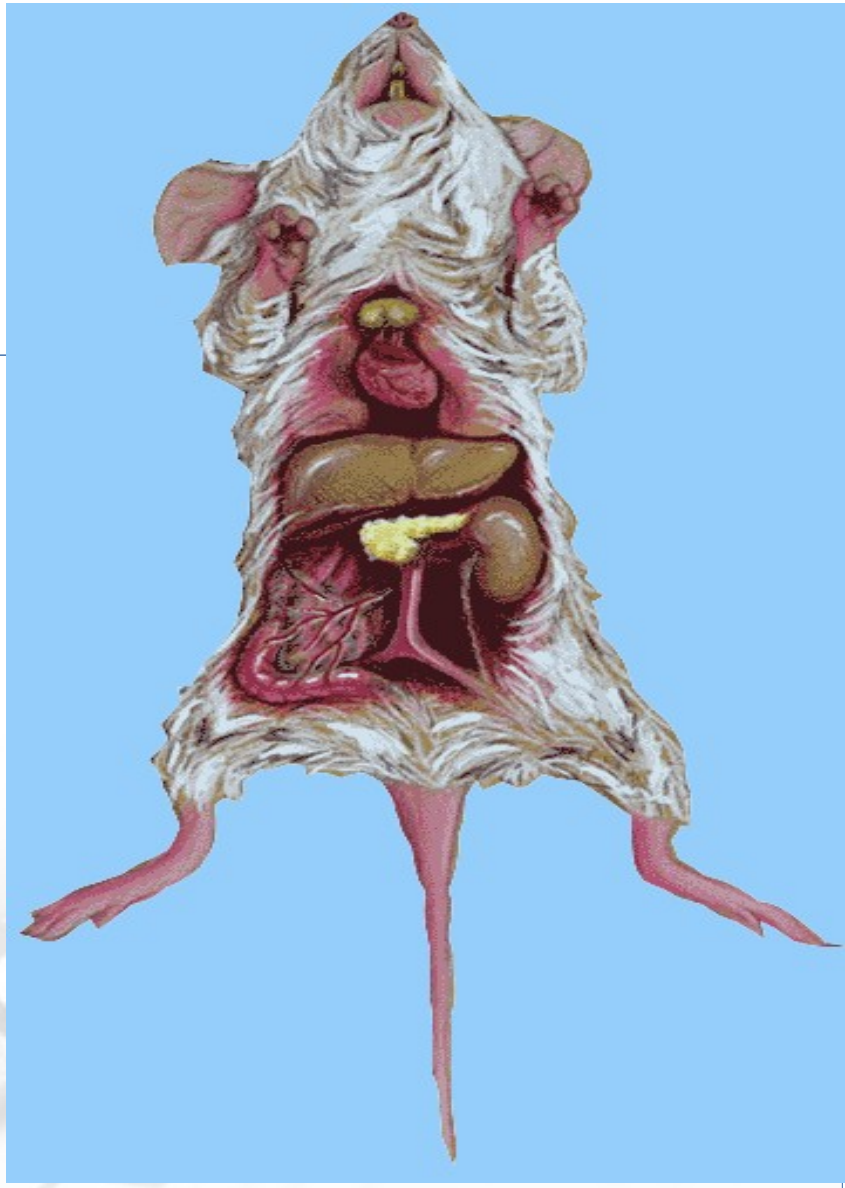
Basic Experiments Protocol

Primary Hepatocytes Isolation

- Theisolation basedon the two- stepscollagenase perfusiontechnique.
- First was developed by**Berry and Friend (1969)**then modified by many authors depending on the formulation of isolating buffers.
- Prior to the introduction of enzymatic digestion with collagenase, different non enzymaticmechanical separation methodswere used to obtain liver cells.
- Collagenaseis widely used for dissociating hepatocytes from the liver.
- Besides**collagenase**, otherdigestive enzymeswere used to acquire a single cell suspension include**trypsin**,**pronase**, and**lysozyme**, but these did not produce large numbers of viable hepatocytes.

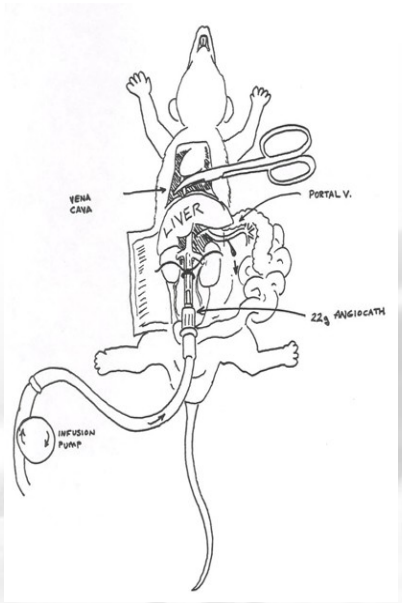
Operative Technique for Hepatocytes Isolation

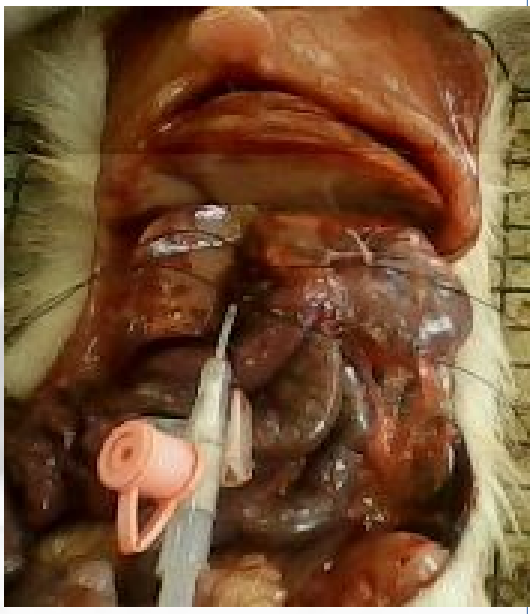
- The rat wasanaesthetizedby intraperitoneal (I/P) injection of ketamine HCl. When it showed unconsciousness, the rat was secured on a clean tray.
- Full anesthesiawas checked bypedal reflex test, pinching the tip of hind legs by fingers, and confirmed by absence of any reflexes.
- “U”-shaped incisionwas made through the skin from the lower abdomen to the lateral aspects of the thorax, after sterilization of the area with 70% ethanol.
- Theliverwasexposedby moving stomach, intestines and any adipose tissue aside, andportal veinwas raised by a hooked forceps.



Operative Technique

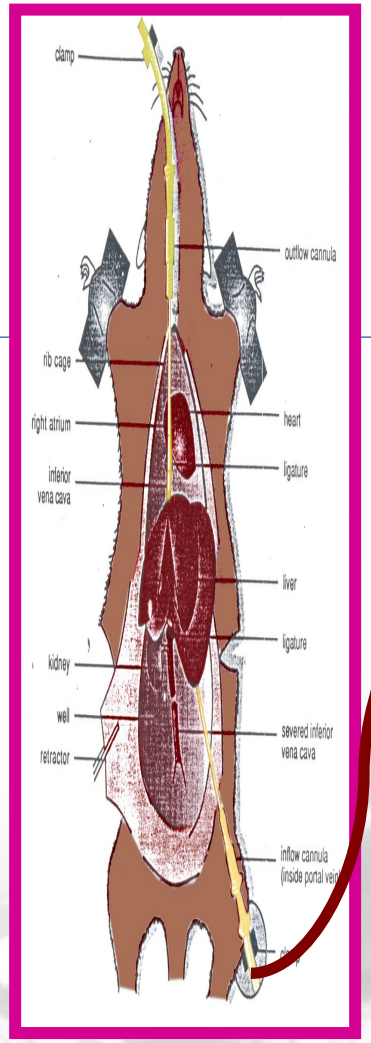
- After exposing the liver andraising the portal veinby a forceps.
- Immediately, acannulawas inserted into the portal vein and secured by two tied-loosely silk ligatures around the vein.
- The shape of the liver was resumed by continuous perfusion of thefirst buffer (Hanks' Ca^{+2}free)(Buffer A)for 8 minutes while the liver was*in situ*.
- Humane terminationof rat's life was achieved by crushing coronary blood supply with a blunt forceps.
- The liver wasloosely freedby dissection of stomach, spleen, and their adherent ligaments from the abdominal cavity.





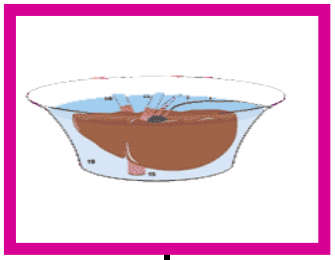
Operative Technique

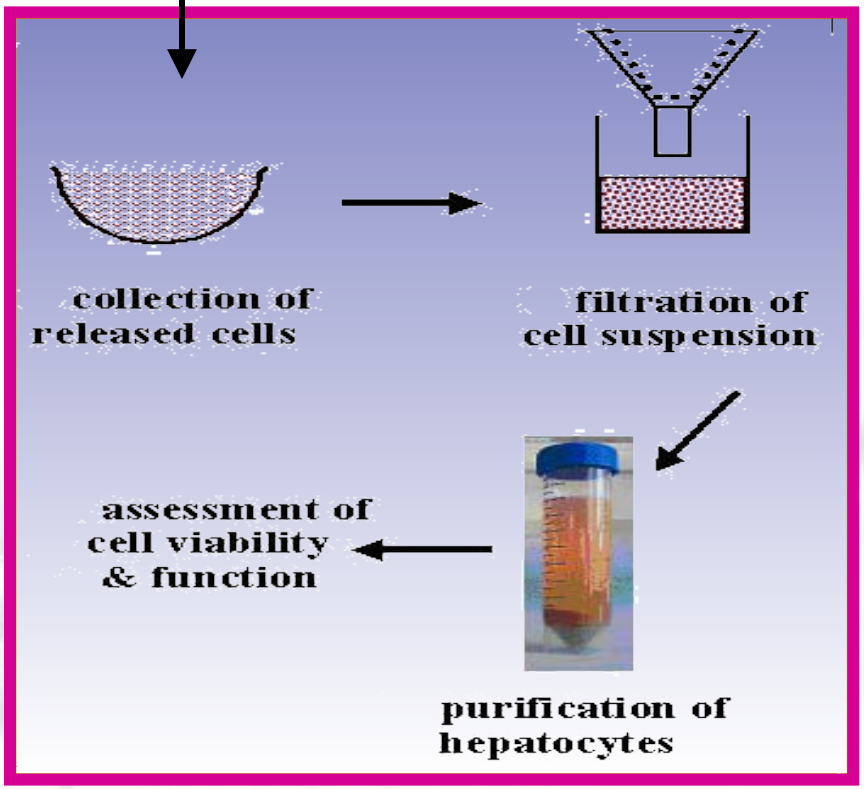
- Isolation of hepatocytes was started by continuous perfusion of liver withbuffer B (Hanks' collagenase buffer) for 15 minutes in order to digest the hepatic connective tissue by collagenase enzymatic activity.
- Full digestionof liver's connective tissue was confirmed by the small indentations left after touching of liver by a cotton bud.
- The entire liver was moved to a plastic bottle containedcollagenase bufferB.
- The isolated hepatocytes weredispersedby gentle stirring for 2 minutes and cellular clumps with connective tissue were removed byfiltrationthrough four layers of cotton gauze.



Isolation Technique

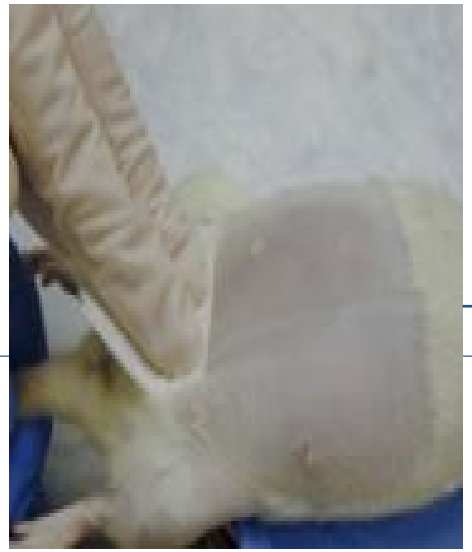
- The filtrate waswashedbyKrebs andHenseleit' buffer (buffer C)and vascular tissue, subcellular debris and broken liver cells were collected in supernatant by centrifugation for 2 minutes at 600 rpm.
- The isolated pellets werewashed twiceand supernatants were discarded after every centrifugation cycle.
- The isolated filtered hepatocytes wereresuspendedin buffer Cand final volume of collected liver cells was measured in graduated plastic tube.
- Cell countandviabilitywere determined usingtrypanblue and ahemocytometer.

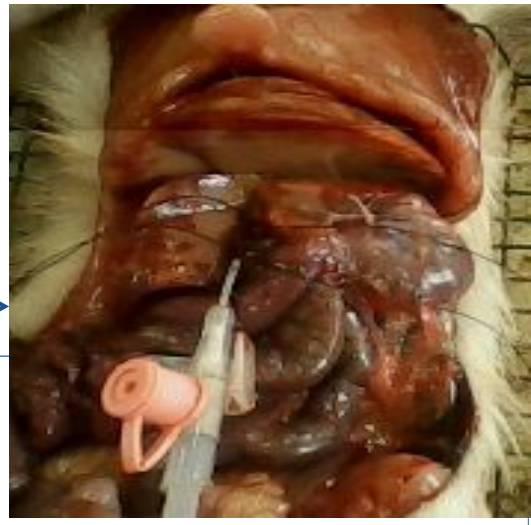


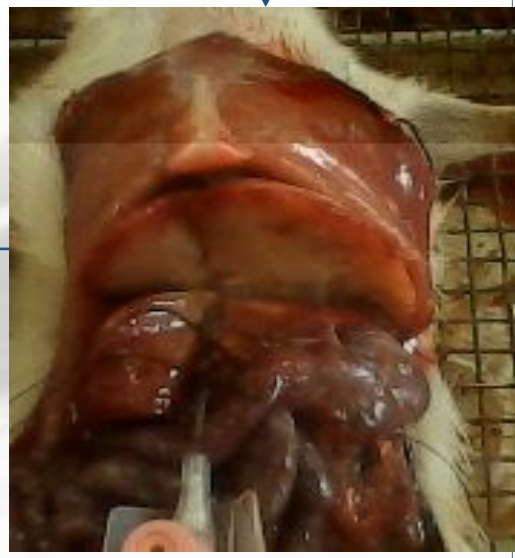


Summary of Isolation Technique

- Anesthesiaand preparation of the rat for thesurgical procedure.
- Cannulation and perfusionwith buffer A.
- Perfusion withbuffer B.
- Filtration.
- Centrifugationand Washing withbuffer C.
- Countingand Viability.







Factors Affecting Efficiency of HepatocyteIsolation

- ***Role of Ca^{2+}:***

The presence of Ca^{2+} is required for the enzymatic activity of collagenase during perfusion.

- ***Concentration and quality of collagenase:***

Collagenase is a key element in hepatocyte isolation.

- ***pH and buffering:***

The pH value of the perfusion medium should be kept around 7.4.

- ***Other factors:***
  - Theperfusion flow rate(optimumforrat 5ml/min)
  - Theforce of centrifugationfollowing isolation (optimum of 600rpm for rat liver cell isolation).

Assessment of Isolated Hepatocytes injury

A variety of endpoints have been developed and applied to quantify hepatocytes toxicity:

Assessment of Hepato-Cytotoxicity

Assessment of Hepatic-Oxidative stress

Assessment of Hepatic-Genotoxicity

Evaluation of the Some Toxicological Mechanisms

Assessment of Hepato-cytotoxicity

Measurement of cytotoxicity is probably themost widely usedaspect of cultured hepatocytes

:Cellviabilitytests

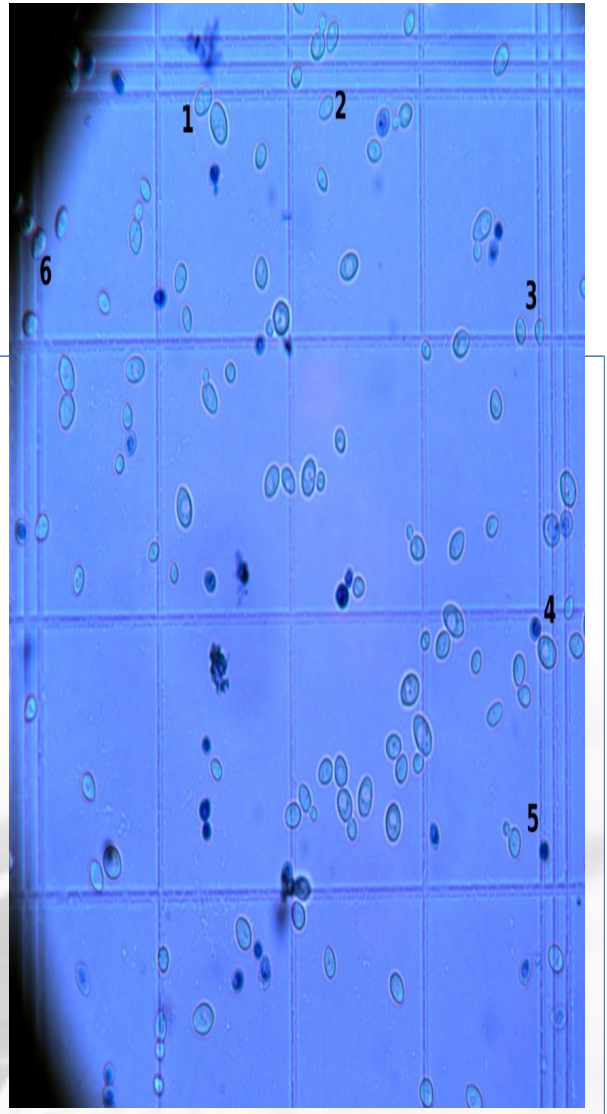
Release ofcytoplasmicenzymes:

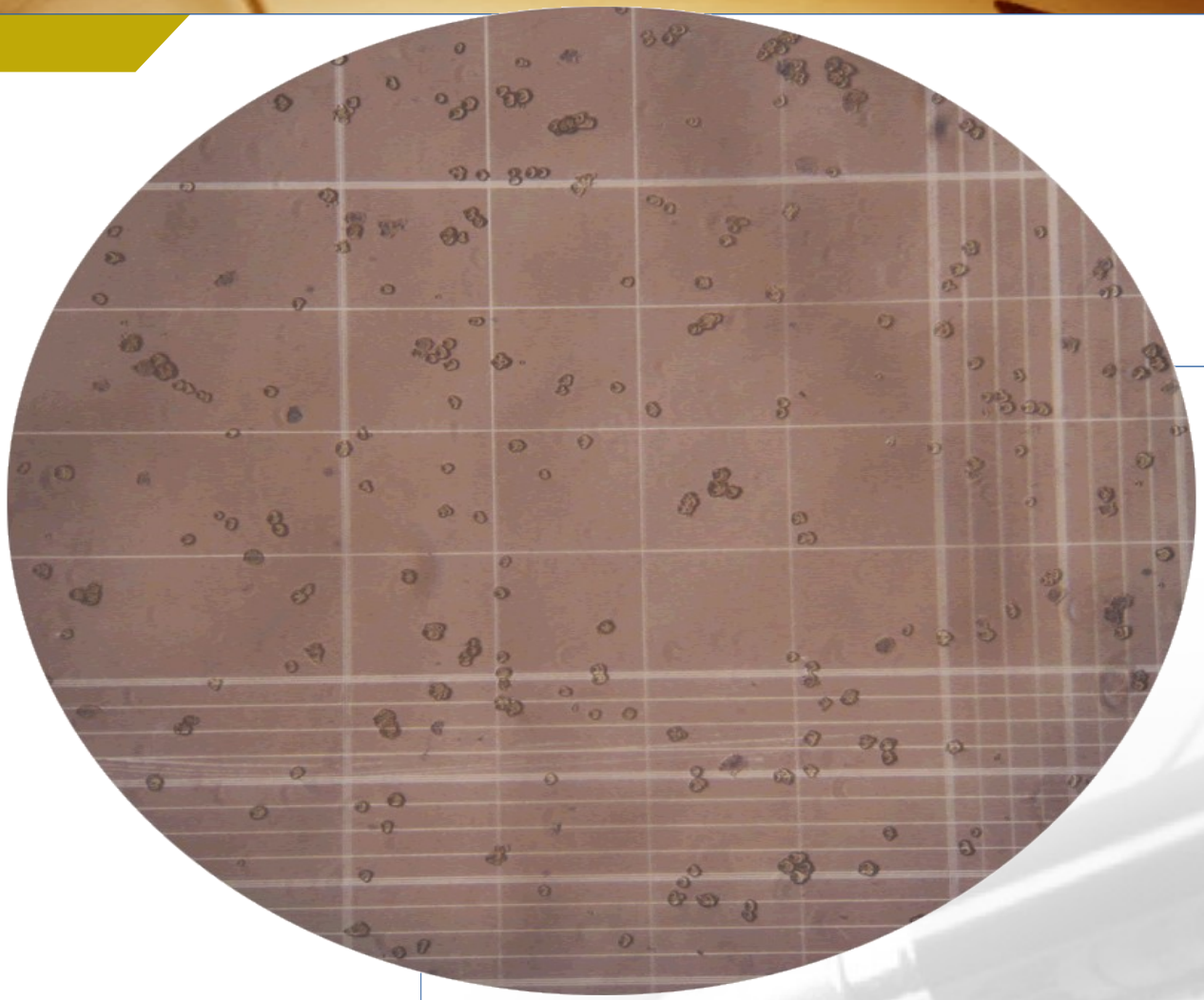
Morphology andCytology:

Cell Viability

ByTrypanblue exclusiontest

- Trypanblueis a high molecular-weight chemical relativelyimpermeableto viable cell.
- Cells withdamagedplasma membrane willallowrapid permeation of the dye into the cytoplasm, therebystainingthe cell nucleiblue.
- Trypanblue uptake is commonly expressed as the number of cellsexhibitingtrypanblue,dividedby thetotal cellpopulation counted.
- A significantincreasein this ratio over untreated or solvent-treated(controls)would indicatecytotoxicity.

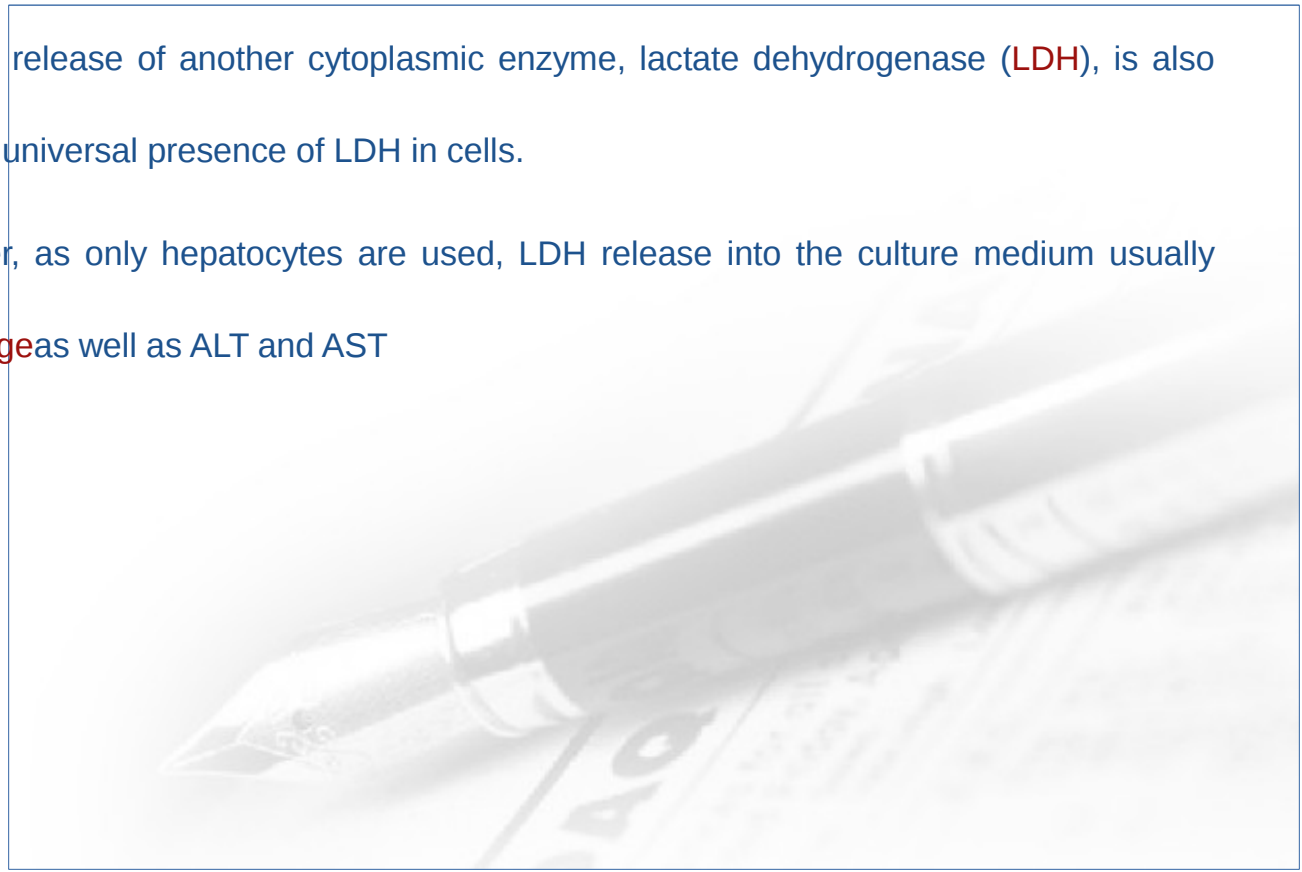




Hepatocytes in hemocytometer under light microscope

Cytosolic enzymatic Leakage

- Analysis ofenzyme releasehas been applied toward cultured hepatocytes.
- Although bothALT and ASTare present in the cytosol, themitochondriaof hepatocytes containonly AST. Analysis of both ALT and AST, therefore, can distinguish plasma membrane damage from mitochondrial damage.
- In addition to ALT and AST, the release of another cytoplasmic enzyme, lactate dehydrogenase (LDH), is also commonly used. Because of the universal presence of LDH in cells.
- Under*in vitro*conditions, however, as only hepatocytes are used, LDH release into the culture medium usually reflects plasmamembrane damageas well as ALT and AST



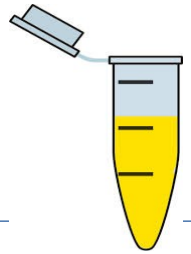
Cytosolic Enzymatic Leakage Percent

(ALT, AST, LDH)

- Enzyme activitieswere monitored in an aliquot ofcell-free mediumandcomparedto the total activity achievedafterlysisof the cells.
- Thecell-free mediumwas obtained by centrifuging 0.2 ml of treated cells and 0.2 ml ofsalineat 2200 rpm for 15 min to obtain the supernatant.
- Thelysatewas obtained by the addition of 0.2 ml of1% Triton X-100to 0.2 ml of treated cells, shaking for 15 min followed by centrifugation at 2200 rpm for 15 min.
- Theleakageis expressed as thepercentage of the total lysateactivity during the indicated time and under the specific treatment.

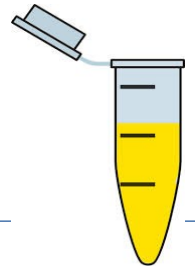
200µl cell suspension

+ 200µl saline



200µl cell suspension

+ 200µl 1% triton-X



Centrifugation 2200 rpm

(15 min.) for cell free medium

Shaking (15 min.)

then centrifugation

(2200rpm 15 min.)

Enzymes Leakage in Supernatants

(cell free and lysate)

were assessed

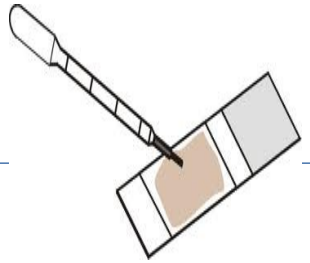
by using enzymatic available kits

Cellular Morphology / Light Microscopy

Thehistologicalappearance of thehepatocytesreflects theirdamageconditions

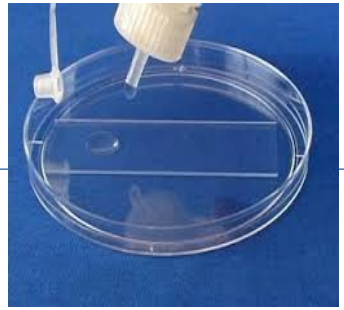
With the use oflight microscopy, cytotoxicity can sometimes be visualized by appearance ofhepatocytes vacuolesand other damages

1



10 µl of cell suspension on a glass slide Leave till dryness In room temperature

2



10 µl of Absolute Ethanol on the dried film

3

Stain with H&E

Cellular Morphology / Electron Microscopy

With the use ofelectron microscopy, cytotoxicity can be observed by alterations inultra-structurefor instanceswelling of mitochondrialmembranes and appearance oflysosomal inclusions bodies

- Cell suspensions werecentrifugedat low speed to obtain apelletof hepatocytes
- Add 1 %paraformaldehydeand 0.5 %glutraldehydein 0.1 mol/L sodium cacodylate buffer (pH 7.4) for 24 hours at 4˚C
- TEM procedures were Proceeded

Assessment of Hepatocytes Oxidative stress

Measurement of Reduced Glutathione

Measurement of lipid peroxide contents

Assessment of Hepatocytes Genotoxicity

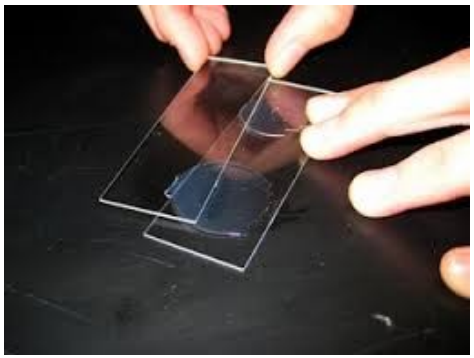
Comet assay

***In vitro*micronucleus assay**

DNA fragmentation assay

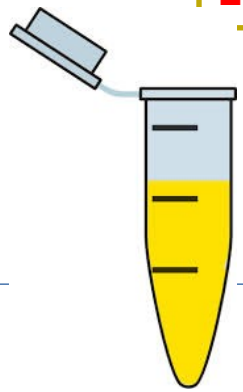
COMET Assay

1



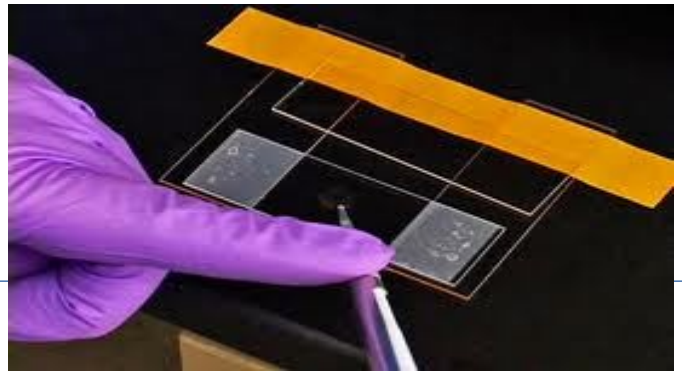
Prepare Slides

2



Suspend Cell in Low Melting Point Agarose (LMP)

3



Pour Cells on Slides

4

Electrophorese Cells

5

Stainslides with Ethidium Bromide

Examineunder fluorescent microscope using COMET Score Software

COMET Assay Scores

Microscopic photograph 1 score 0

Microscopic photograph 2 score 1

Microscopic photograph 3 score 2

Microscopic photograph 4 score 3

DNAFragmentationAssay

1

Extract DNA using

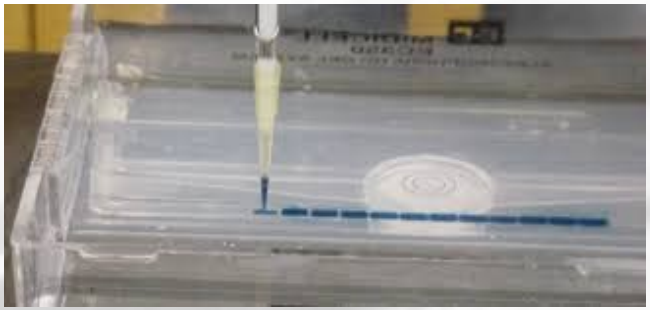
Commercially Available Kits

2

Mix DNA sample with

Loading Dye

3



Run samples on Agarose Gel

Electrophoresis

Some Experimental Protocols

using isolated hepatocytes to study mechanistic pathways of

environmental pollutants

- Previous studies for dust analysis using X-Ray spectromicroscopy and X-ray Fluorescence indicates thecomposition of dust:
  - Metals(Aluminum, iron, silver,…etc.)
  - Organic chemicals(Acrylonitrile, CCL4)
  - Somepesticides(cypermethrin, Lambadacyhalothrin).
  - The aimof our approach is to examine thehepatotoxic effectsof the detected pollutants using isolated cells.

Assessment of the different mechanistic pathways

- Role ofcytochrome oxidase enzyme(CYP450) in the metabolism of different pesticides , in order to determine the source of toxicity, whereas fromparentcompounds or itsmetabolites.
- Role ofGlutathione modulation, antioxidants and free radical scavengers on the toxicity of different metals (Vanadium, Iron)
- Role of differenthepatoprotective plant extracts(either crude or fractionated extracts) on induced hepatotoxicity by acetaminophen or CCL_4
- Comparison between the effects of metalnanoparticles and microparticleson isolated hepatocytes.
- Role of differentchelating agentson hepatotoxicity induced by aluminum chloride
- Role ofGenderdifference on the susceptibility of isolated hepatocytes to cypermethrin insecticide.

Some Discoveries and Findings

RoleofCytochrome P450 on hepatotoxicity (cypermethrinandLambda-cyhalothrin

The role of cytochrome P450 in the hepatotoxicity of bothcypermethrinandLambda-cyhalothrininsecticides was investigated in freshly hepatocytes isolated eitherfrom:

- Phenobarbitalpretreated rats (a well-known cytochrome P450inducer)

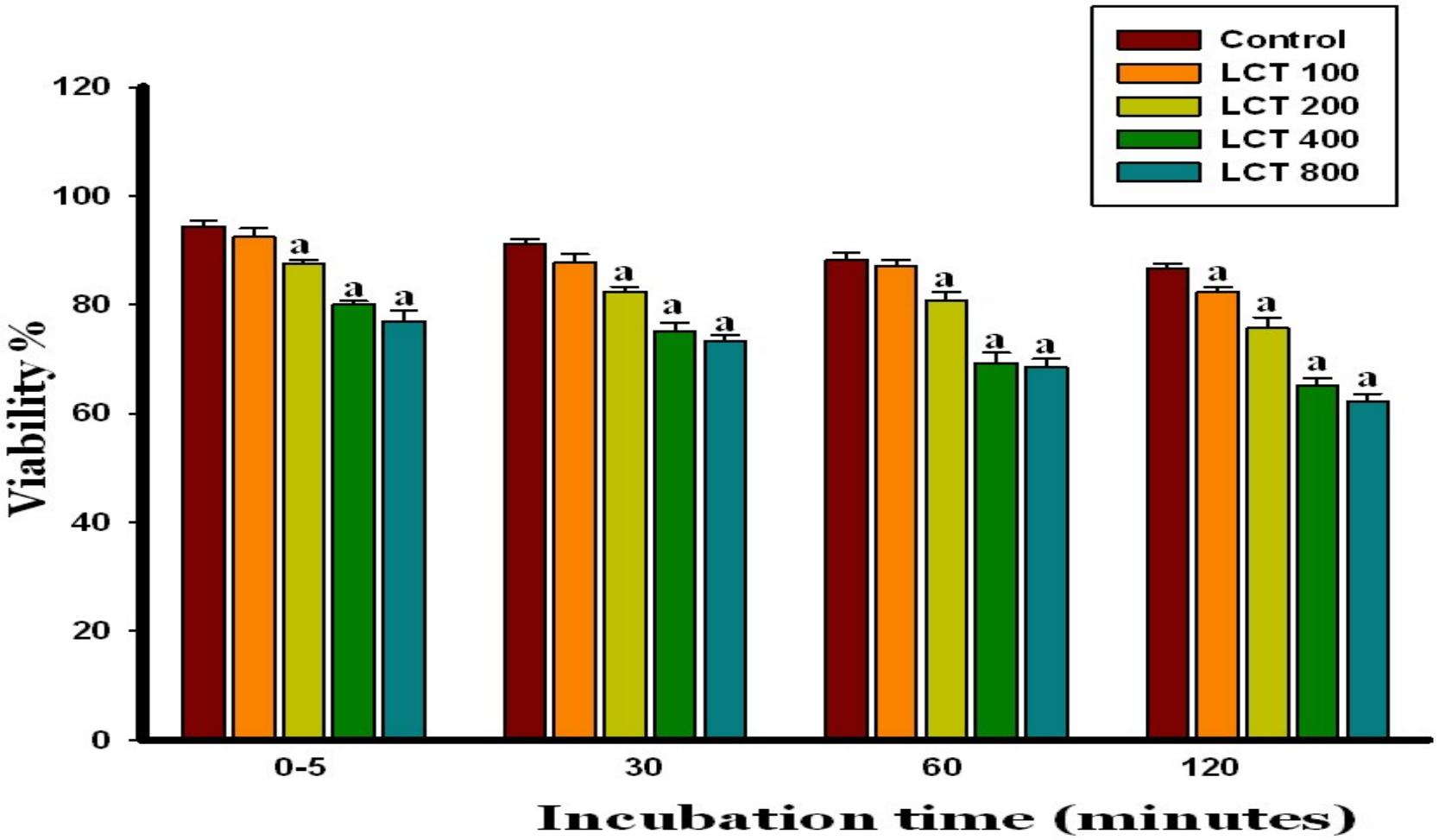
or

- Controlrats andcoincubatedwithSKF525A(a well-known cytochrome P450 inhibitor).

Role of Cytochrome P450

- Pretreatment withPhenobarbitalstronglyprotectedthe hepatocytes against both insecticides induced loss of cell viability and increased enzymes leakages.
- Coincubationof the hepatocytes withSKF525A, substantiallypotentiatedthe effect of cypermethrin andLambda-cyhalothrinon cell viability and enzyme leakages.
- The hepatotoxicity ofcypermethrinandLambda-cyhalothrincould be attributed toits parent compoundand at least partly owed to its induced cytotoxicity and oxidative stress.
- Moreover, phenobarbital and other cytochrome inducers could be of therapeutic value

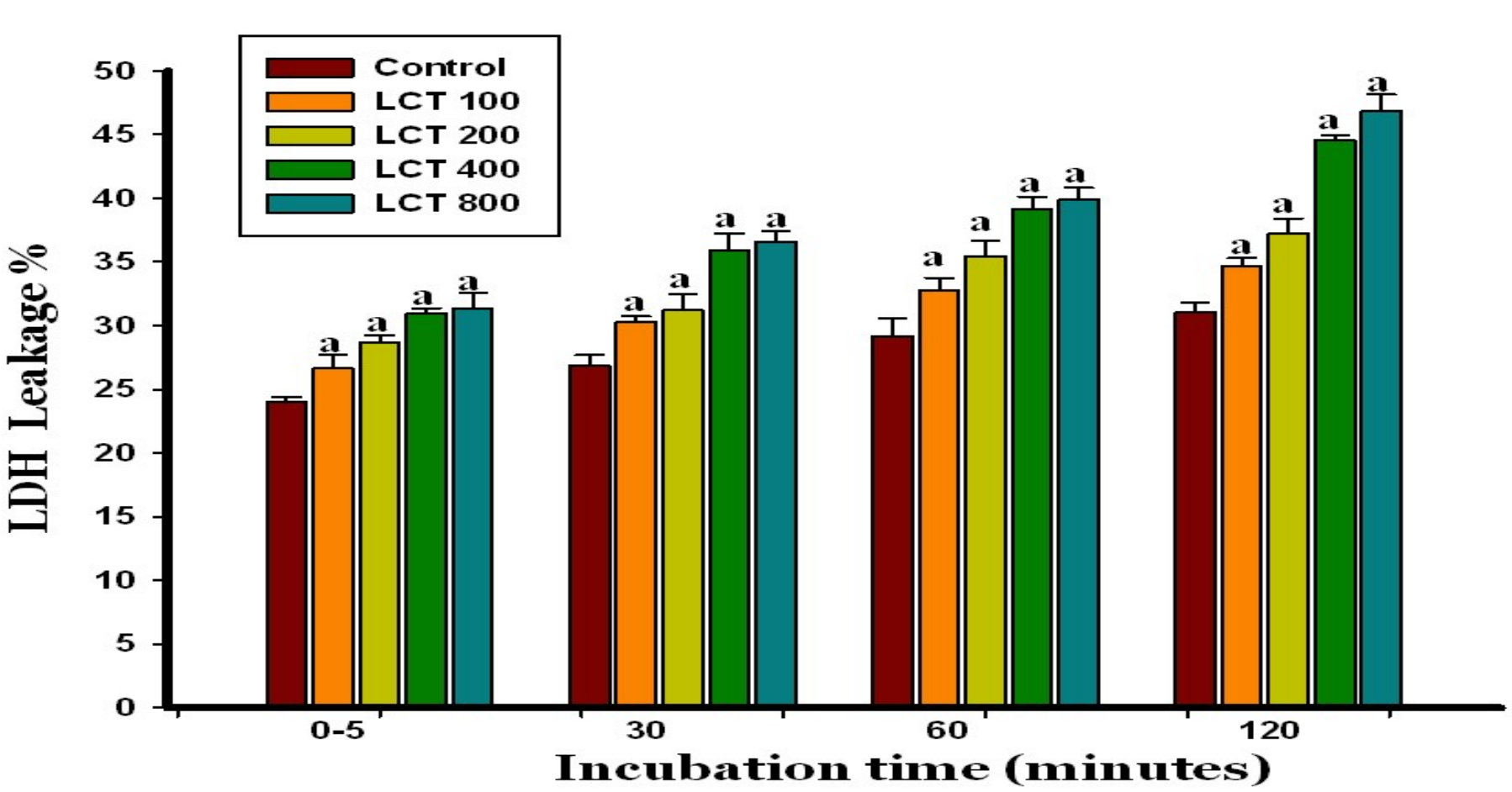
Viability % of isolated rat hepatocytes



Data expressed as Mean ± S.E. (n= 10 replicates).

(a) Significantly different from the respective control (DMSO) group (one-way ANOVA) (P<0.05).

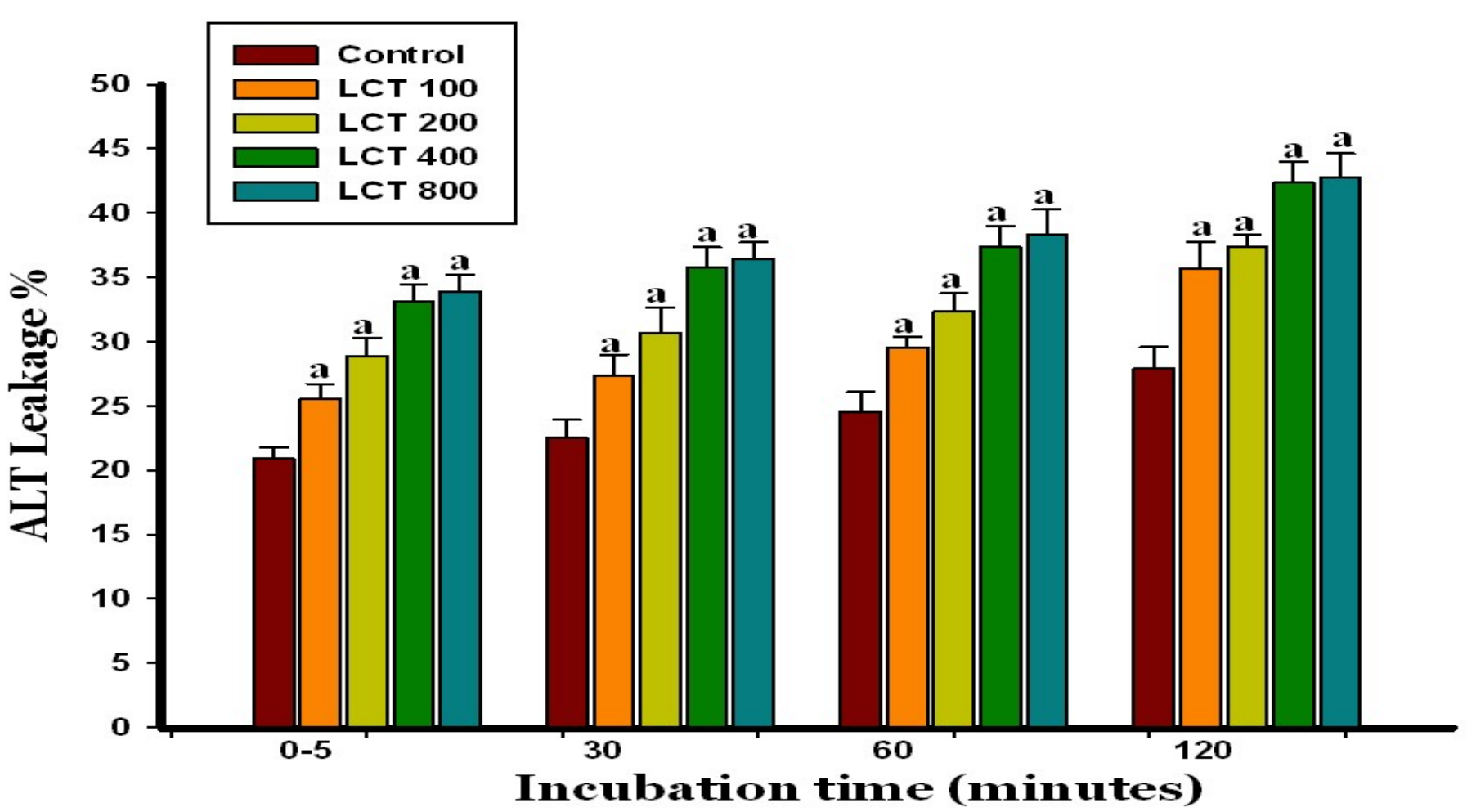
Lactate Dehydrogenase (LDH) leakage%:



Data expressed as Mean ± S.E. (n= 10 replicates).

(a) Significantly different from the respective control (DMSO) group (one-way ANOVA) ($P<0.05$).

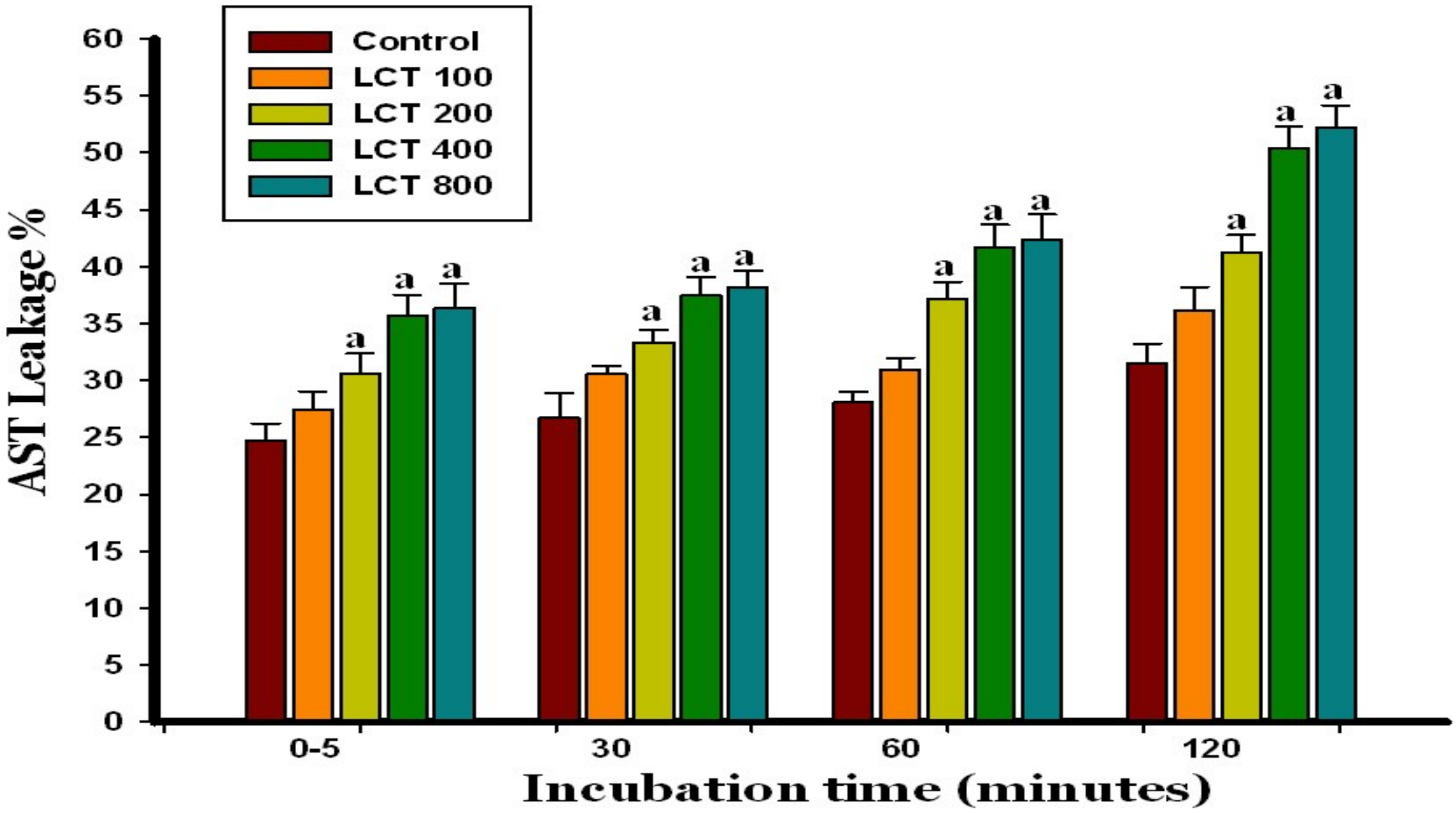
ALT leakage %:



Data expressed as Mean ± S.E. (n= 10 replicates).

(a) Significantly different from the respective control (DMSO) group (one-way ANOVA) (P<0.05).

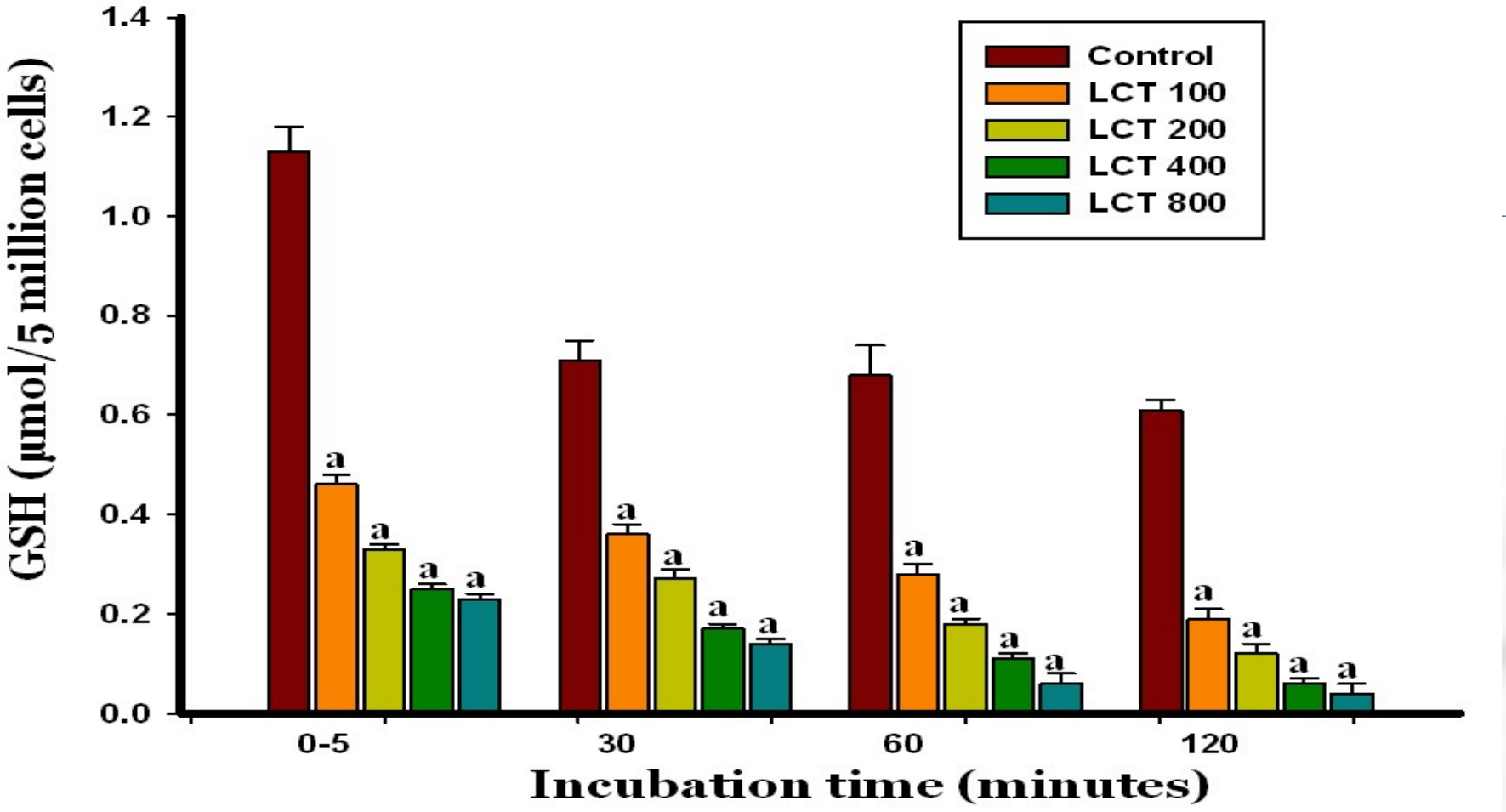
AST leakage %:



Data expressed as Mean ± S.E. (n= 10 replicates).

(a) Significantly different from the respective control (DMSO) group (one-way ANOVA) (P<0.05).

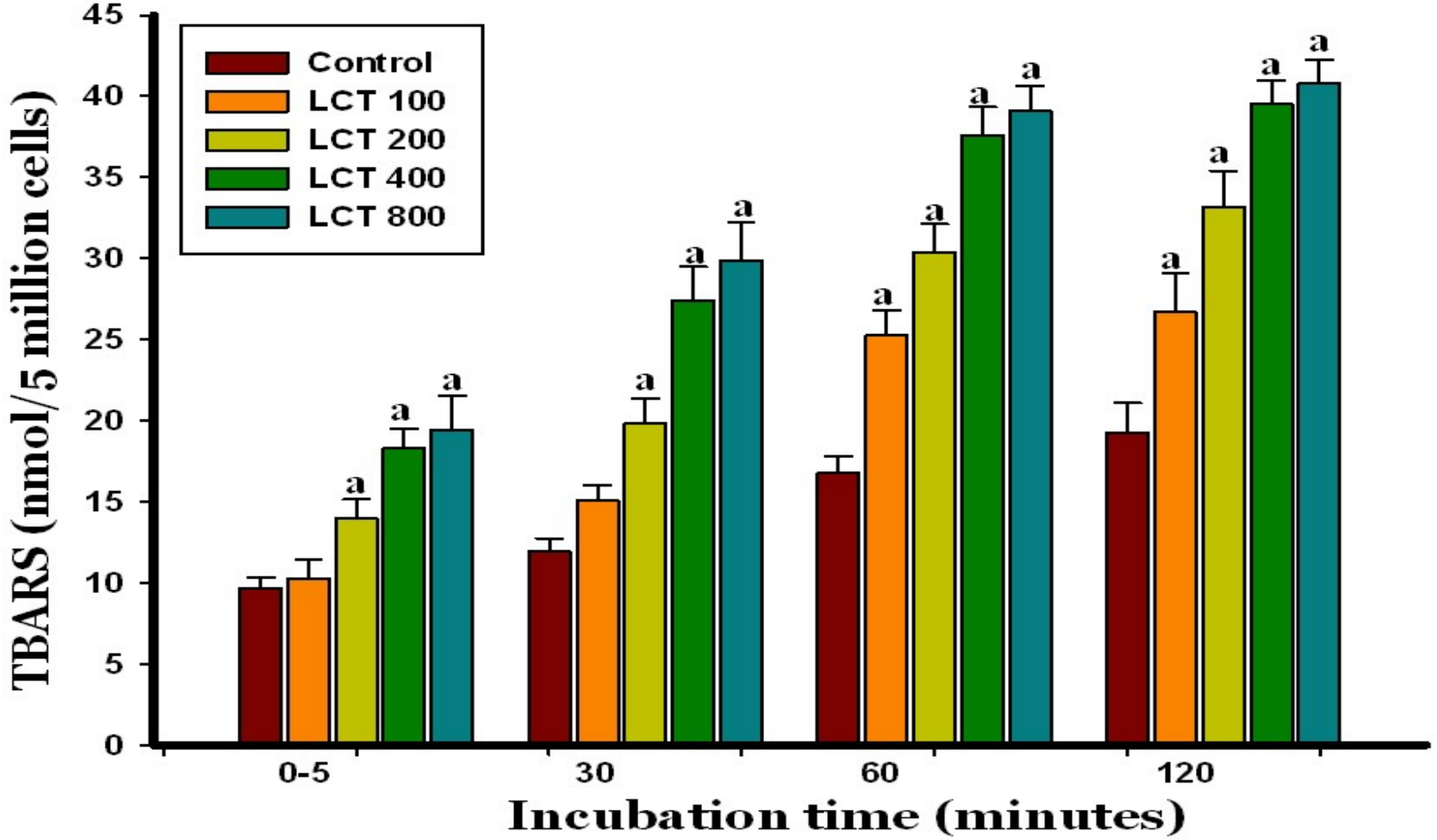
GSH contents of isolated rat hepatocytes:



Data expressed as Mean ± S.E. (n= 10 replicates).

(a) Significantly different from the respective control (DMSO) group (one-way ANOVA) ($P<0.05$).

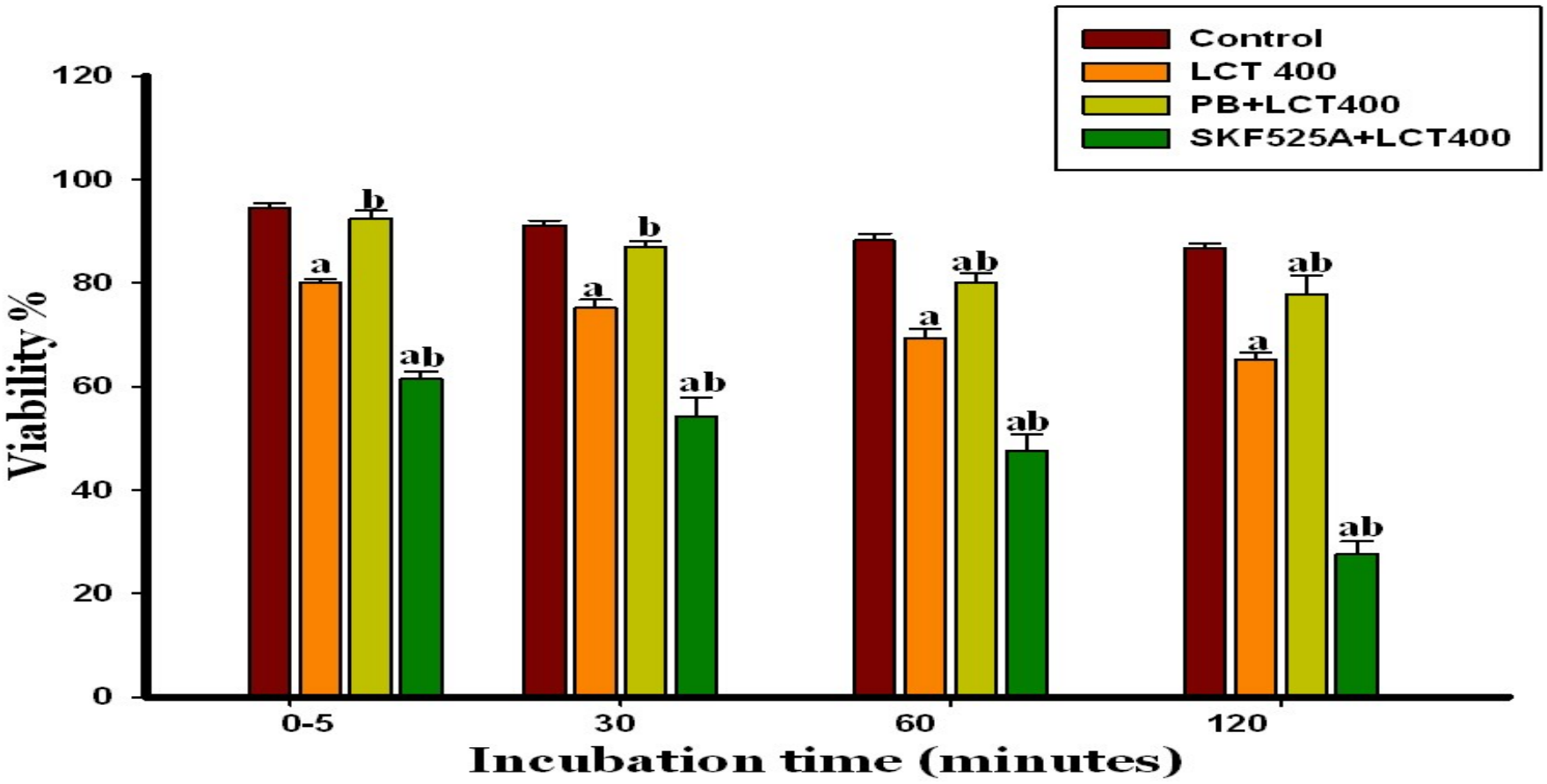
Lipidperioxidationproducts of isolated rat hepatocytes:



Data expressed as Mean ± S.E. (n= 10 replicates).

(a) Significantly different from the respective control (DMSO) group (one-way ANOVA) (P<0.05).

Effects of Enzyme induction and inhibition on Viability %



PB → **Phenobarbital"Cytochrome inducer".**

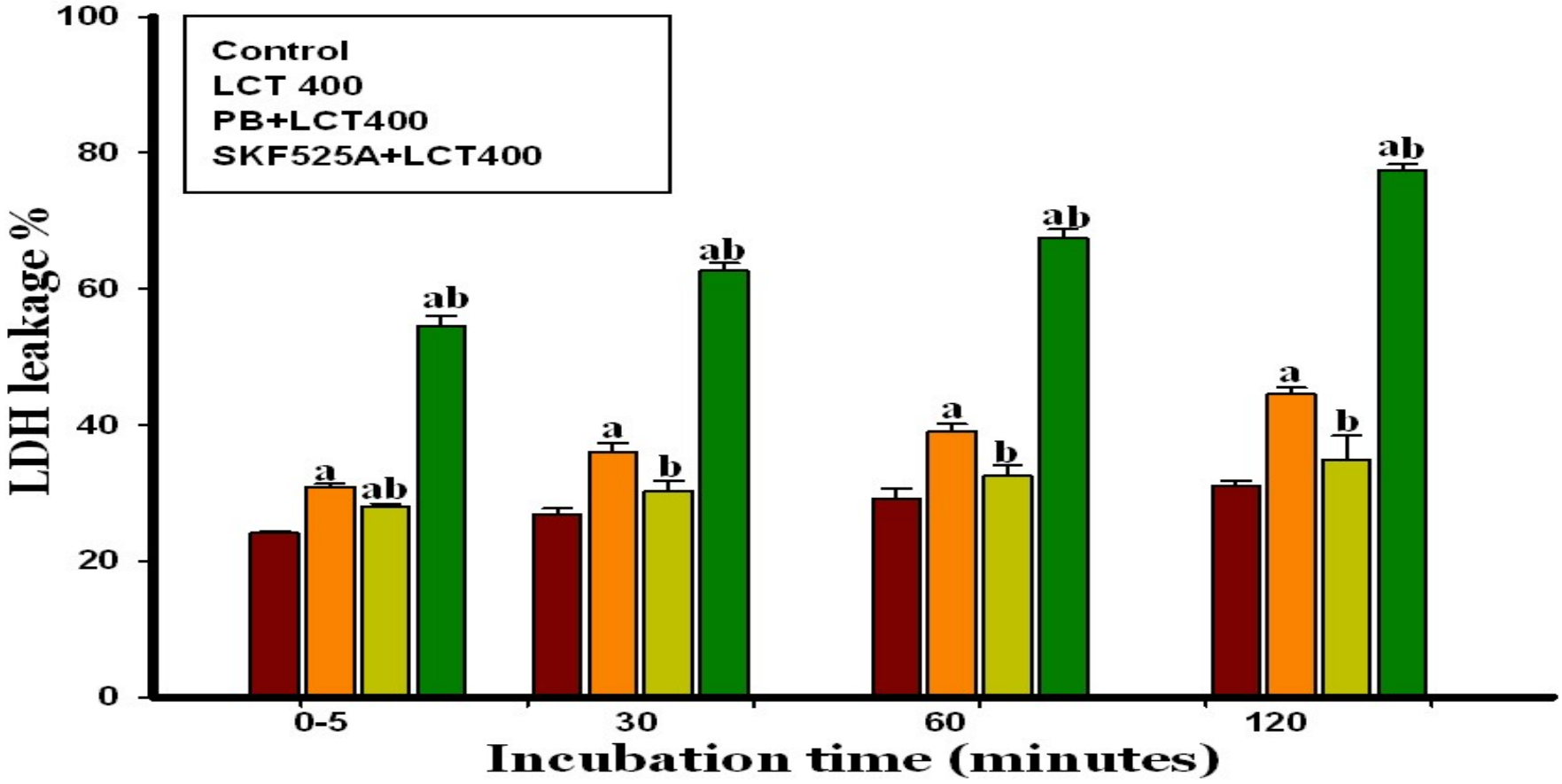
SKF525AProadifen"Cytochrome inhibitor"

Data expressed as Mean ± S.E. (n= 10 replicates).

(a) Significantly different from the respective control (DMSO) group (one-way ANOVA)

(b) Significantly different from 400 ng LCT / ml treated group (one-way ANOVA)

Effects of Enzyme induction and inhibition on LHD Leakage %



PB → **Phenobarbital"Cytochrome inducer".**

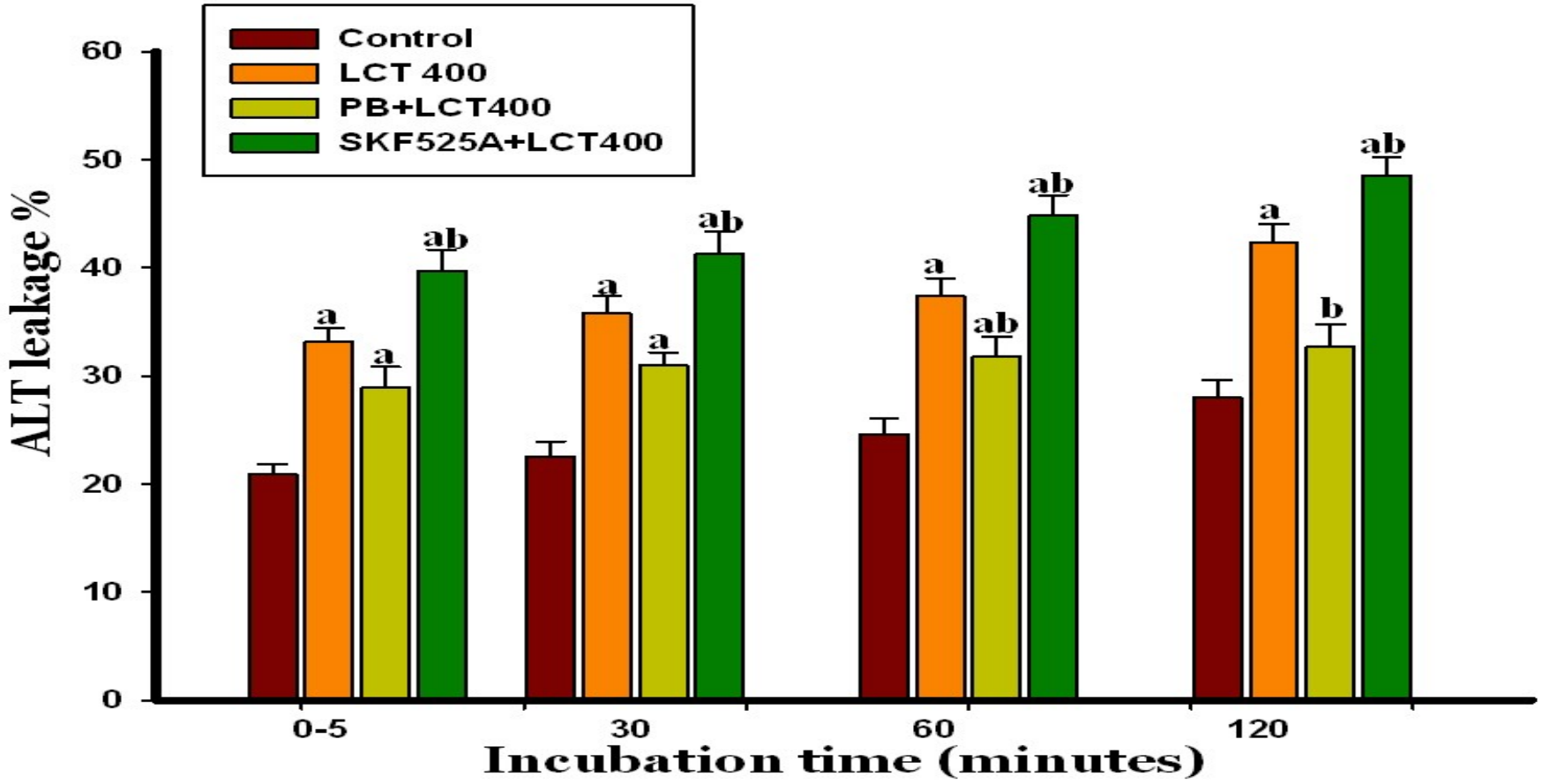
SKF525ACytochrome inhibitor

Data expressed as Mean ± S.E. (n= 10 replicates).

(a) Significantly different from the respective control (DMSO) group (one-way ANOVA)

(b) Significantly different from 400 ng LCT / ml treated group (one-way ANOVA)

Effects of Enzyme induction and inhibition on ALT Leakage %



PB → **Phenobarbital"Cytochrome inducer".**

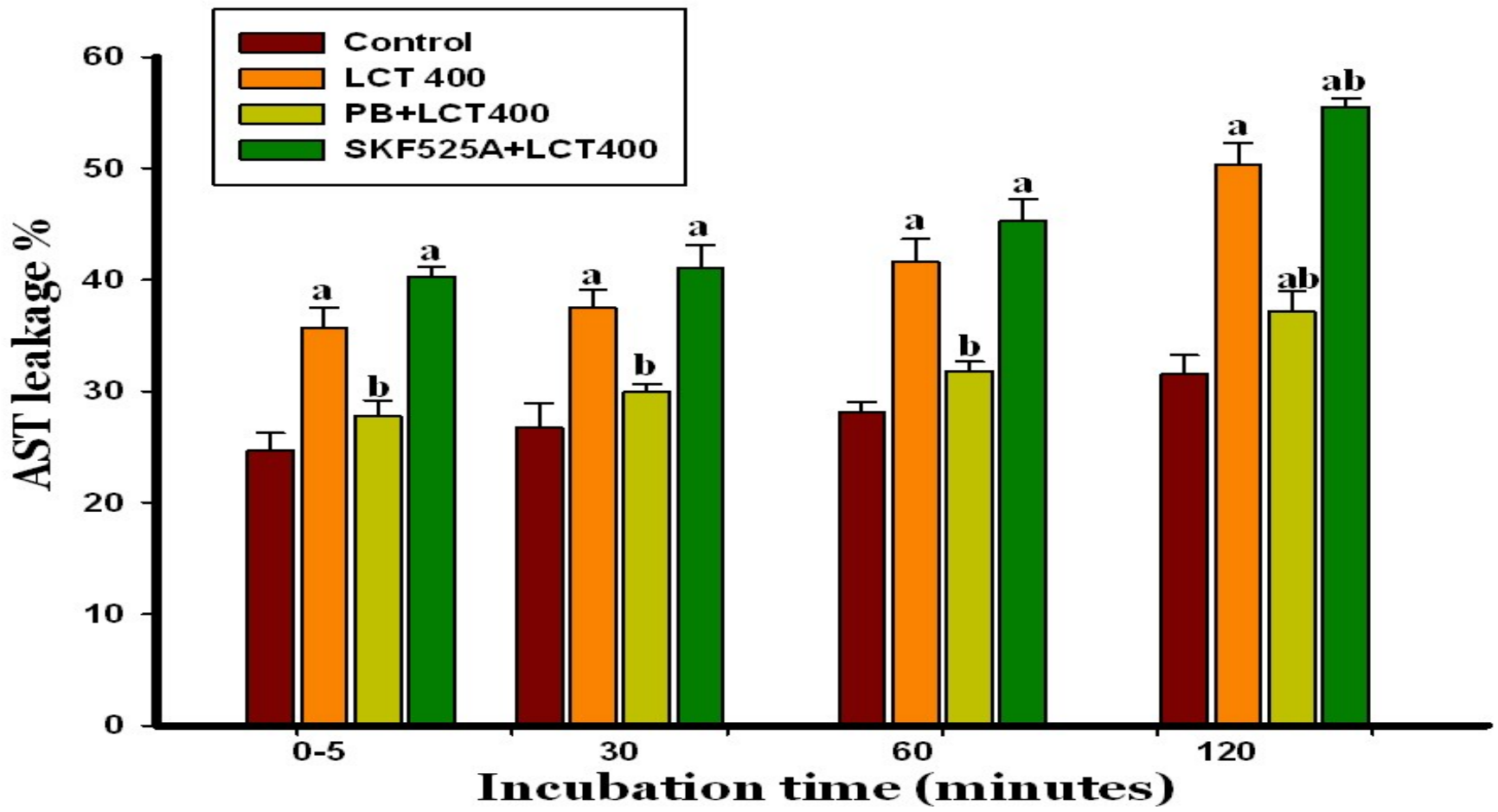
SKF525ACytochrome inhibitor

Data expressed as Mean ± S.E. (n= 10 replicates).

(a) Significantly different from the respective control (DMSO) group (one-way ANOVA)

(b) Significantly different from 400 ng LCT / ml treated group (one-way ANOVA)

Effects of Enzyme induction and inhibition on AST Leakage %



PB Phenobarbital"Cytochrome inducer".

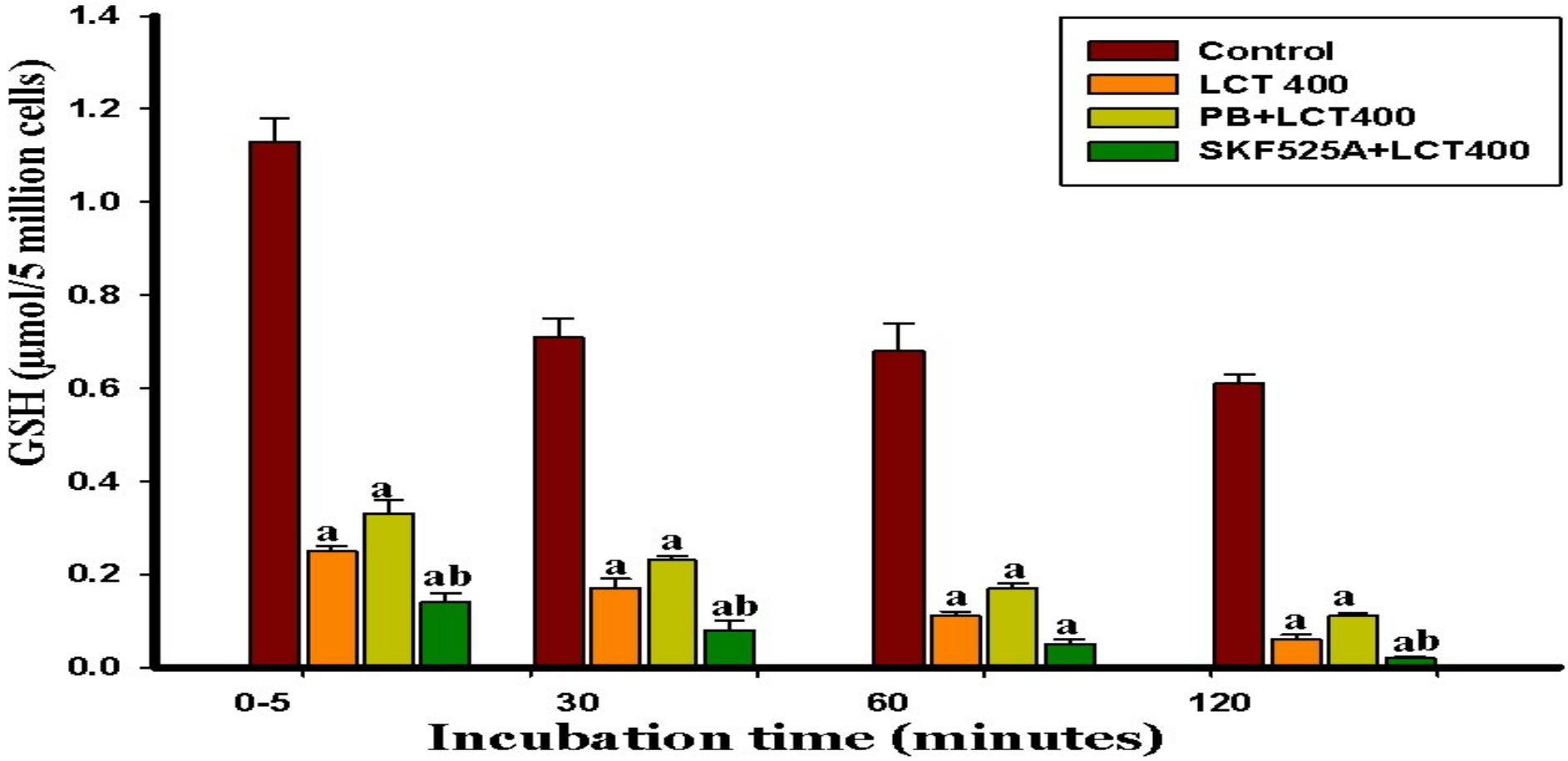
SKF525ACytochrome inhibitor

Data expressed as Mean ± S.E. (n= 10 replicates).

(a) Significantly different from the respective control (DMSO) group (one-way ANOVA)

(b) Significantly different from 400 ng LCT / ml treated group (one-way ANOVA)

Effects of Enzyme induction and inhibition on GSH



PB → **Phenobarbital"Cytochrome inducer".**

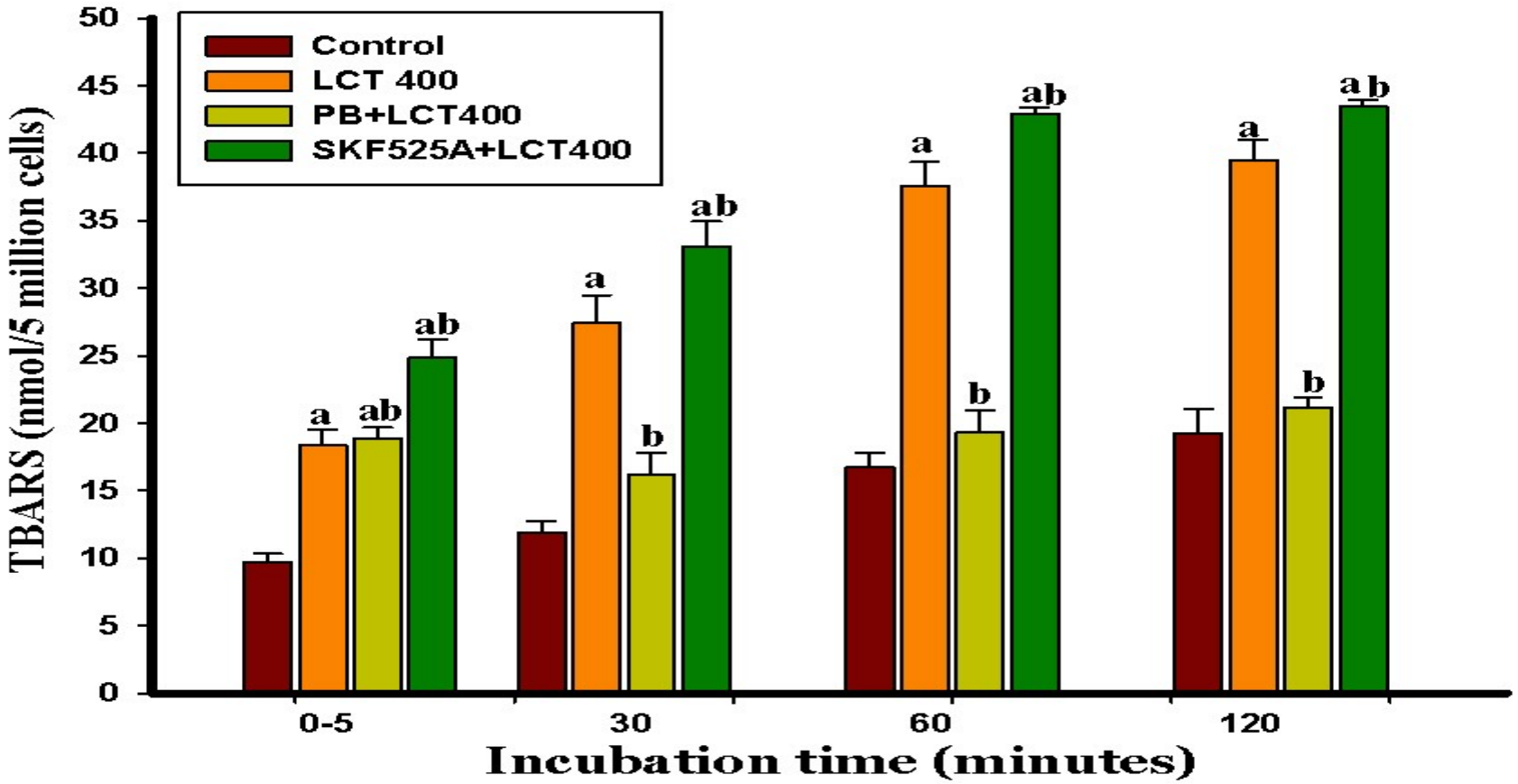
SKF525ACytochrome inhibitor

Data expressed as Mean ± S.E. (n= 10 replicates).

(a) Significantly different from the respective control (DMSO) group (one-way ANOVA)

(b) Significantly different from 400 ng LCT / ml treated group (one-way ANOVA)

Effects of Enzyme induction and inhibition on Lipid Peroxidation Contents



PB → **Phenobarbital"Cytochrome inducer".**

SKF525ACytochrome inhibitor →

Data expressed as Mean ± S.E. (n= 10 replicates).

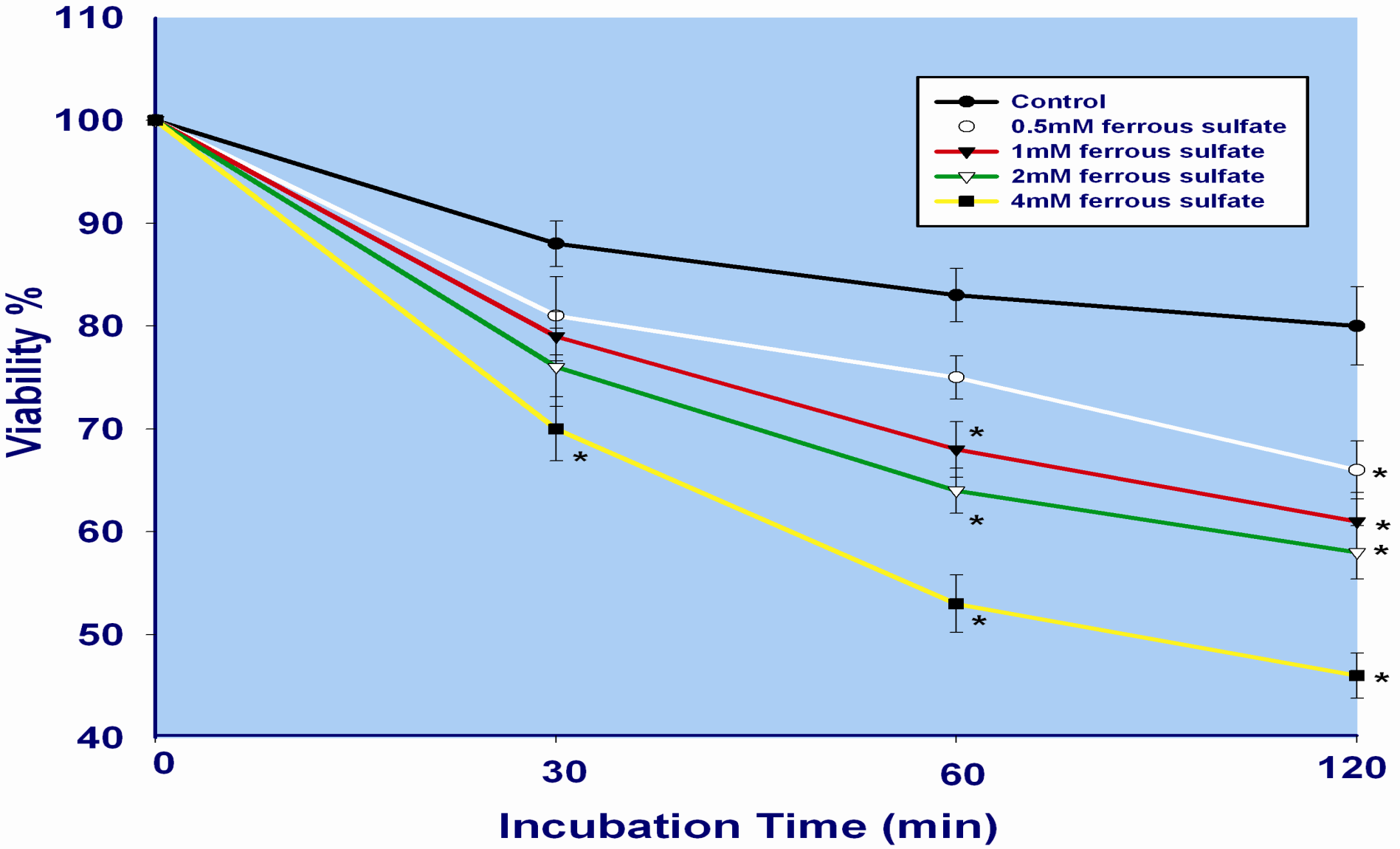
(a) Significantly different from the respective control (DMSO) group (one-way ANOVA)

(b) Significantly different from 400 ng LCT / ml treated group (one-way ANOVA)

Role of Glutathione modulation onhepatotoxicity (Doxorubicn, Ferrous sulfate and Vanadium)

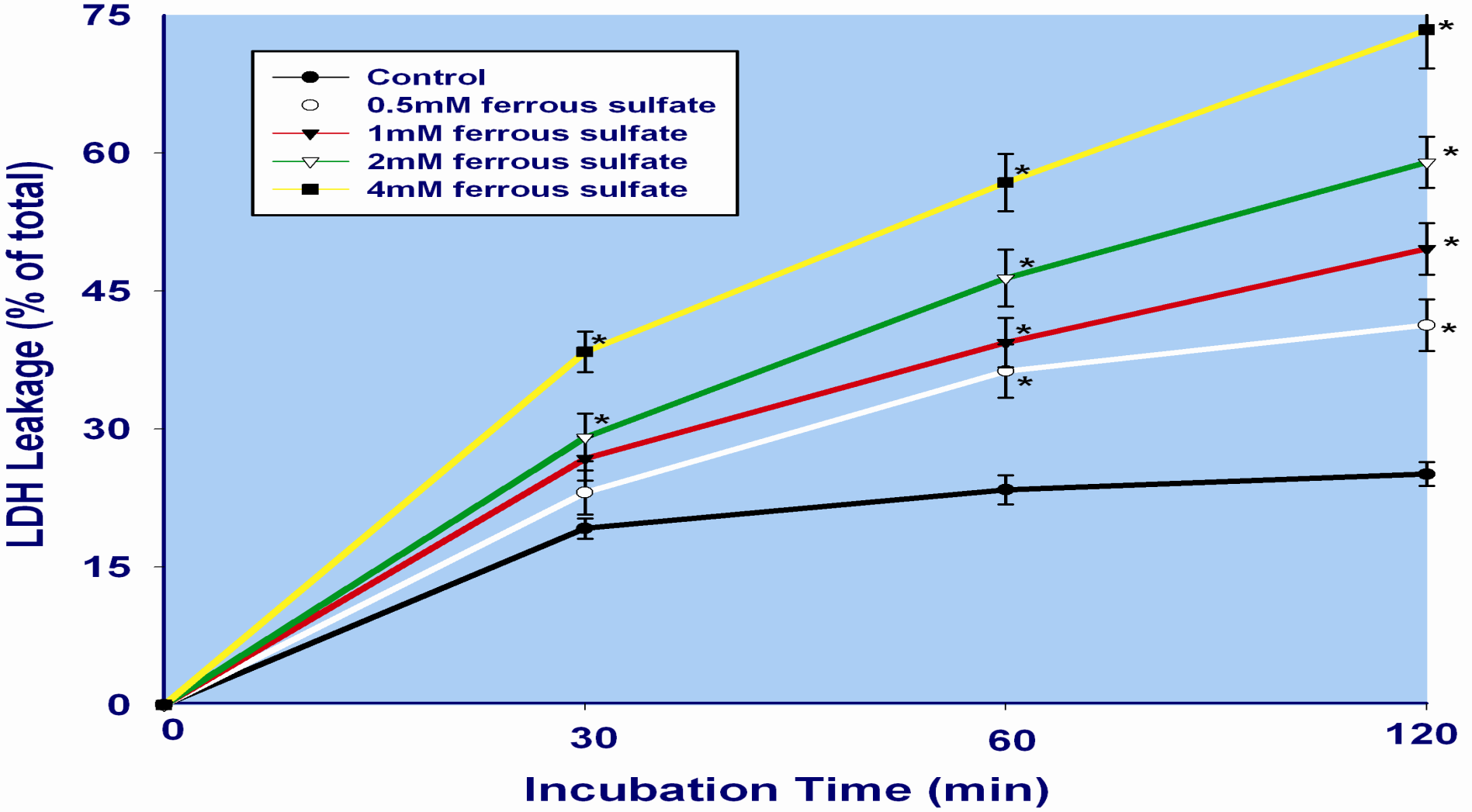
- Cytotoxicity as well as the oxidative stress induced bydoxorubicin and Some metals (Ferroussulfate and Vanadium)in isolated rat hepatocytes and the role of Glutathione (GSH) modulation and free radical scavengers on their cytotoxicity were evaluated
- GSH depletionand oxidative stress play an important role inenhancing hepatotoxicpotential of Doxorubicin or Ferrous sulfate in isolated rat hepatocytes.
- However,GSH boosting,antioxidant enzymesandfree radical scavengersmarkedlyprotectedthe cells from doxorubicin or Ferrous sulfate cytotoxicity.

Fig.1. Effects of Ferrous Sulfate on Viability % of isolated rat hepatocytes



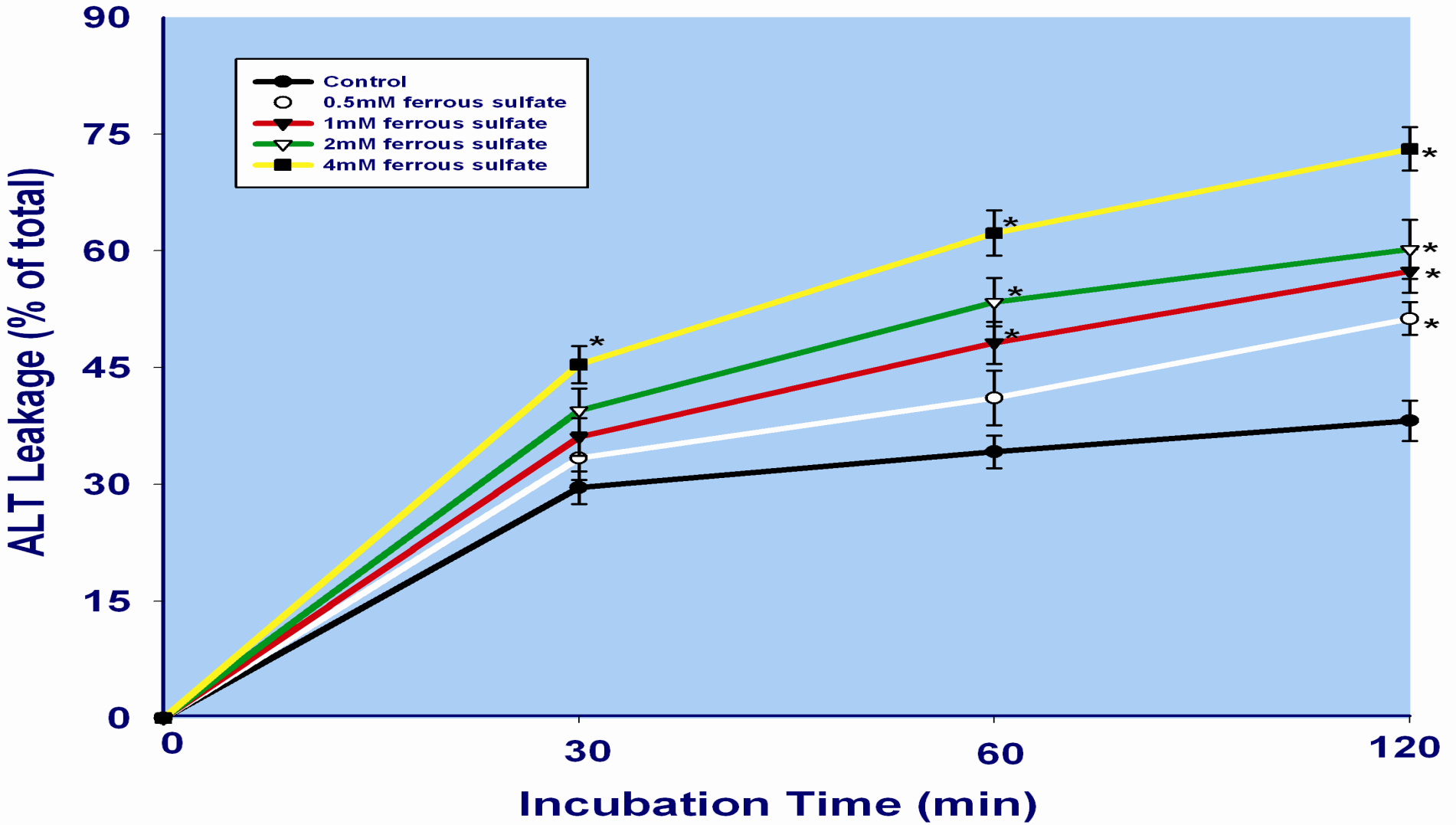
Data expressed as mean ± S.E.M. of six hepatocyte replicates.
(*) Significantly different from control group at $p<0.05$.

Fig. 2. Effects of Ferrous Sulfate on LDH leakage % from isolated rat hepatocytes



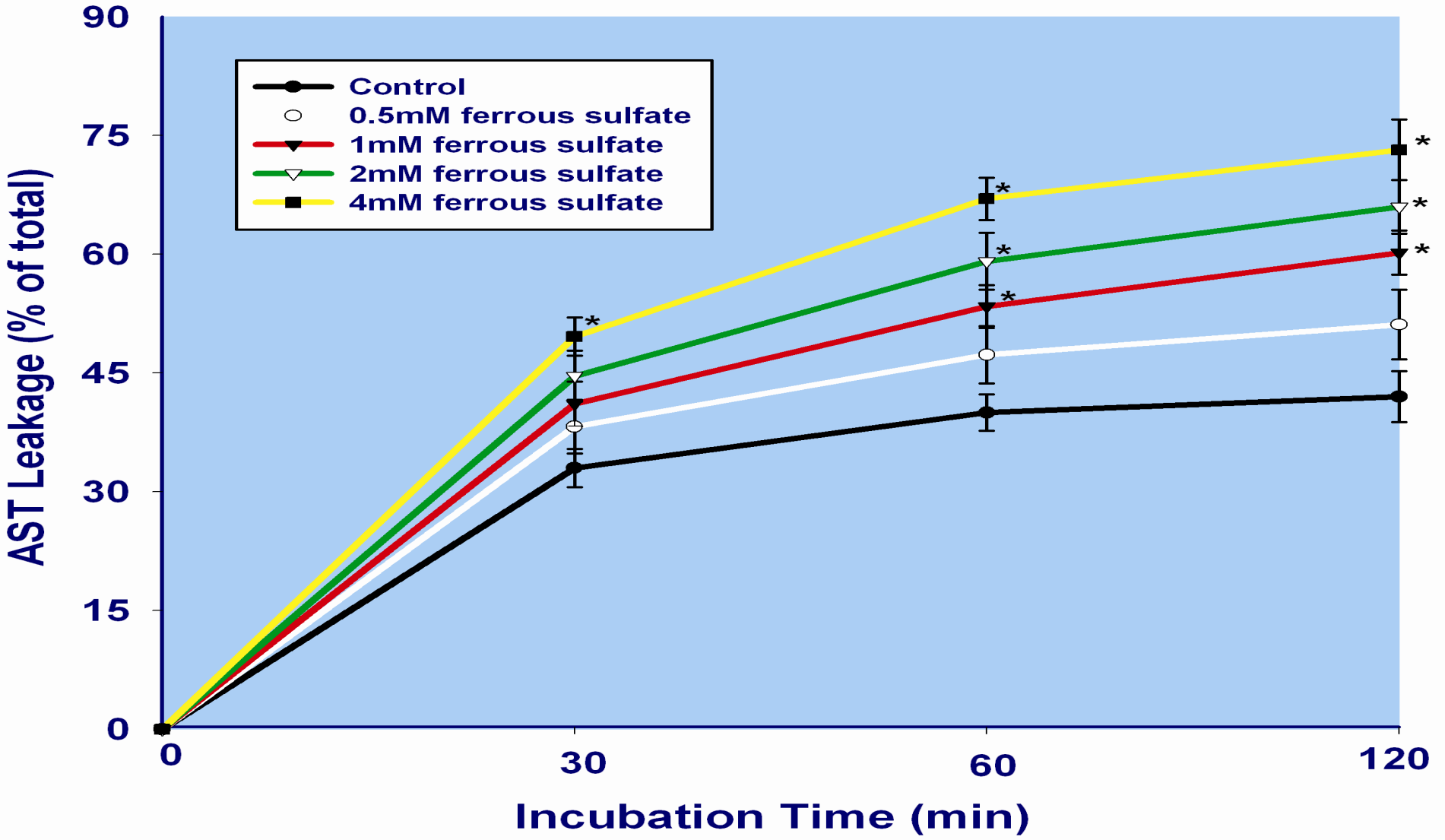
Data expressed as mean ± S.E.M. of six hepatocyte replicates.
(*) Significantly different from control group at $p<0.05$.

Fig. 3. Effects of Ferrous Sulfate on ALT leakage % from isolated rat hepatocytes



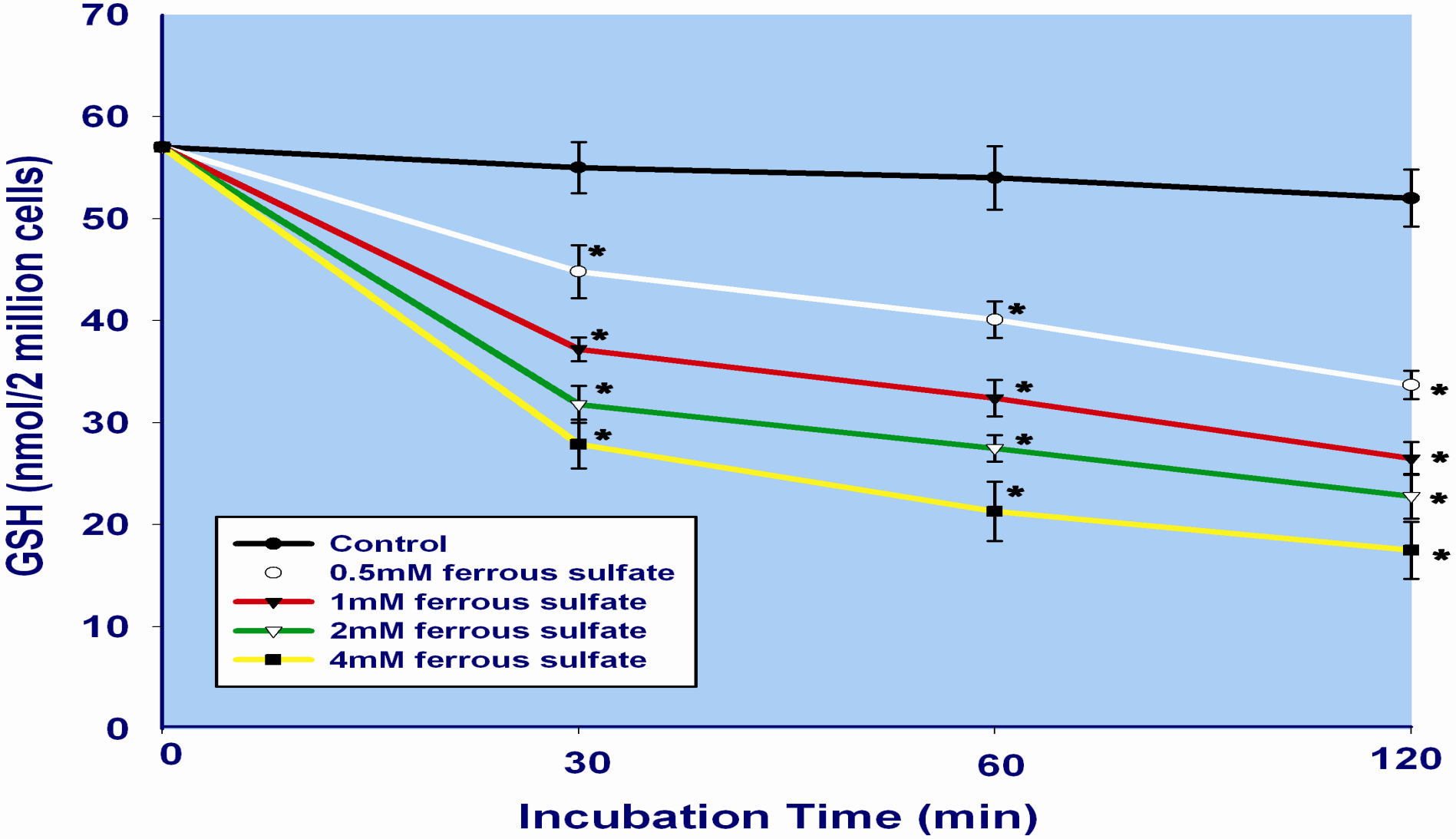
Data expressed as mean ± S.E.M. of six hepatocyte replicates.
(*) Significantly different from control group at $p<0.05$.

Fig. 4. Effects of Ferrous Sulfate on AST leakage % from isolated rat hepatocytes



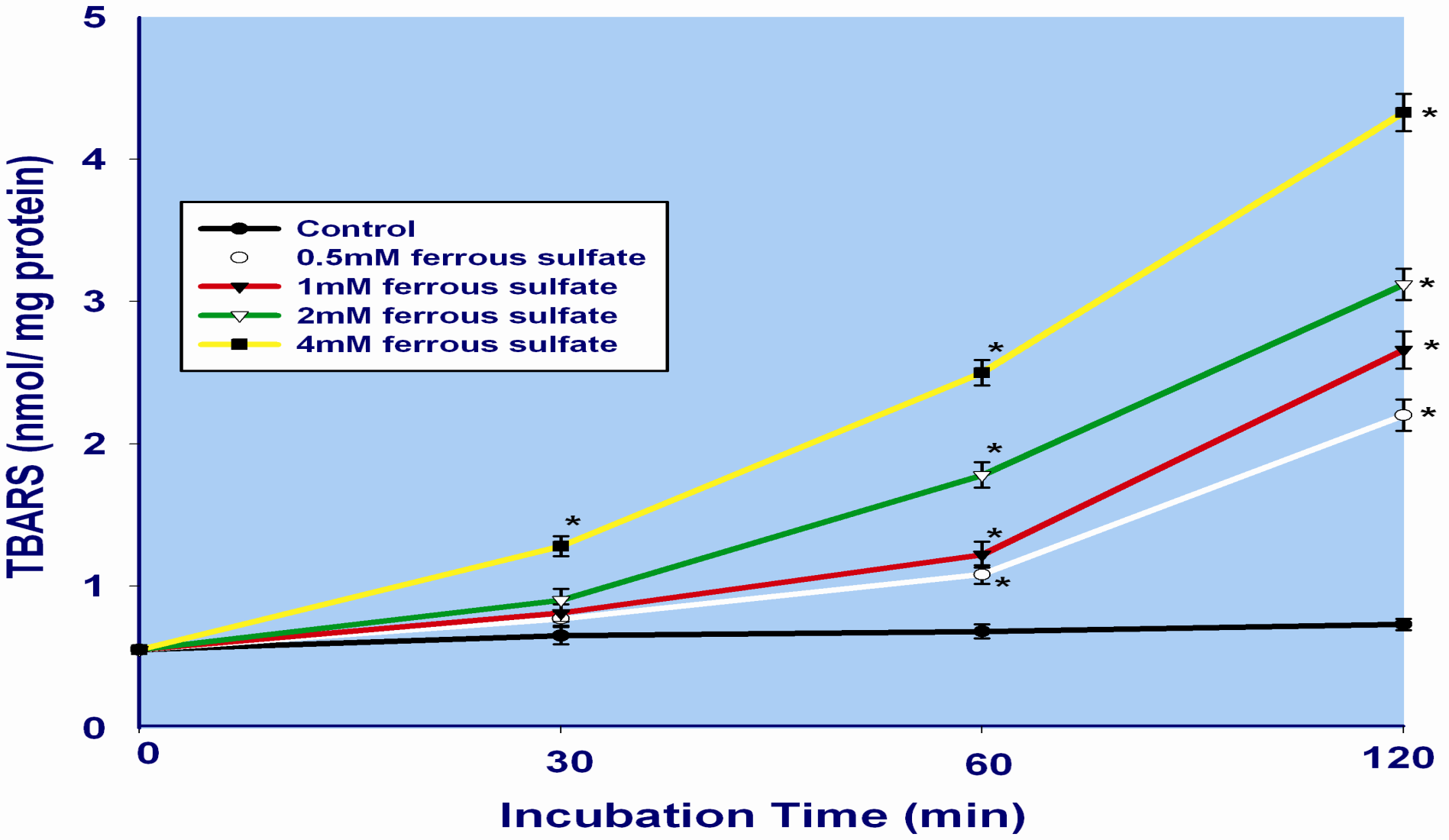
Data expressed as mean ± S.E.M. of six hepatocyte replicates.
(*) Significantly different from control group at $p<0.05$.

Fig. 5. Effects of Ferrous Sulfate on GSH level of isolated rat hepatocytes



Data expressed as mean ± S.E.M. of six hepatocyte replicates.
(*) Significantly different from control group at $p<0.05$.

Fig. 6. Effects of Ferrous Sulfate on lipid peroxidation of isolated rat hepatocytes



Data expressed as mean ± S.E.M. of six hepatocyte replicates.
(*) Significantly different from control group at $p<0.05$.

Protective effects ofthiol-containing compounds on ferrous sulfate - induced LDH leakage and lipid peroxidation in isolated rat hepatocytes

TBARS (nmol/mg protein)	LDH leakage (% of total)	Co-incubation
0.73±0.01	25.1±1.10	Non (control)
4.33±0.12[a]	73.4±4.20[a]	Ferrous sulfate (4mM)
2.12±0.09[a, b]	37.2±1.50[a, b]	Ferrous sulfate + GSH (0.5 mM)
1.55±0.16[a, b]	47.6±3.21[a, b]	Ferrous sulfate + MT (0.5mM
1.64±0.09[a, b]	38.6±1.02[a, b]	Ferrous sulfate + NAC (0.5mM
2.14±0.12[a, b]	41.3±2.10[a, b]	Ferrous sulfate + DTT (0.5mM)

Data are presented as mean±SEM of six replicates.

(a) Significantly different from corresponding control group at $p < 0.05$

(b) Significantly different from ferrous sulfate alone-treated group at $p < 0.05$.

GSH, reduced glutathione; MT, methionine; NAC, N-acetyl-l-cystiene; DTT,dithiothreitol.

Allthiolcompounds were added 30 min before the addition of ferrous sulfate.

LDH and TBARS were determined 120 min after the addition of ferrous sulfate.

Effects of GSH depleting agents on Ferrous Sulfate –induced LDH leakage and Lipid peroxidation in isolated rat hepatocytes

TBARS (nmol/mg protein)	LDH leakage (% of total)	Co-incubation
0.73±0.01	25.1±1.10	Non (control)
4.33±0.12[a]	73.4±4.20[a]	Ferrous sulfate (4 mM)
5.41±2.31[a, b]	97.6±3.4[a, b]	Ferrous sulfate + BCNU (0.5mM)
5.82±3.44[a, b]	89.4±4.6[a, b]	Ferrous sulfate + BSO (5 mM)
6.81±4.15[a, b]	99.8±5.2[a, b]	Ferrous sulfate + CDNB (0.25mM)

Data are presented as mean±SEM of six replicates.

(a) Significantly different from corresponding control group at $p < 0.05$

(b) Significantly different from ferrous sulfate alone-treated group at $p < 0.05$.

BCNU,bis(chloroethyl)-nitrosurea(GSSGreductaseinhibitor);

BSO,buthioninesulfoximine(selective inhibitor of γ-glutamylcysteinesynthetase);

CDNB,chlorodinitrobenzene(glutathione-S-transferaseinhibitor).

All compounds were added 30 min before the addition of ferrous sulfate.

LDH and TBARS were determined 120 min after the addition of ferrous sulfate.

Protective Effects of SOD, CAT, DMSO and DFO on ferrous sulfate –induced LDH leakage and lipid peroxidation in isolated rat hepatocytes

TBARS (nmol/mg protein)	LDH leakage (% of total)	Co-incubation
0.73±0.01	25.1±1.10	Non (control)
4.33±0.12[a]	73.4±4.20[a]	Ferrous sulfate (4 mM)
3.11±0.25[a, b]	50.2±3.60[a, b]	Ferrous sulfate + SOD (100 U/ml)
2.28±0.14[a, b]	39.4±1.70[a, b]	Ferrous sulfate + CAT (100 U/ml)
2.69±0.11[a, b]	51.4±2.47[a, b]	Ferrous sulfate + DMSO (100µM)
2.41±0.15[a, b]	46.1±3.21[a, b]	Ferrous sulfate + DFO (20mM)

Data are presented as mean±SEM of six replicates.

(a) Significantly different from corresponding control group at $p < 0.05$

(b) Significantly different from ferrous sulfate alone-treated group at $p < 0.05$.

SOD, superoxide dismutase; CAT, catalase; DMSO, dimethylsulfoxide; DFO,desferrioxamine. All antioxidants were added 30 min before the addition of ferrous sulfate. LDH and TBARS were determined 120 min after the addition of ferrous sulfate.


Hepatoprotective Effects of Plant Extracts

Hepatoprotective Effects of Plant Extracts

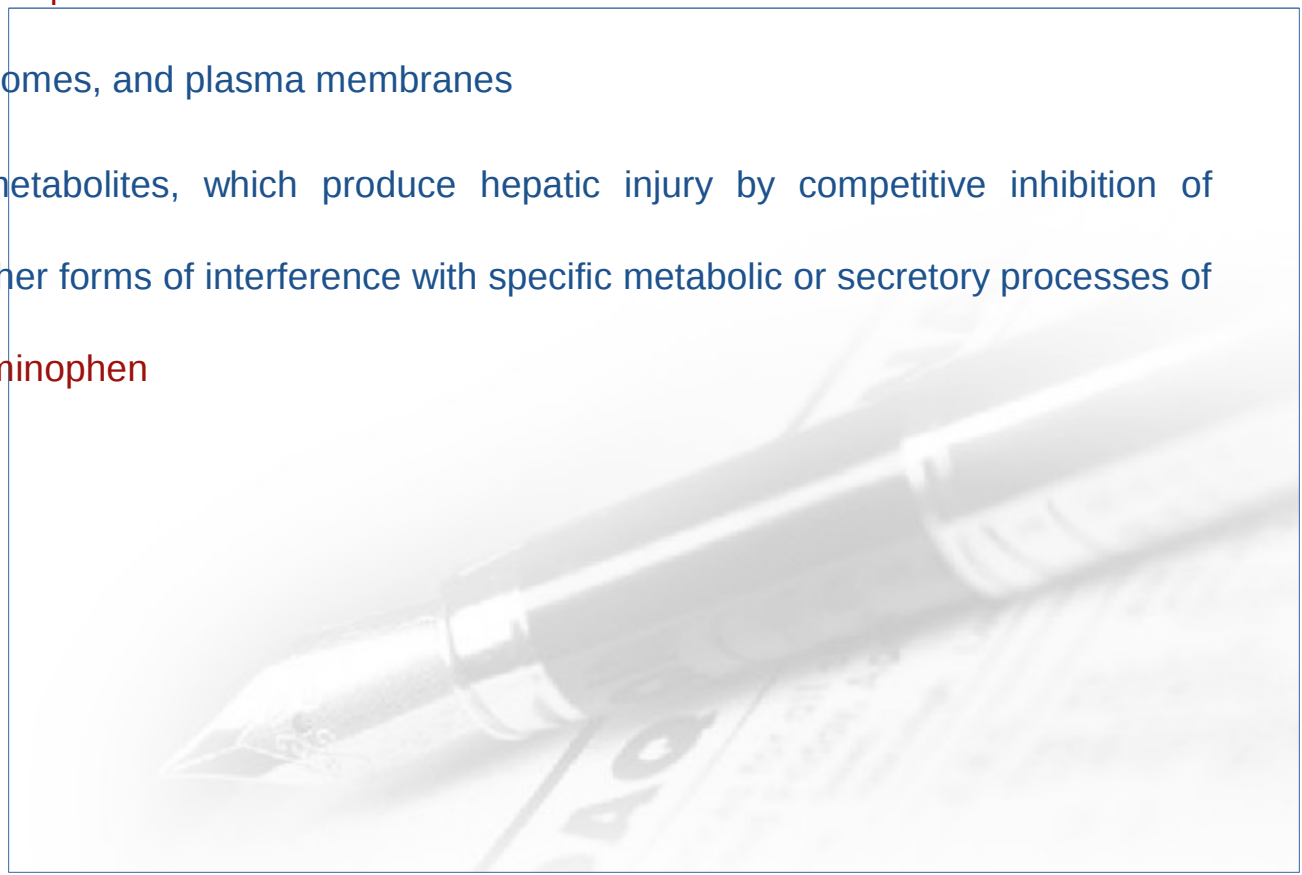
- In the absence ofreliable liver protective drugsin modern medicine, there is a challenge for pharmaceutical scientists to explore the potential of hepatoprotective activity ofplants. In order to develop more precise, safe, cheap and effectivetreatment of liver disorders.

- Isolated hepatocyteshave gained growing interest in pharmacological and toxicological researches for studying thehepatoprotectiveeffects of plant extracts because:-

  - Mostof these studies in laboratory animalsentail large quantitiesof plant extracts.
  - Inaddition,*in vivo*animal modeldo notclearly indicate themechanismof action.

Known Hepatotoxins Models

- Intrinsic hepatotoxins appear to include at least two subcategories, direct and indirect.
- **Direct hepatotoxins** are protoplasmic poisons capable of injuring many tissues, particularly the liver. Carbon tetrachloride (CCL_4) disrupts all elements of the hepatocyte including the endoplasmic reticulum, mitochondria, lysosomes, and plasma membranes
- **Indirect hepatotoxins** are metabolites, which produce hepatic injury by competitive inhibition of essential metabolites or by other forms of interference with specific metabolic or secretory processes of the hepatocyte such as acetaminophen



Hepatoprotective Effects of Plant Extracts

- The aim of our studies was to investigate the protective effects of different plant extracts at different concentrations against hepatotoxicity induced by acetaminophen or CCL_4 in a primary isolated rat hepatocytes
- The predictive protective effect was compared with Silymarin which is one of the most known potent hepatoprotective agents.
- The benefits of these studies is the discovery of active compounds which will be developed to successful drugs for protection of liver disorders.

List of Some Proved Hepatoprotective Plant Extracts

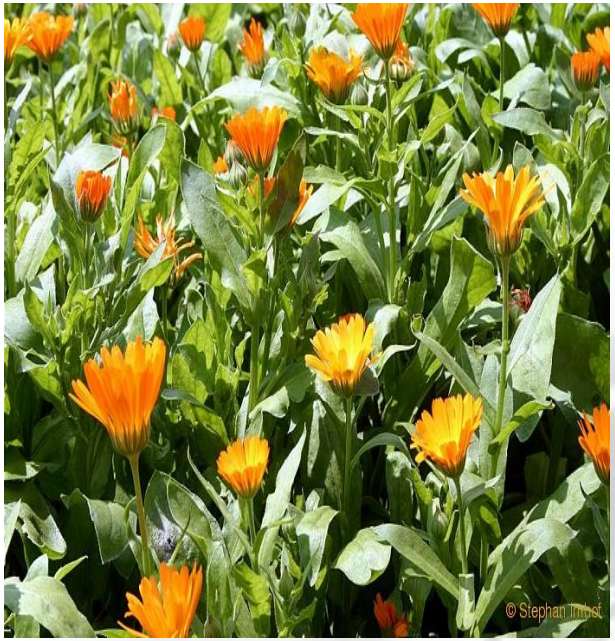
- *Olive Leaves*
- *Chichorium endivia plant*
- *Rubus sanctus*
- *Morus alba leaves and fruits*
- *Magnolia grandiflora*
- *Calendula officinalis Flower*
- *Mentha longifolia plant leaves*
- *Nigella sativa (Thymoquinone)*

Chichorium endivia plant

Mentha longifolia leaves

Rubus sanctus

Morus alba leaves and fruits



Calendula officinalis Flower

Olive Leaves

Distribution ofRubusSanctus in Saint Catherine's Monastery, Sinai, Egypt

RubusSanctus inside Saint Catherine's Monastery

Olive Leaves Findings

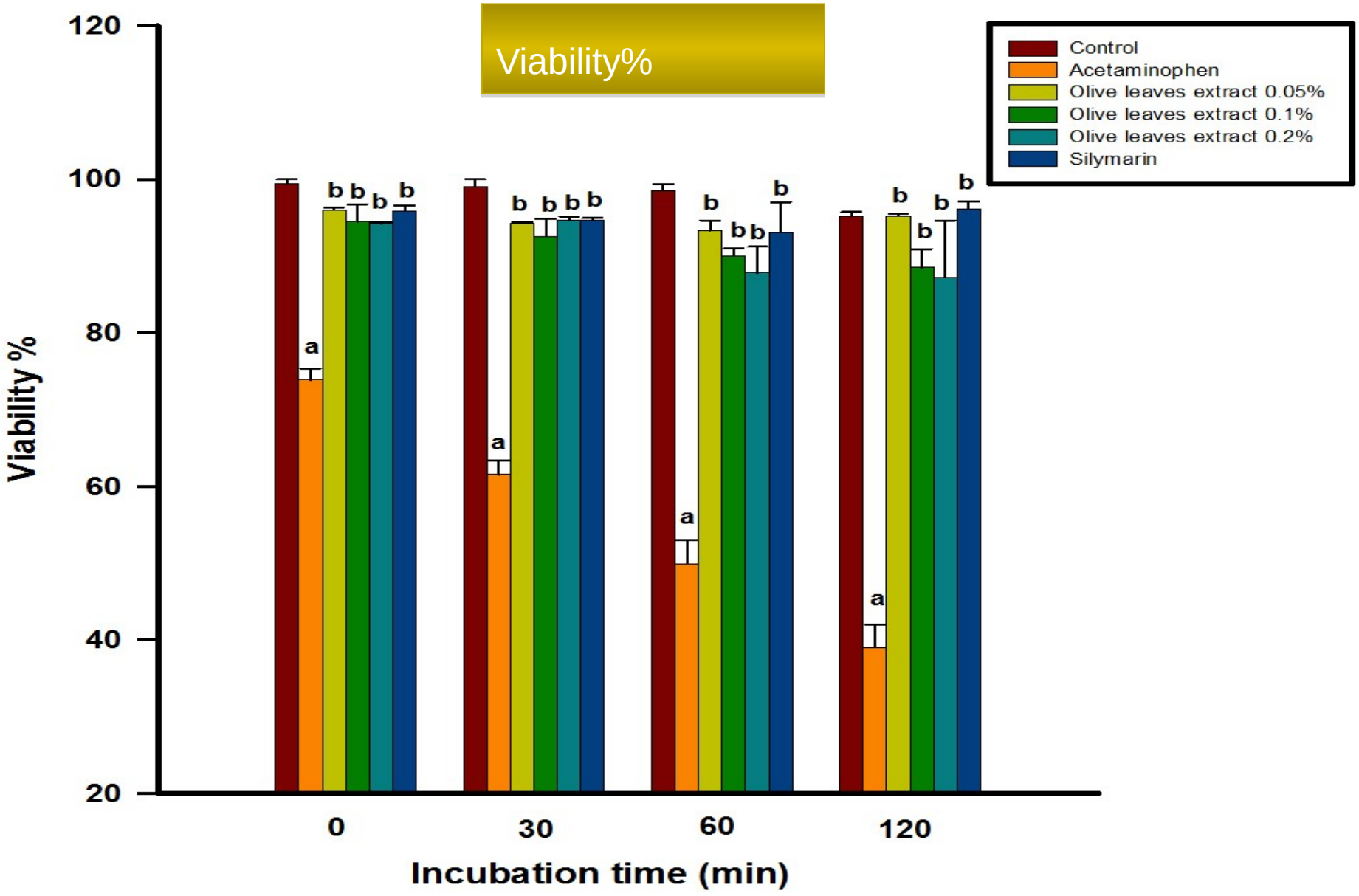
as an examples of Hepatoprotective Plant Extracts

Olive leavesextract has apromisinghepatoprotective effects:

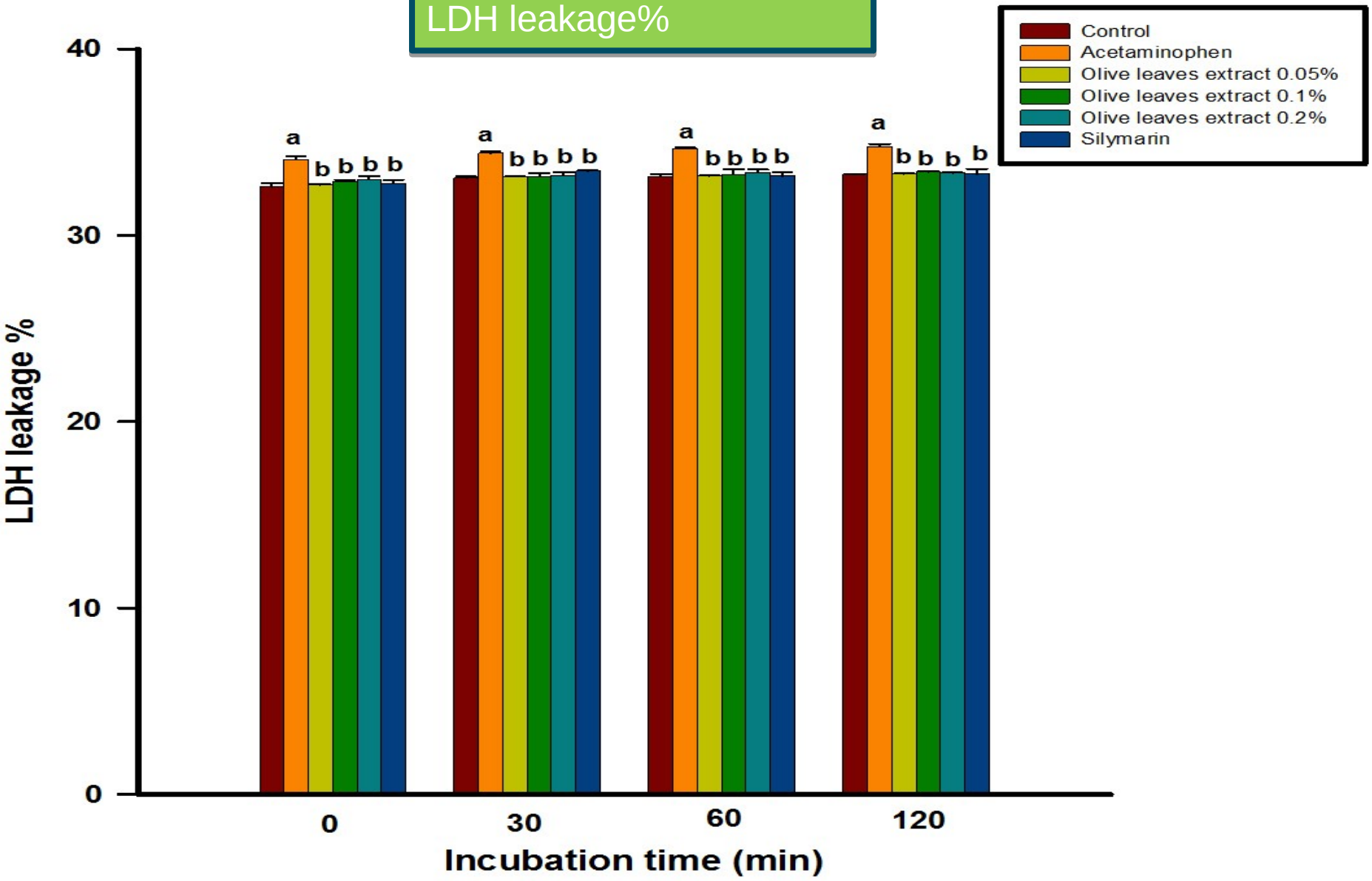
- Improvescell survival.
- Helpsto maintain the integrity of cellular membranes.
- Preservesthe intracellular antioxidant defense system.
- Preventsthe development of sever cyto-pathological effects of acetaminophen.

Therefore our studyrecommendeda rational basis for the use of olive leaves against hepatotoxicity.

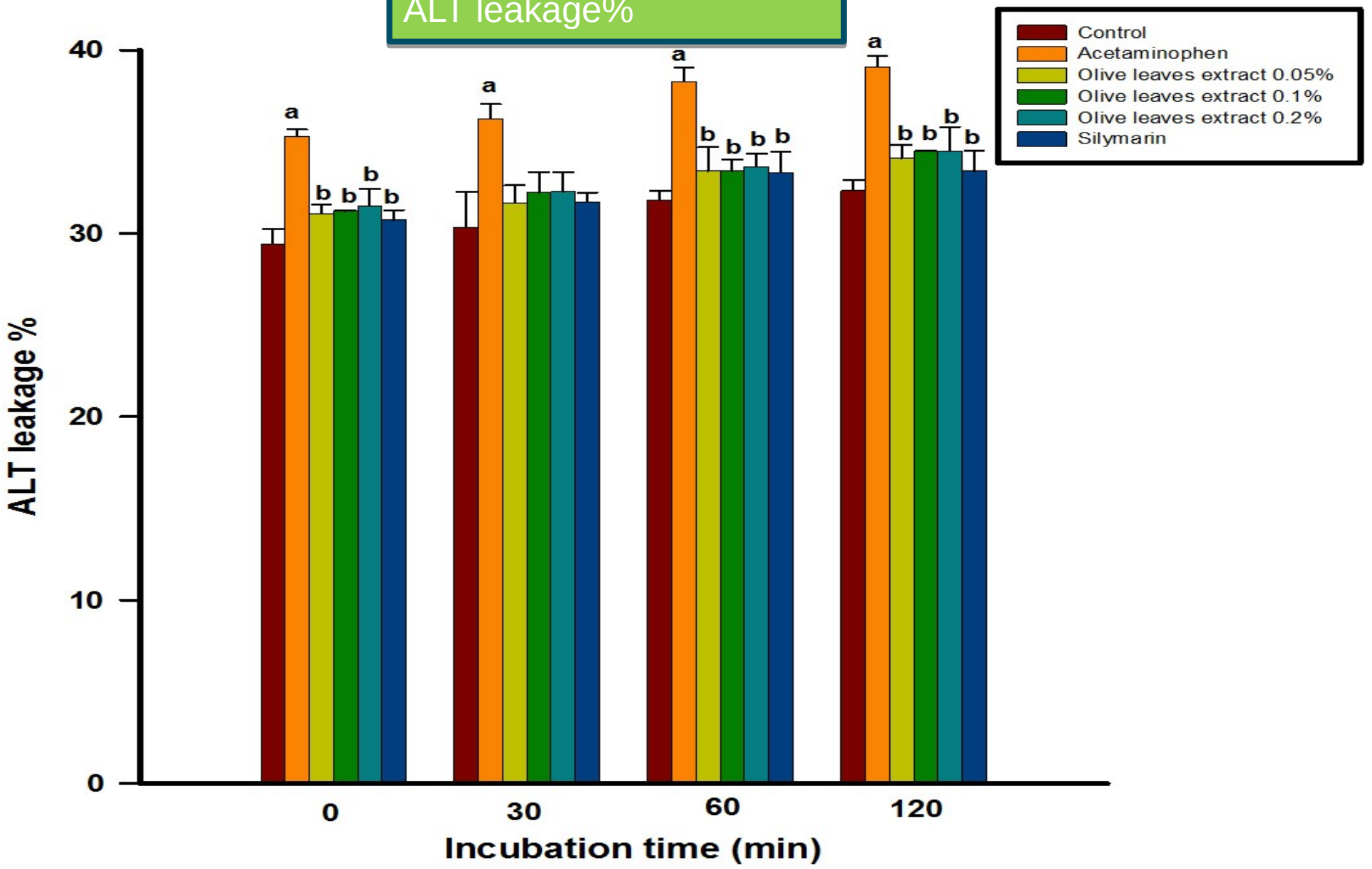
Olive leavescanprovidean effective, cheap and accessible nativesourceof human and animalfeed additiveto conventional treatment of hepatic injurers complicated by xenobiotics.

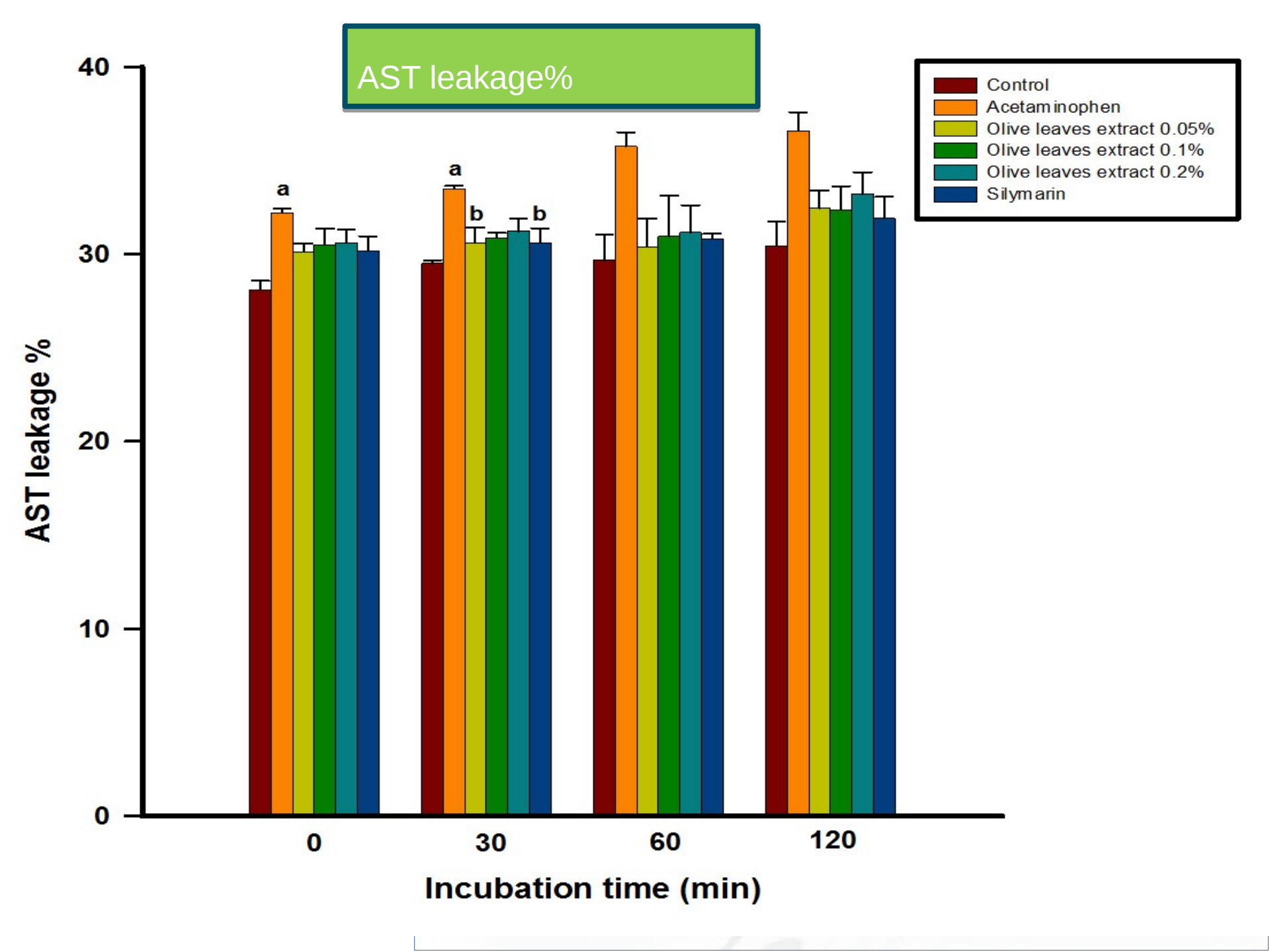

Viability%
Control
Acetaminophen
Olive leaves extract 0.05%
Olive leaves extract 0.1%
Olive leaves extract 0.2%
Silymarin
Viability %
120
100
80
60
40
20
0
30
60
120
Incubation time (min)
a
b

LDH leakage%

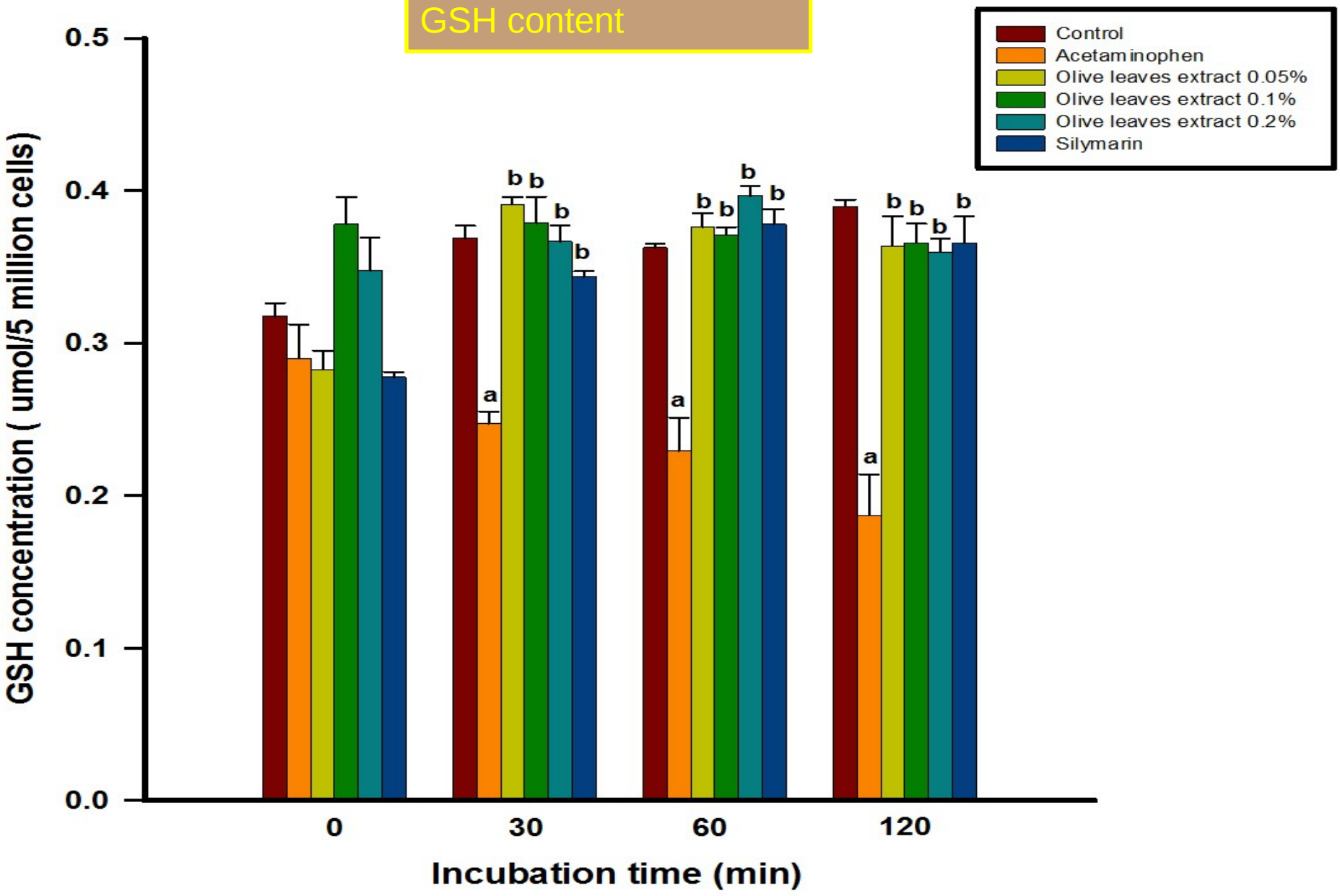

Control
Acetaminophen
Olive leaves extract 0.05%
Olive leaves extract 0.1%
Olive leaves extract 0.2%
Silymarin
LDH leakage %
0
10
20
30
40
a
b b b b
a
b b b b
a
b b b b
a
b b b b
0
30
60
120
Incubation time (min)

ALT leakage%

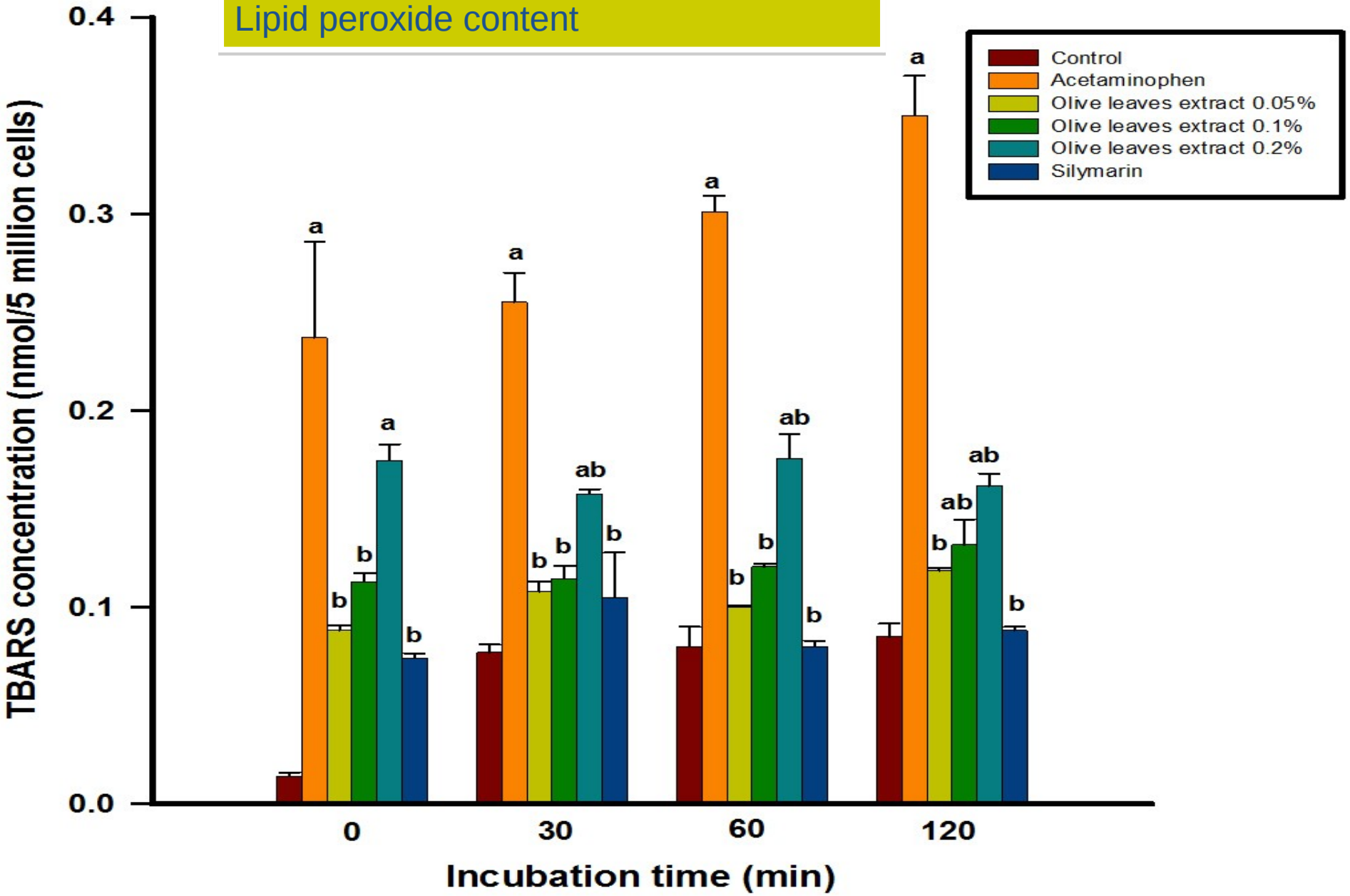

Control
Acetaminophen
Olive leaves extract 0.05%
Olive leaves extract 0.1%
Olive leaves extract 0.2%
Silymarin
ALT leakage %
0
10
20
30
40
0
30
60
120
Incubation time (min)
a
b


AST leakage%
Control
Acetaminophen
Olive leaves extract 0.05%
Olive leaves extract 0.1%
Olive leaves extract 0.2%
Silymarin
AST leakage %
40
30
20
10
0
a
a
b
b
0
30
60
120
Incubation time (min)

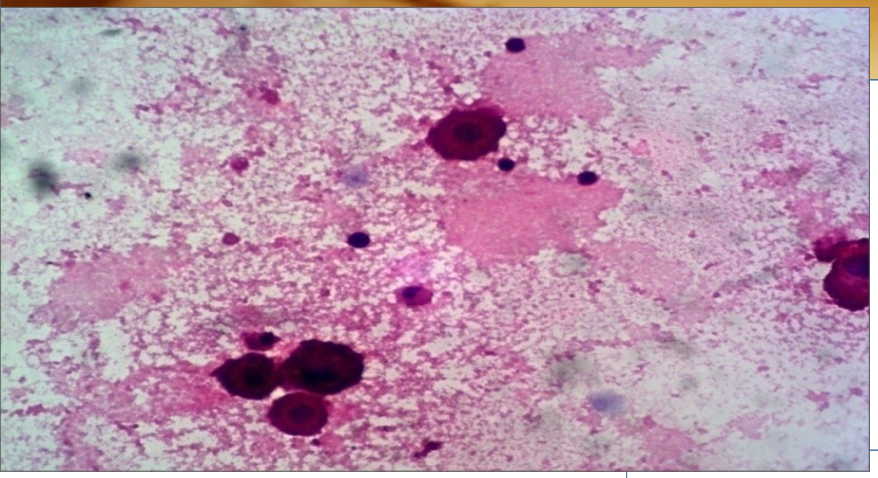
GSH content


Control
Acetaminophen
Olive leaves extract 0.05%
Olive leaves extract 0.1%
Olive leaves extract 0.2%
Silymarin
GSH concentration (umol/5 million cells)
0.0
0.1
0.2
0.3
0.4
0.5
a
b
0
30
60
120
Incubation time (min)

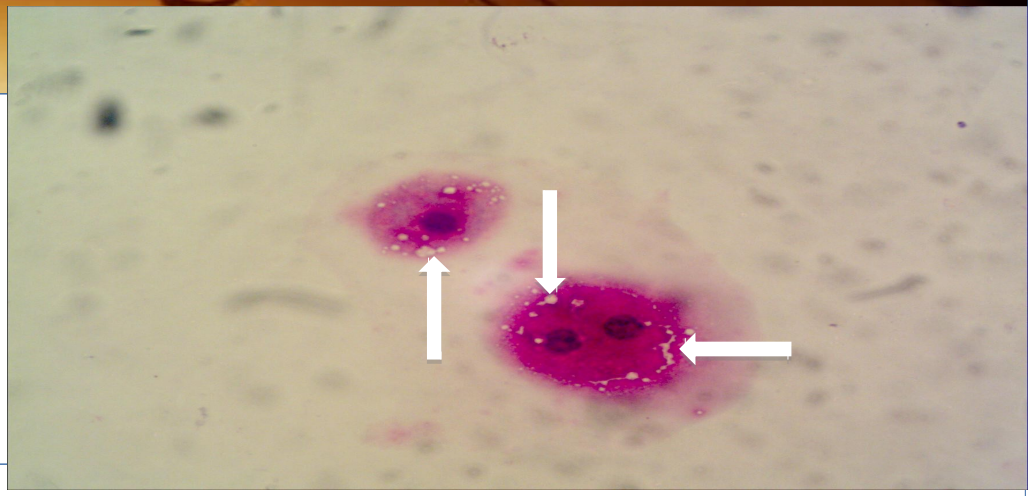
Lipid peroxide content



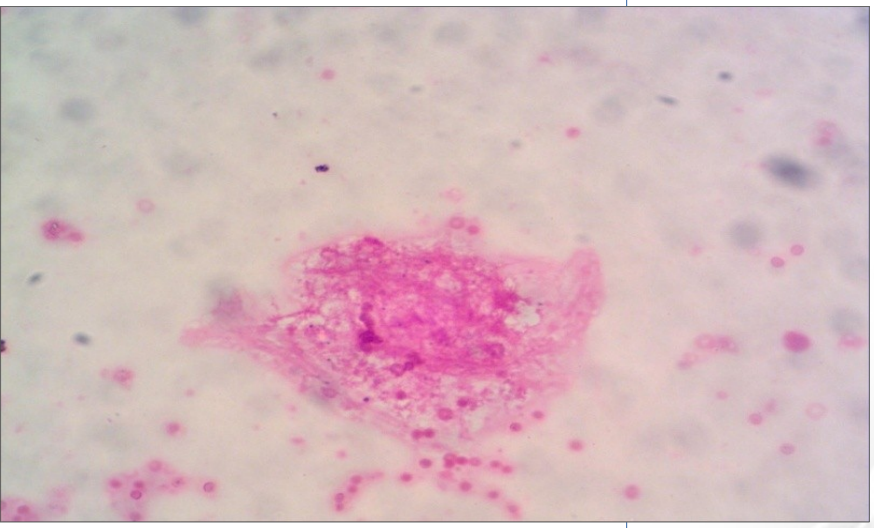
Light microscopical examination



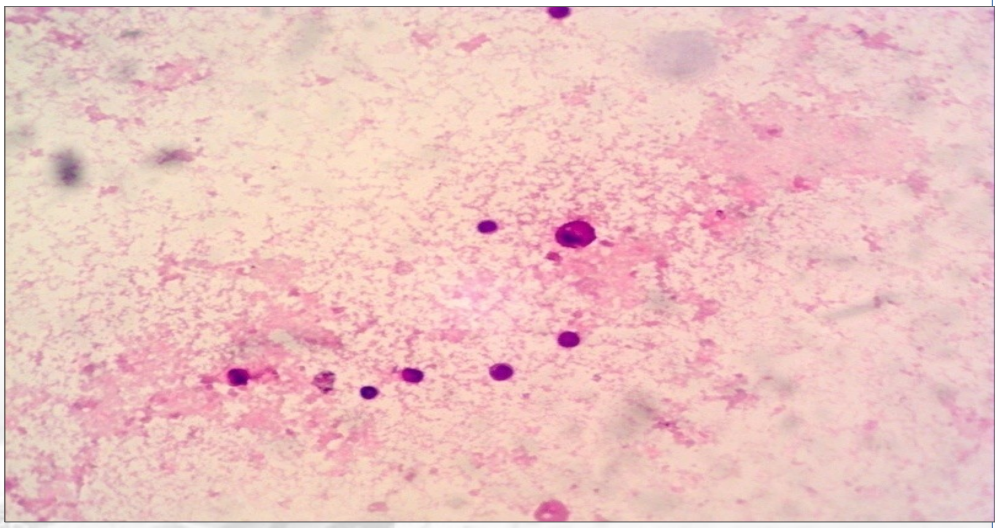
Hepatocytes of control group revealed normal structure of hepatocytes (H&EX400).



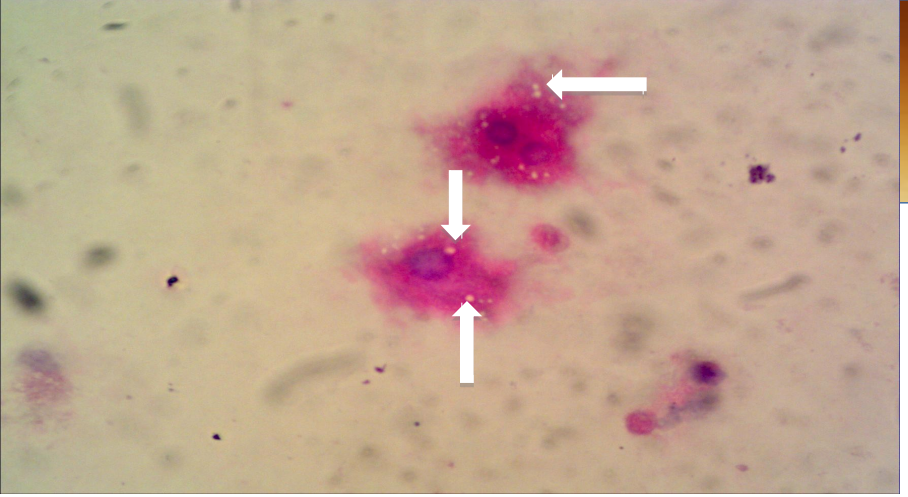
hepatocytes of acetaminophen group at 60 min of the incubation period revealed the presence of high number of large sized vacuoles (H&EX400)



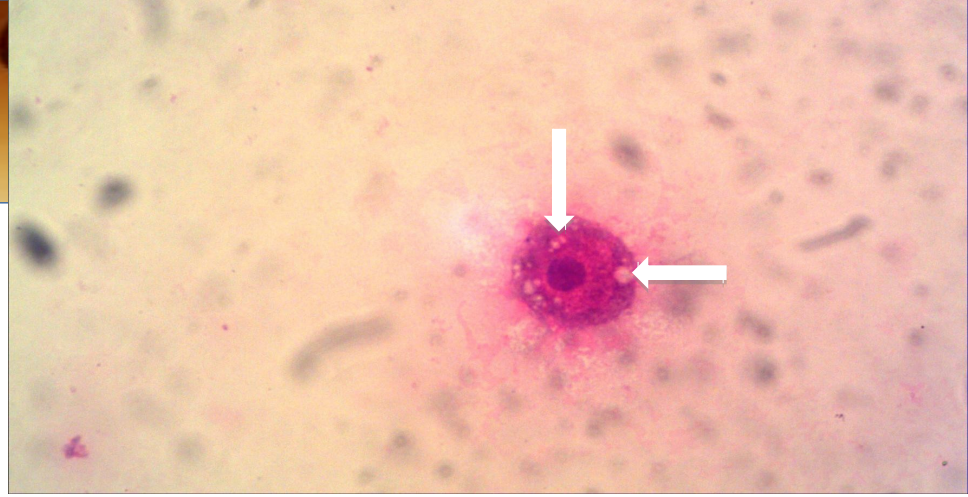
hepatocytes of acetaminophen group at 120 min of the incubation period revealed loss of cells nuclei (H&EX400).



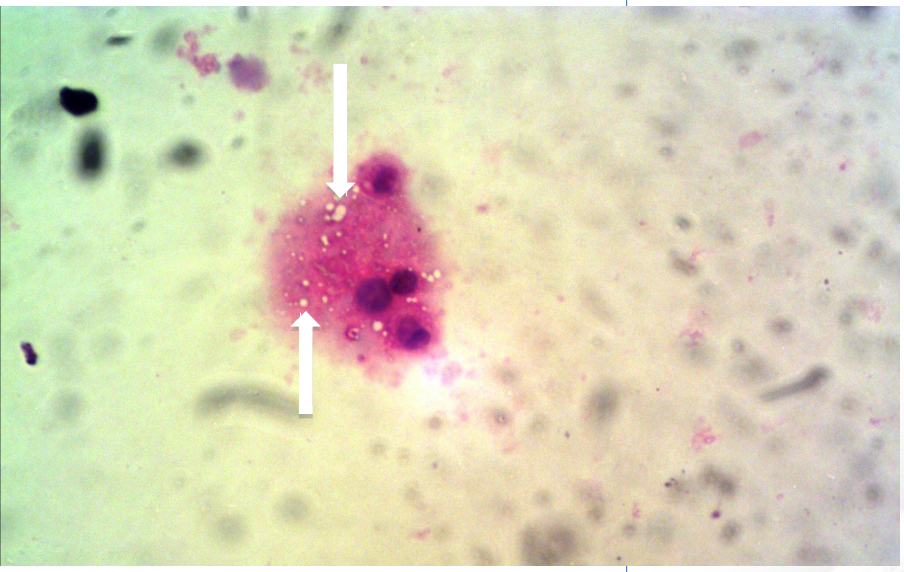
Hepatocytes of acetaminophen group at 120 min of the incubation period revealed the presence of multiple mononuclear cells around the necrotic cells (H&EX400).



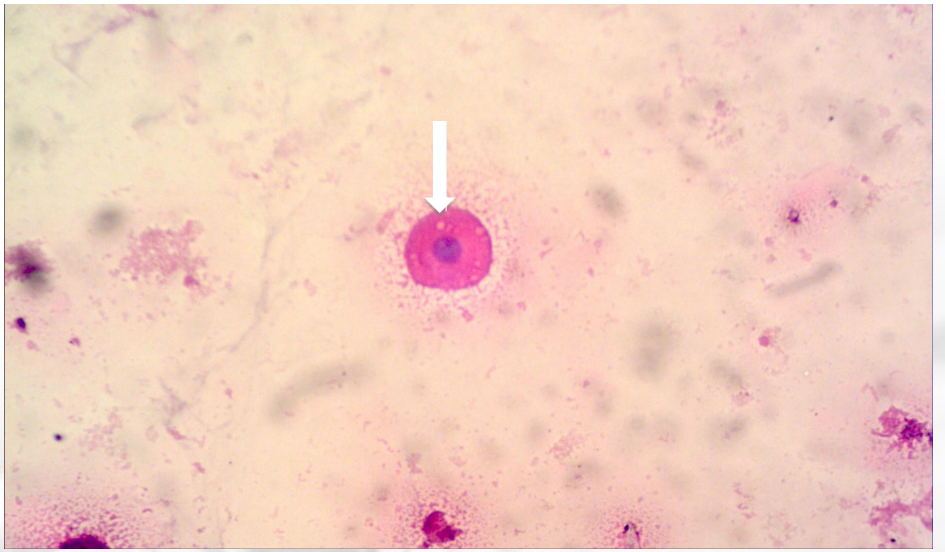
hepatocytes of 0.05% w/v olive leaves extract revealed the presence of few small to medium sized vacuoles in the cytoplasm(H&EX400).



hepatocytes of 0.1 % w/v olive leaves extract group revealed the presence of many small, medium and large sized vacuoles in the cytoplasm (H&EX400).

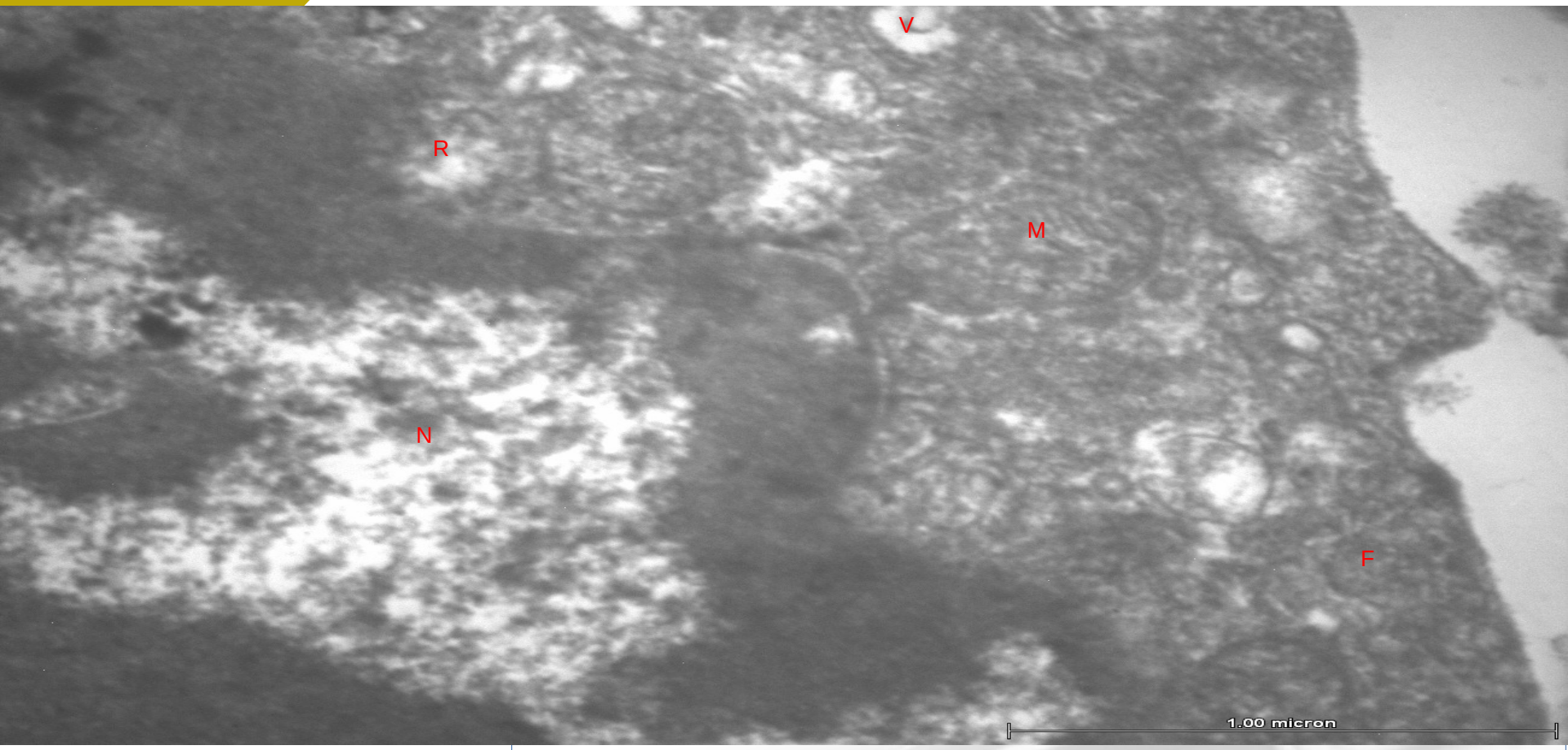


hepatocytes of 0.2 % w/v olive leaves extract group revealed the presence of high numberofsmall, medium and large sized vacuoles in the cytoplasm (H&EX400)



hepatocytes of silymarin group at 30 min of the incubation period revealed the presence of few small to medium sized cytoplasmic vacuoles(H&EX400).

Electronmicroscope examination



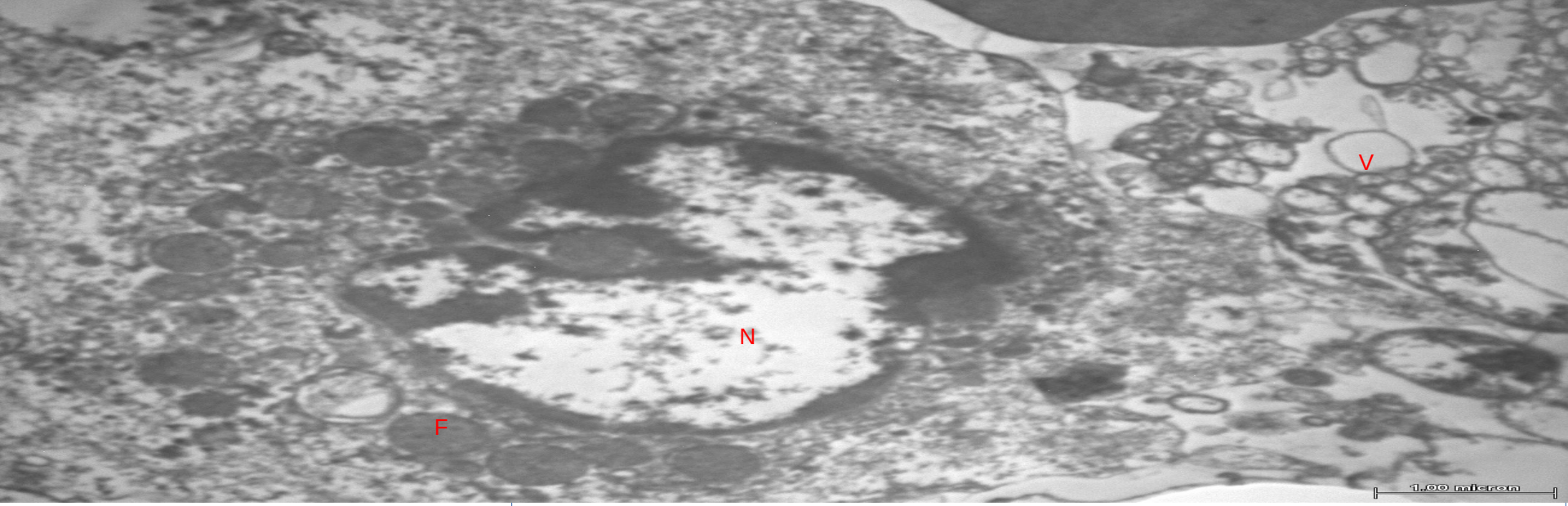
hepatocytes ofcontrol groupshowing relatively preserved organelles.

The nucleus (N) has a marginal condensation of chromatin.

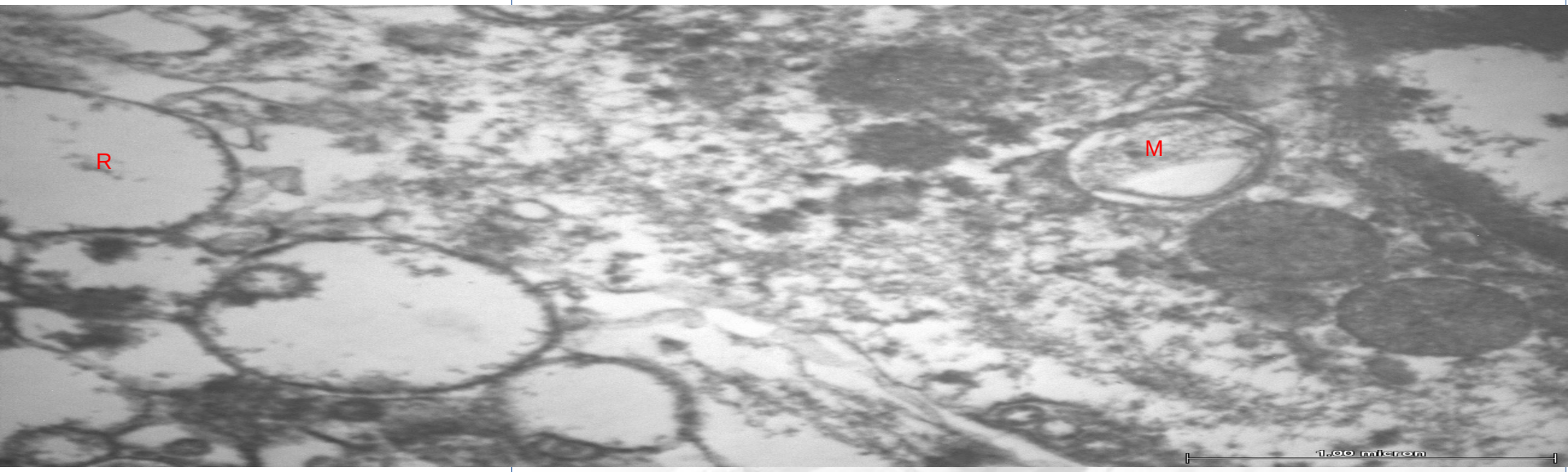
The mitochondria (M) are slightly dilated with relatively preserved cristae.

The rough endoplasmic reticulum (R) is slightly dilated.

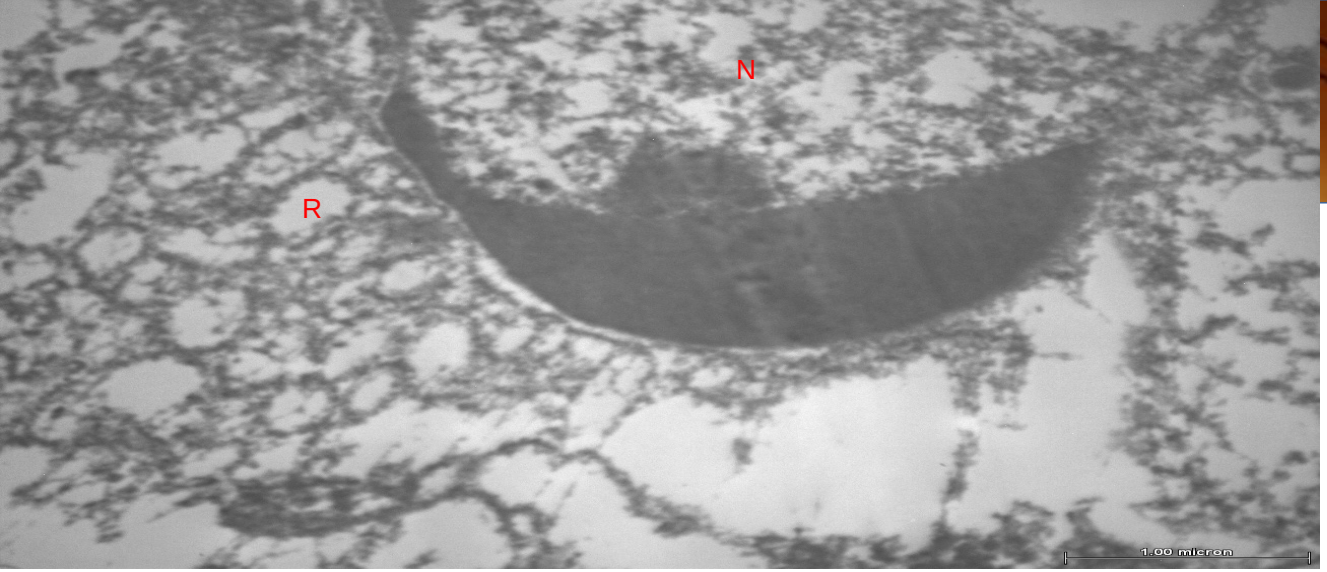
Vacuoles (V) and fat globules (F) are very limited in number and size.



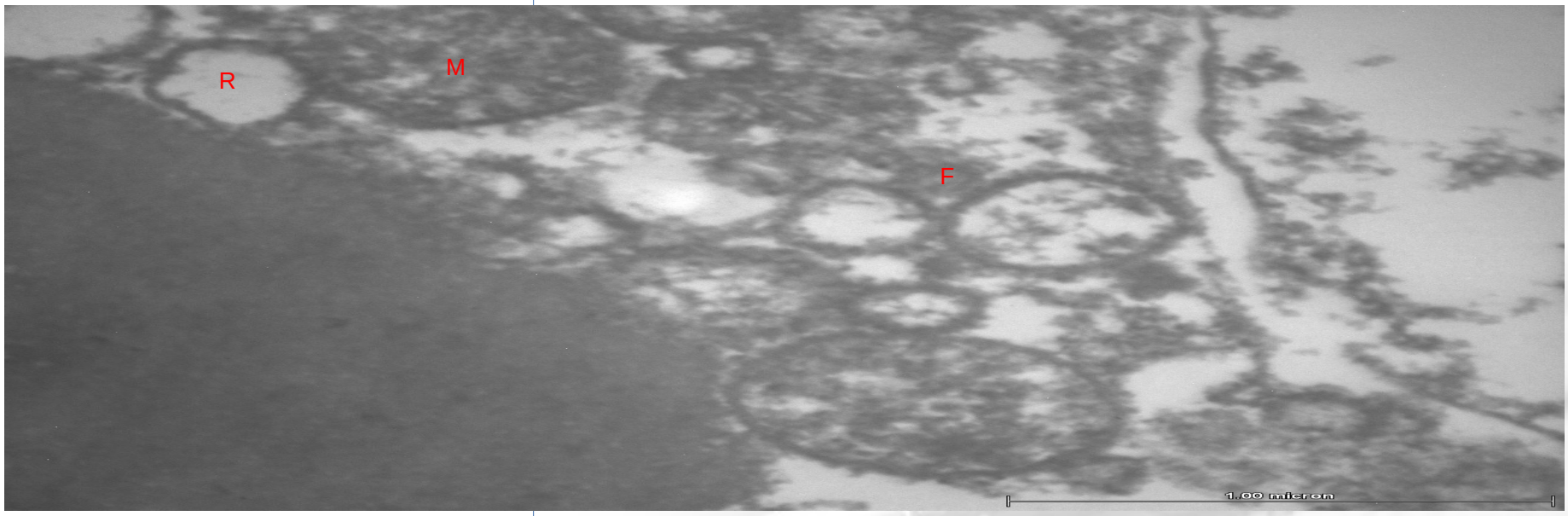
hepatocytes of acetaminophen group showing karyolysis of the nucleus (N). Many moderate to large vacuoles (V) and fat globules (F) are seen.



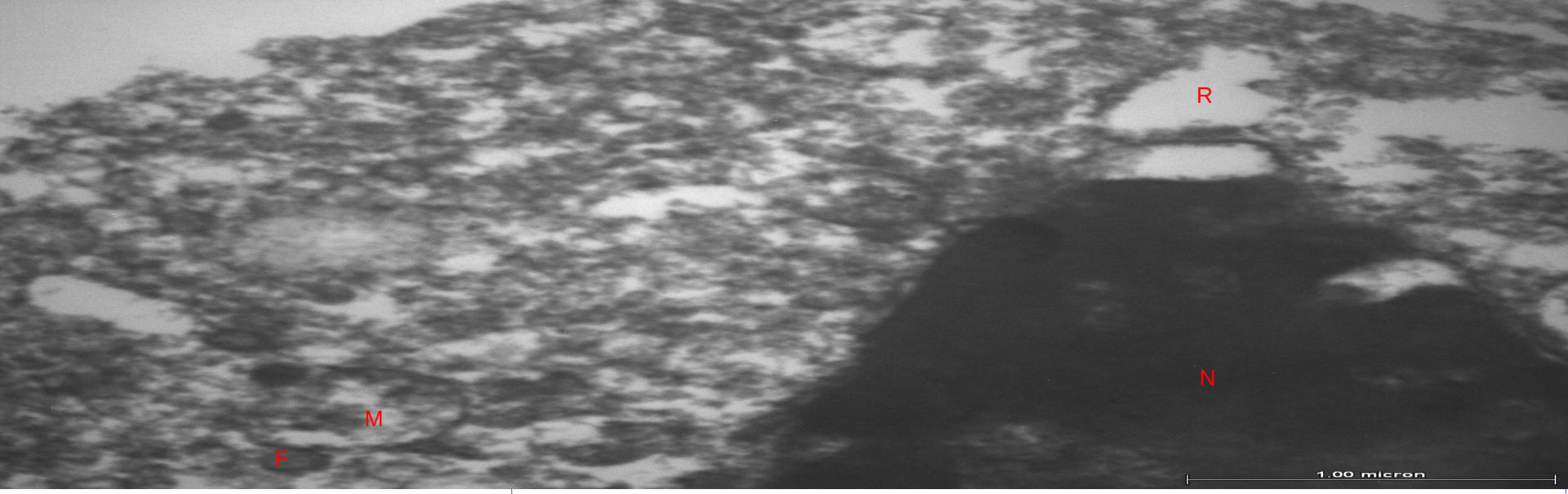
hepatocytes of acetaminophen group showing degeneration and sever dilatation of mitochondria (M) and rough endoplasmic reticulum (R). Remnant of cristae are seen.



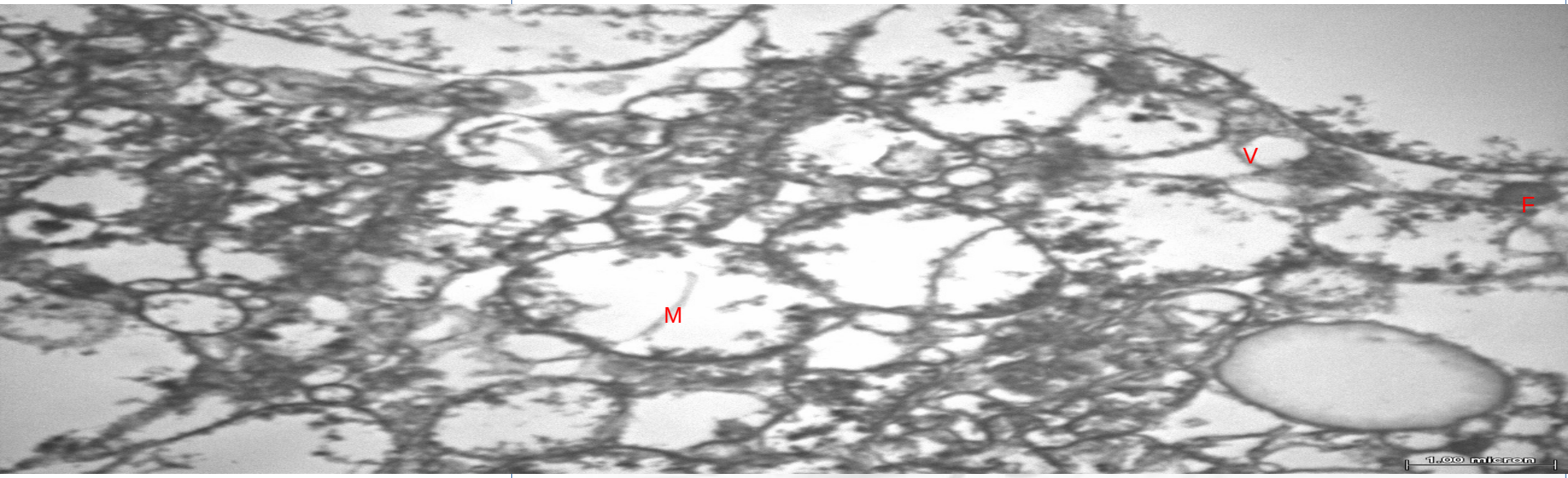
hepatocytes of 0.05 % w/v olive leaves extract group showing
partial condensation of the chromatin of their nuclei (N).
The rough endoplasmic reticulum (R) is moderately dilated.



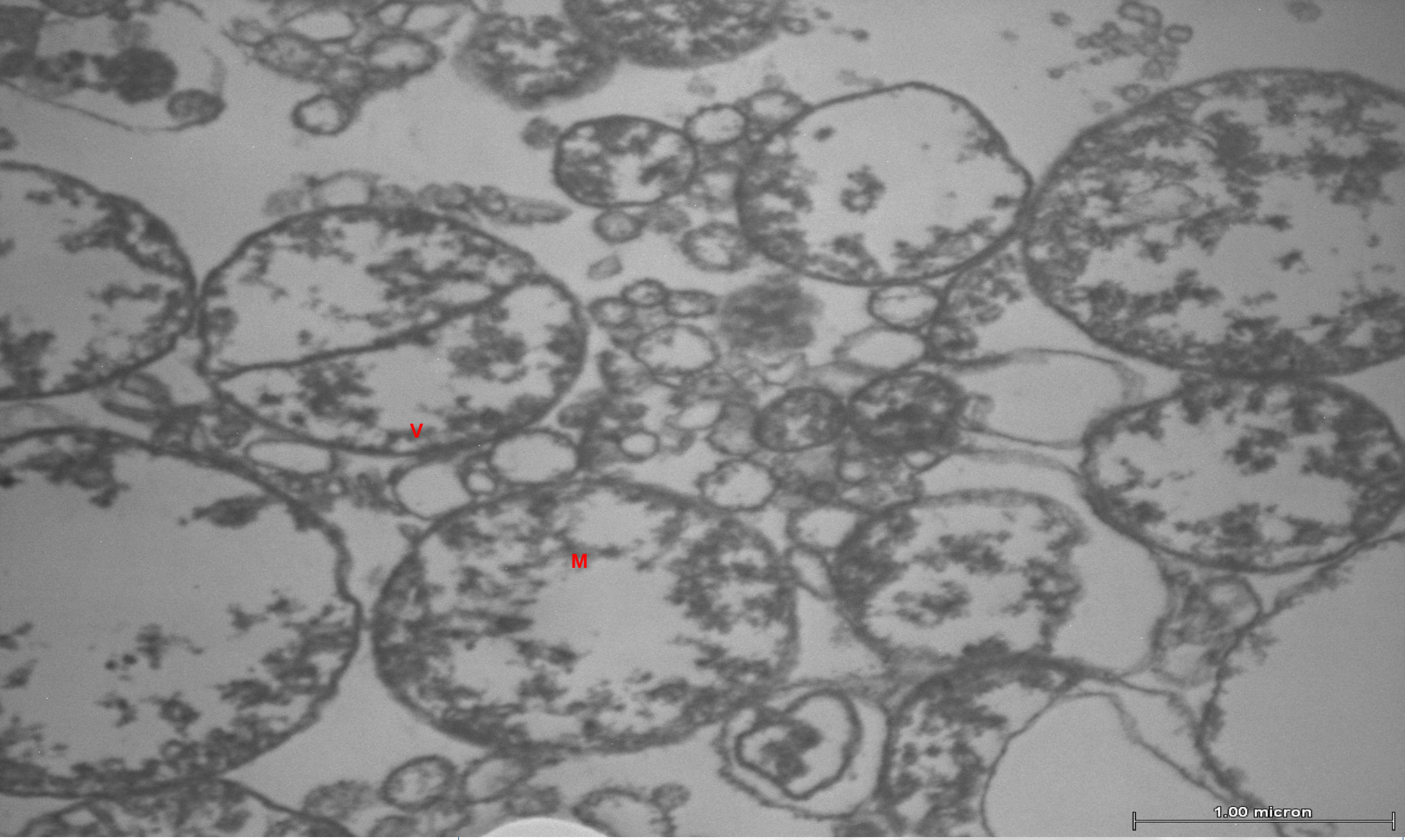
hepatocytes of 0.05 % w/v olive leaves extract group showing moderately dilated mitochondria (M) with some relatively preserved cristae. The rough endoplasmic reticulum (R) is moderately dilated.
Many small fat globules (F) are seen.



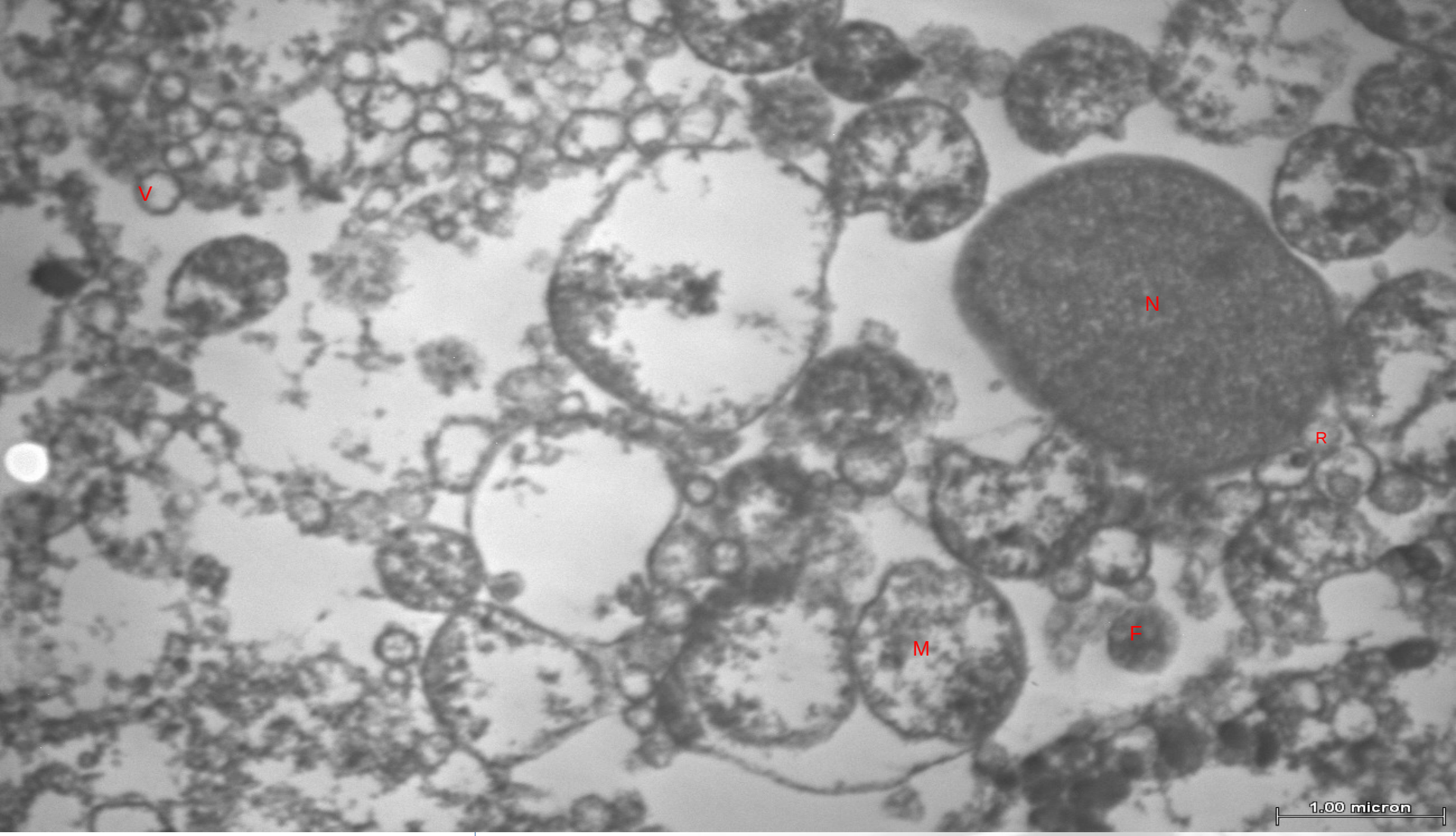
hepatocytes of 0.1 % w/v olive leaves extract group showing complete condensation of the chromatin of the nucleus (N). The mitochondria (M) are moderately dilated with some relatively preserved cristae. The rough endoplasmic reticulum (R) is moderately dilated. Many small fat globules (F) are seen.



hepatocytes of 0.1 % w/v olive leaves extract group showing moderately dilated mitochondria (M) with some relatively preserved cristae. Many small to large vacuoles (V) and fat globules (F) are seen.



hepatocytes of 0.2 % w/v olive leaves extract group showing highly dilated mitochondria (M) with some relatively preserved cristae. Many small to large vacuoles (V) and small fat globules (F) are seen.



hepatocytes of silymarin group showing homogenously distributed chromatin in their nuclei (N).

The mitochondria (M) are moderately dilated with relatively preserved cristae.

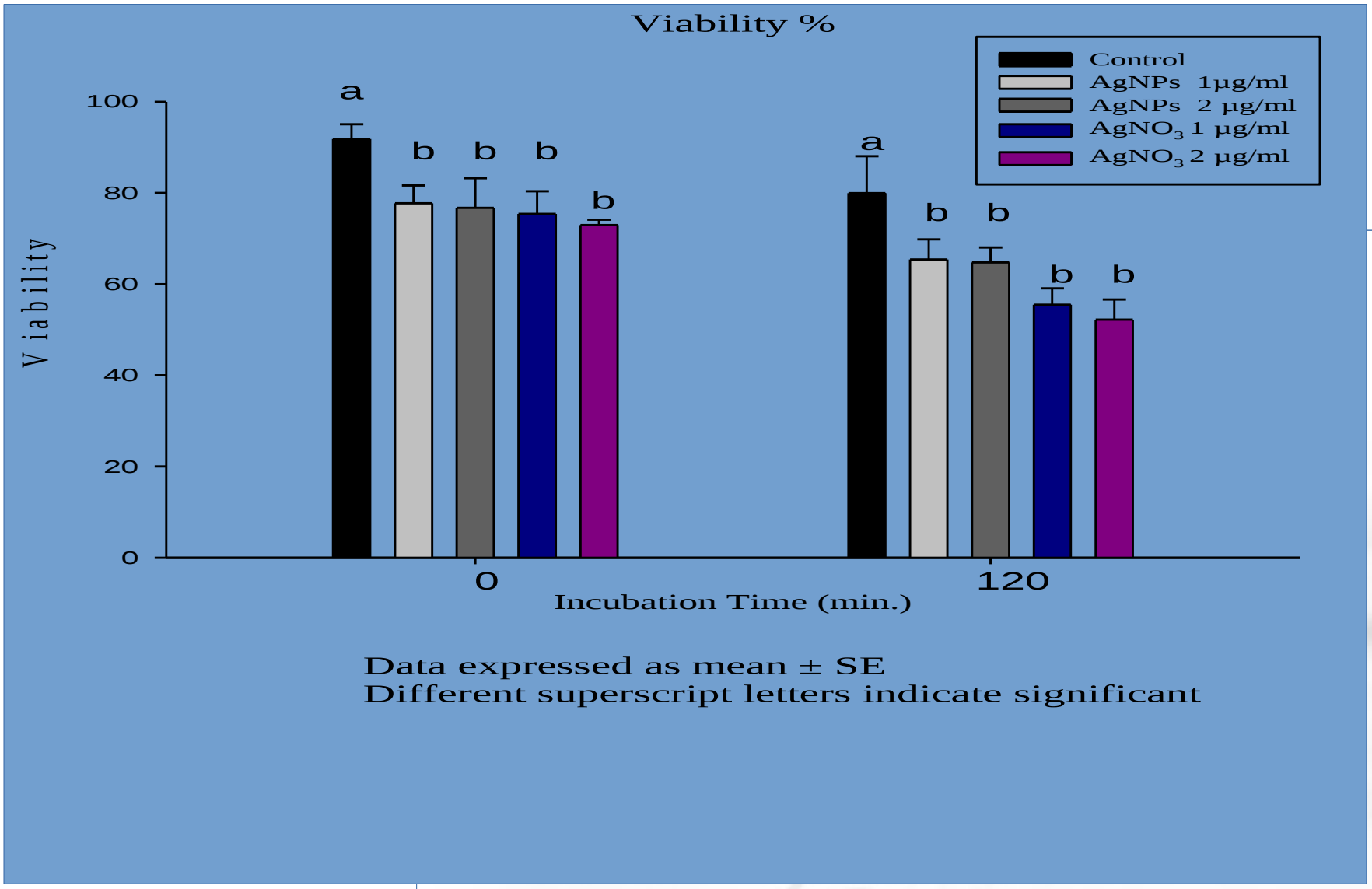
The rough endoplasmic reticulum (R) is slightly dilated.

Many small vacuoles (V) and fat globules (F) are seen.

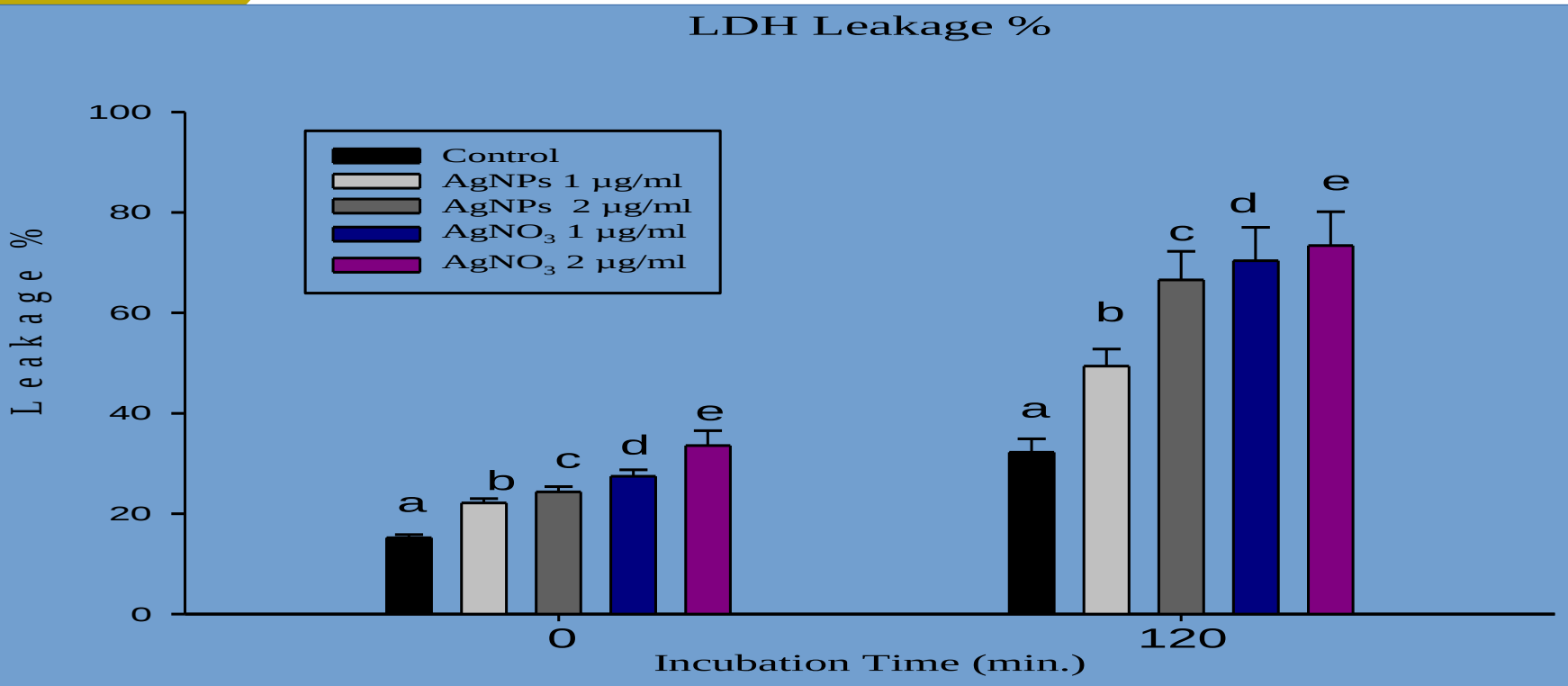
Comparison between Cytotoxicity andGenotoxicityof SilverNanoparticlesandMicroparticleson Isolated hepatocytes

- Silver nanoparticles(AgNPs) have a wide array ofapplicationsincluding food packaging, odor-resistant textiles, antimicrobial agents, household appliances and medical devices
- There is limitednanotoxicologicalinformation ofsilver nanoparticles on isolated hepatocytes
- The cytotoxic and Genotoxic effects ofsilver microparticleswere more noticeable than in nanoparticles in dose- and time-dependent manner.
- Findings recommend thesafety of silver nanoparticlesin its low-dose form, if we wereurged to useit in novel industrial applications.
- However, moreinvestigations are neededto assess the risk of higher doses of AgNPs and/or the safety of longer exposures to silver nanoparticles before their extensive use in future industries

Effect of AgNPs & $AgNO_3$ on cell viability

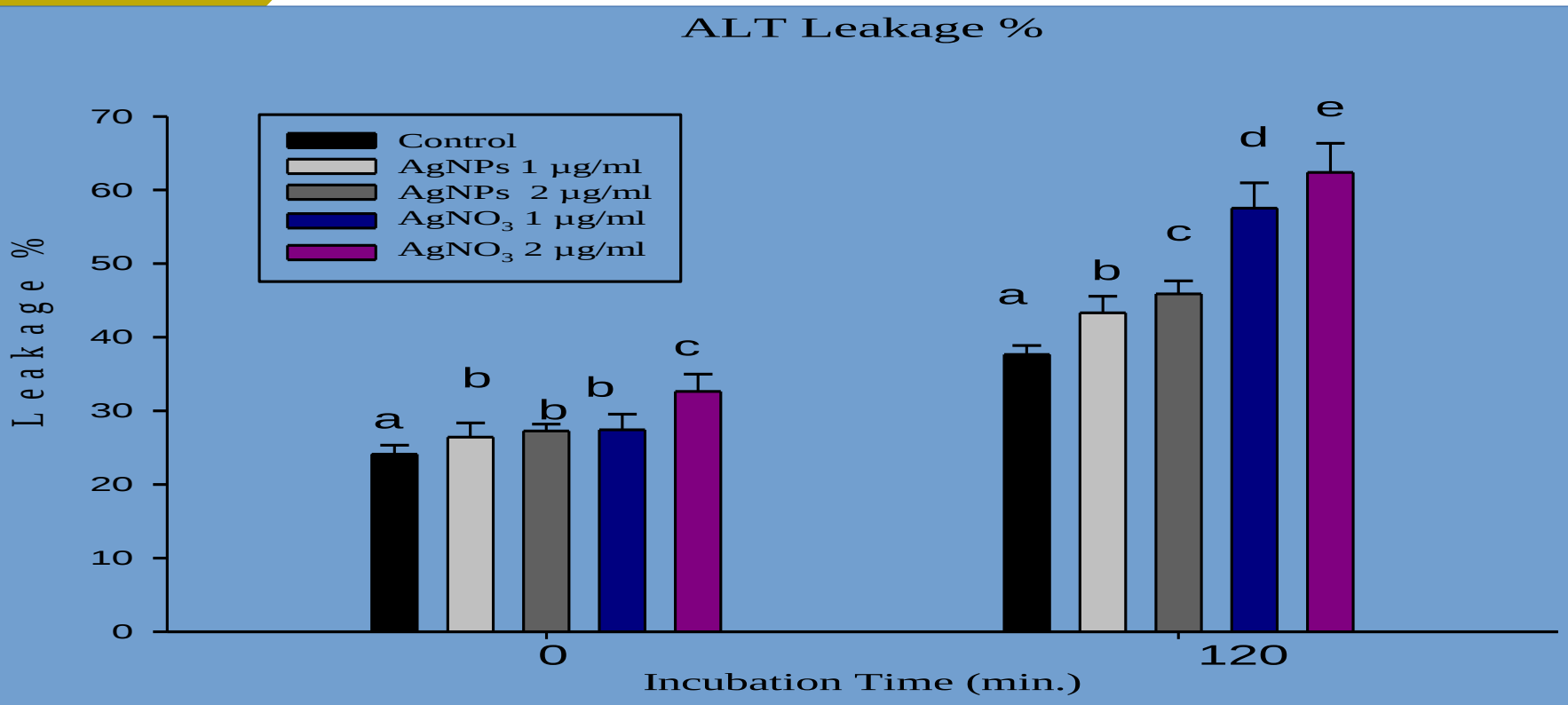


Effect of AgNPs & $AgNO_3$ on LDH Leakage



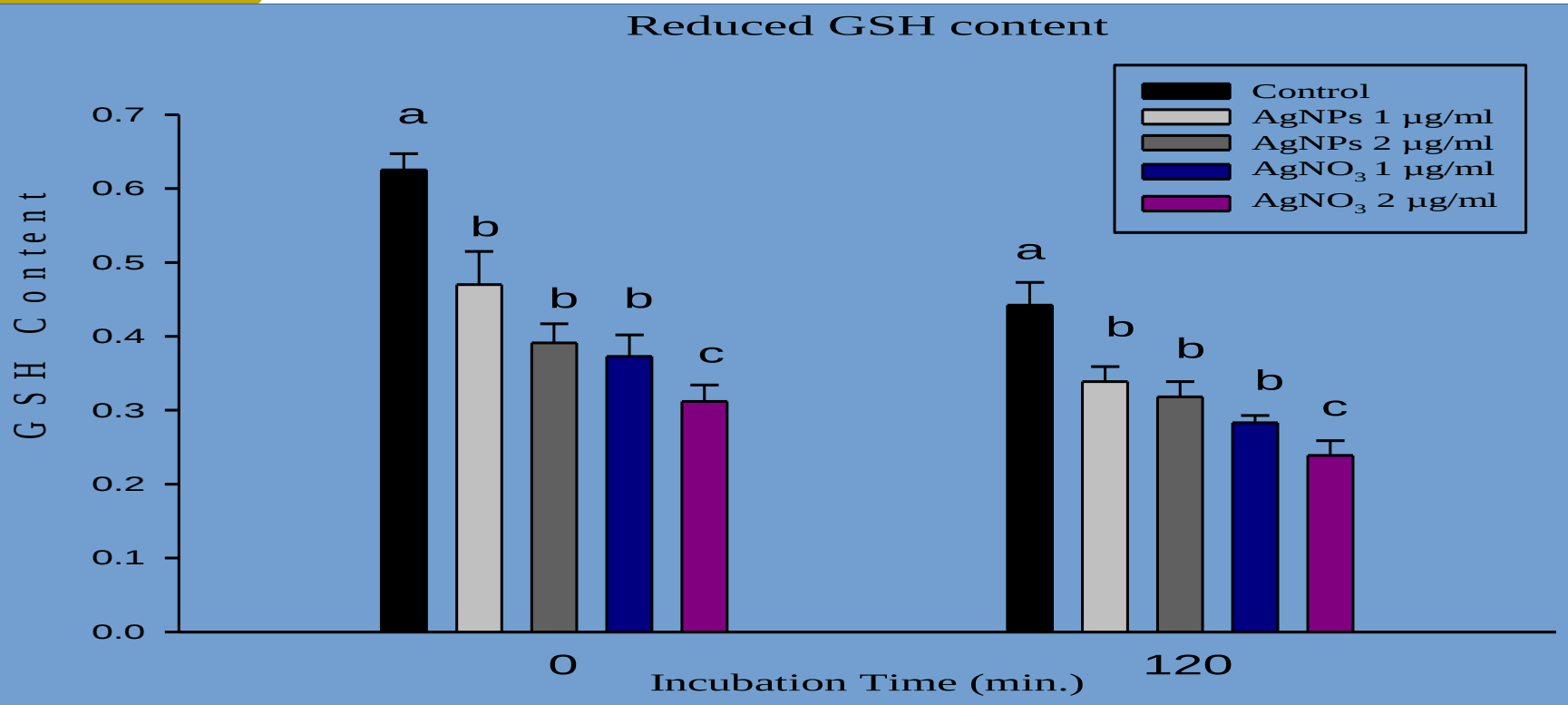
Data expressed as mean ± SE
Different superscript letters indicate significant

Effect of AgNPs & $AgNO_3$ on ALT Leakage



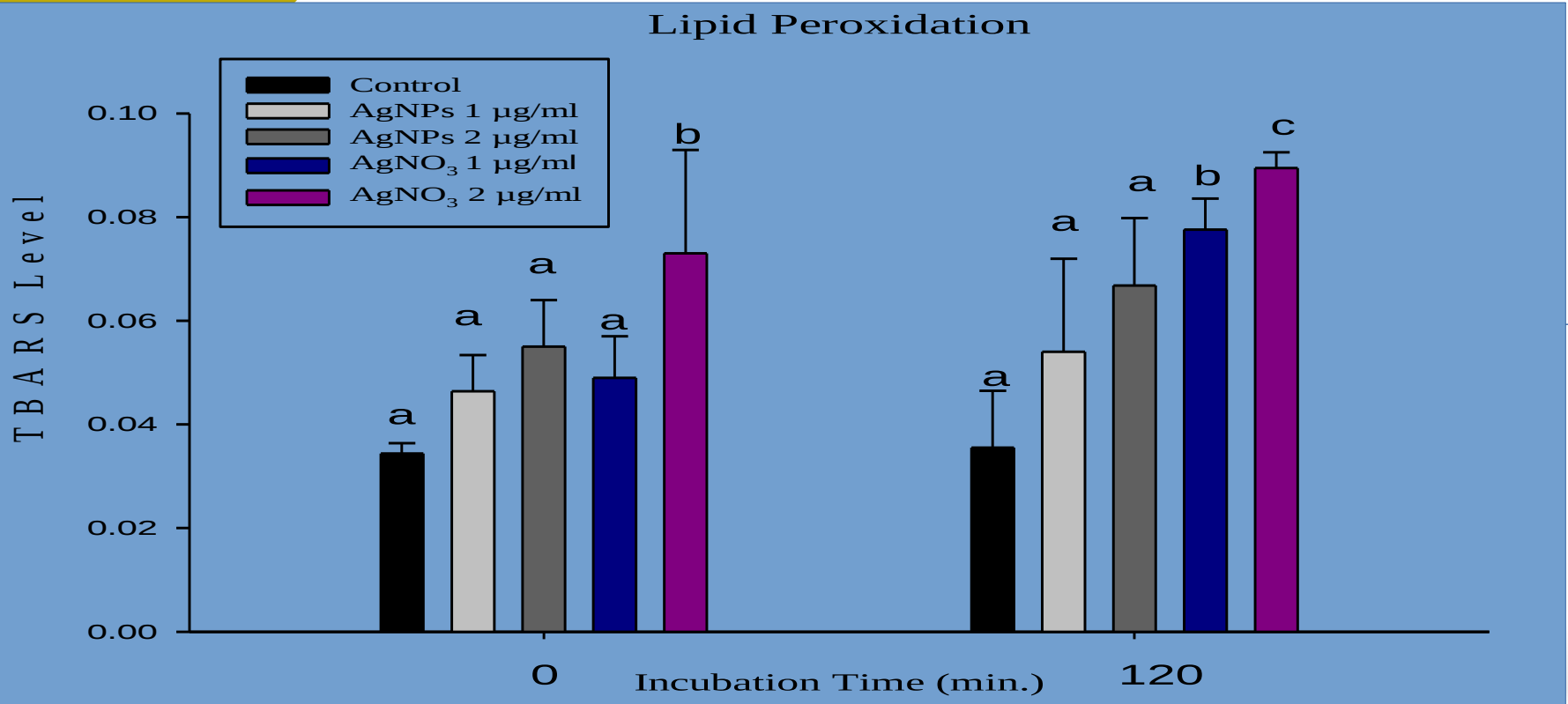
Data expressed as mean ± SE
Different superscript letters indicate significant

Effect of AgNPs & $AgNO_3$ on cellular GSH Content



Data expressed as mean ± SE
Different superscript letters indicate significant

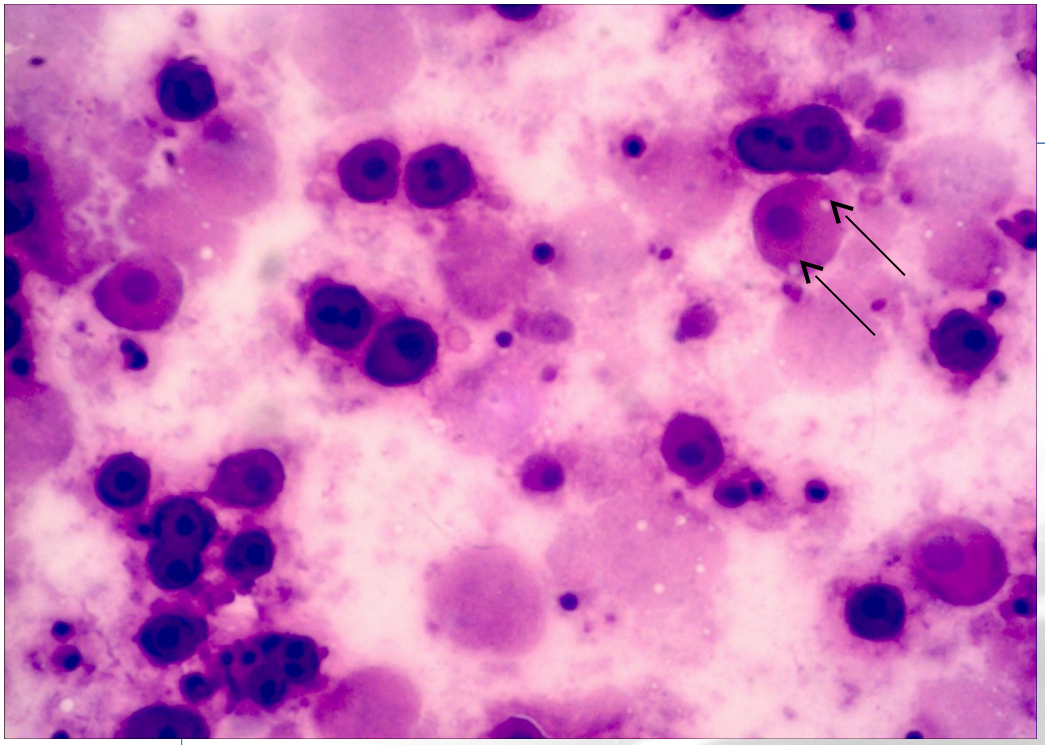
Effect of AgNPs & $AgNO_3$ on Lipid Peroxidation



Data expressed as mean ± SE
Different superscript letters indicate significant

Effects on cell morphology

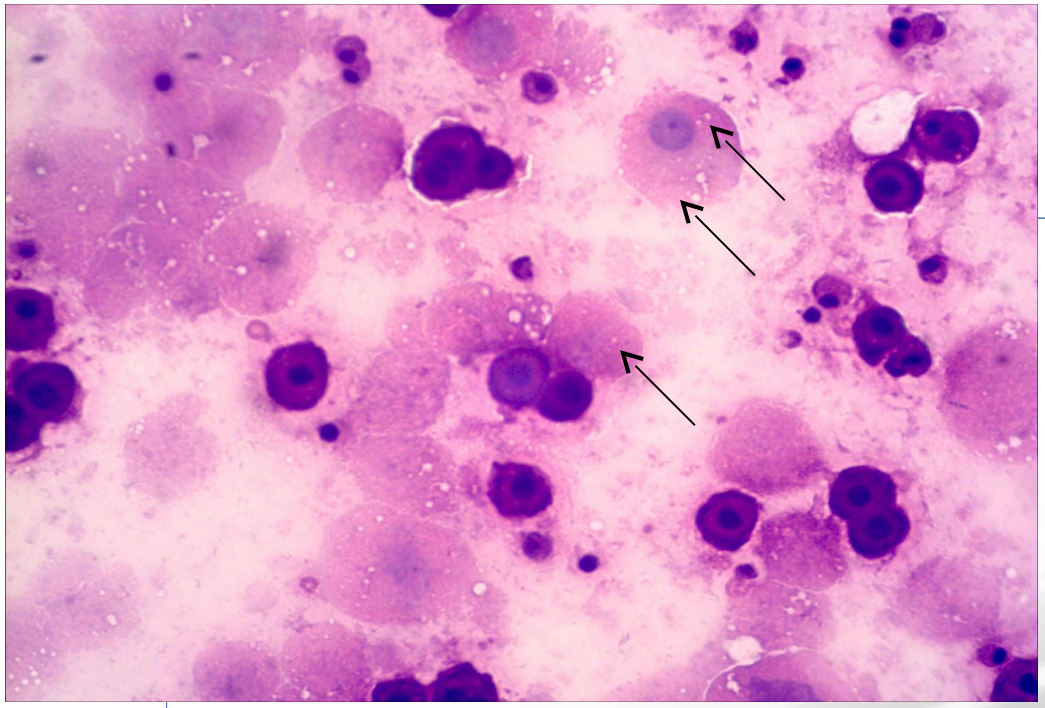
1µg/ml AgNPs



small vacuoles in the cytoplasm of most of hepatocytes

Effects on cell morphology

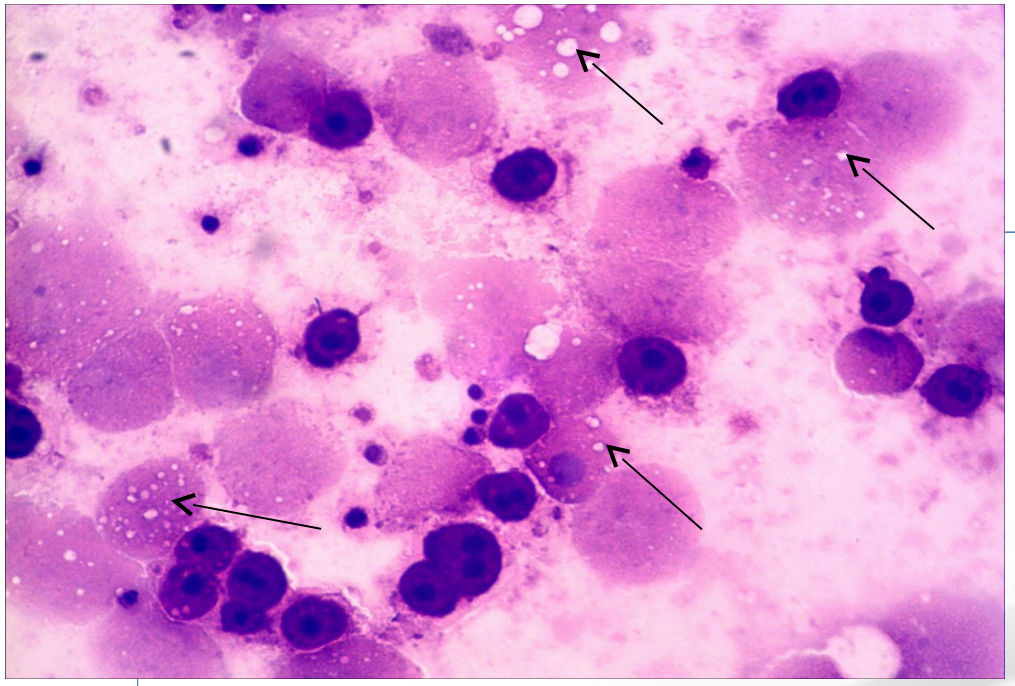
2µg/ml AgNPs



multiple vacuoles in the cytoplasm of some hepatocytes

Effects on cell morphology

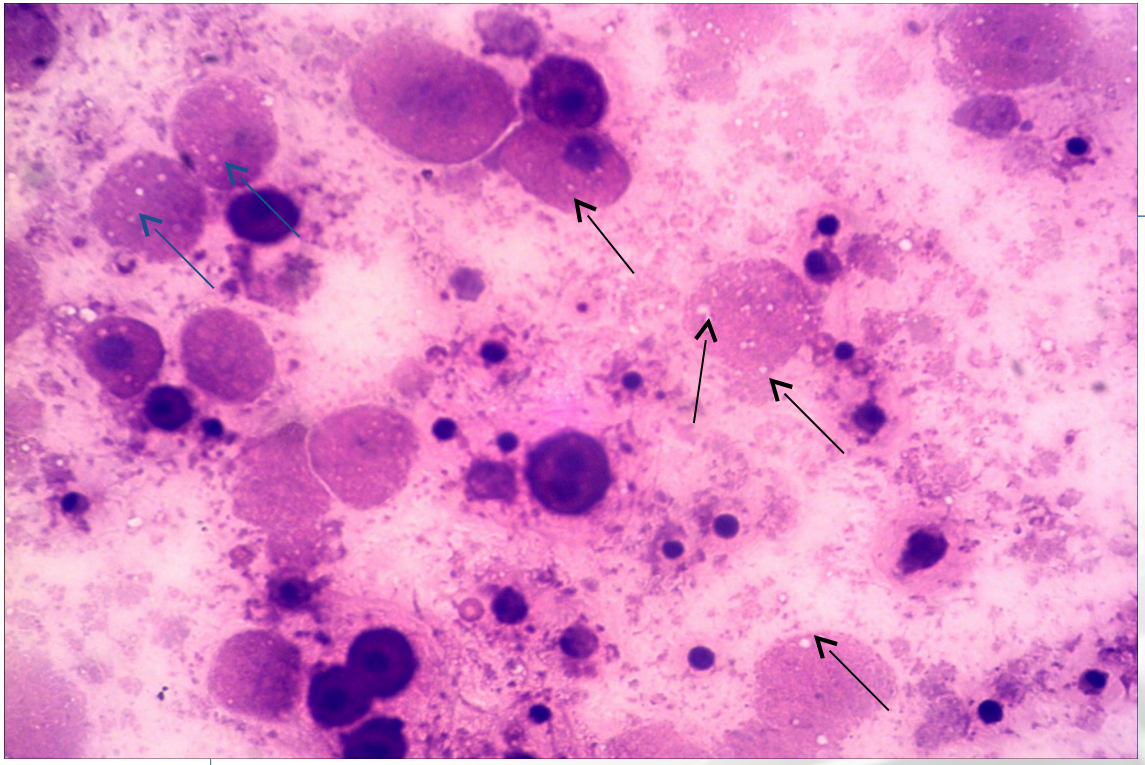
1µg/ml $AgNO_3$



Multiple large vacuoles in the cytoplasm of most of hepatocytes

Effects on cell morphology

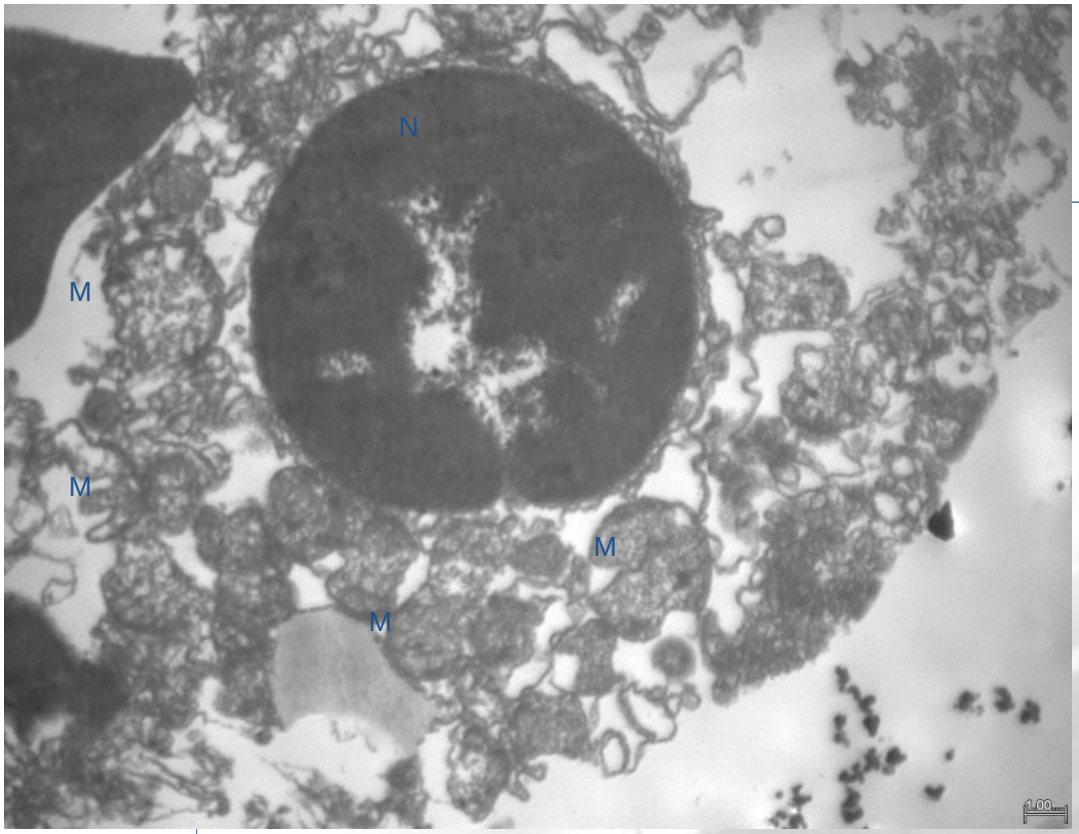
2µg/ml $AgNO_3$



Large vacuoles in the cytoplasm of most of hepatocytes

Effects on cell morphology / Electron microscope

1µg/ml AgNPs

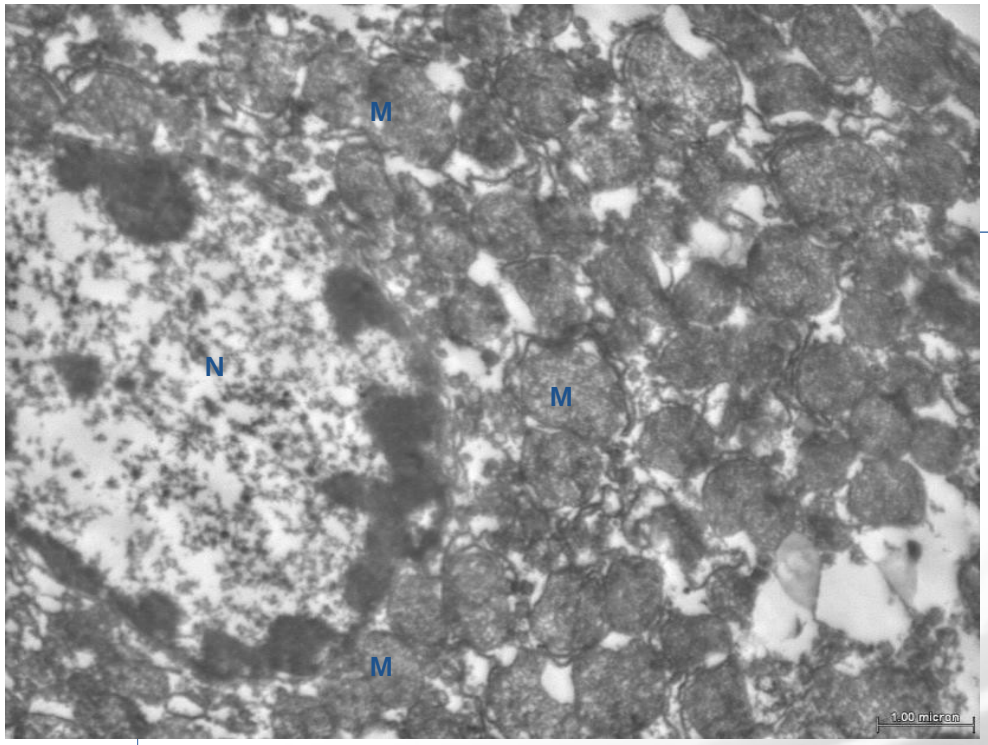


Low numbers of cytoplasmic vacuoles, degeneration of some mitochondria (M) and nuclear(N) pyknosis

(irreversible condensation of chromatin in the nucleus

Effects on cell morphology

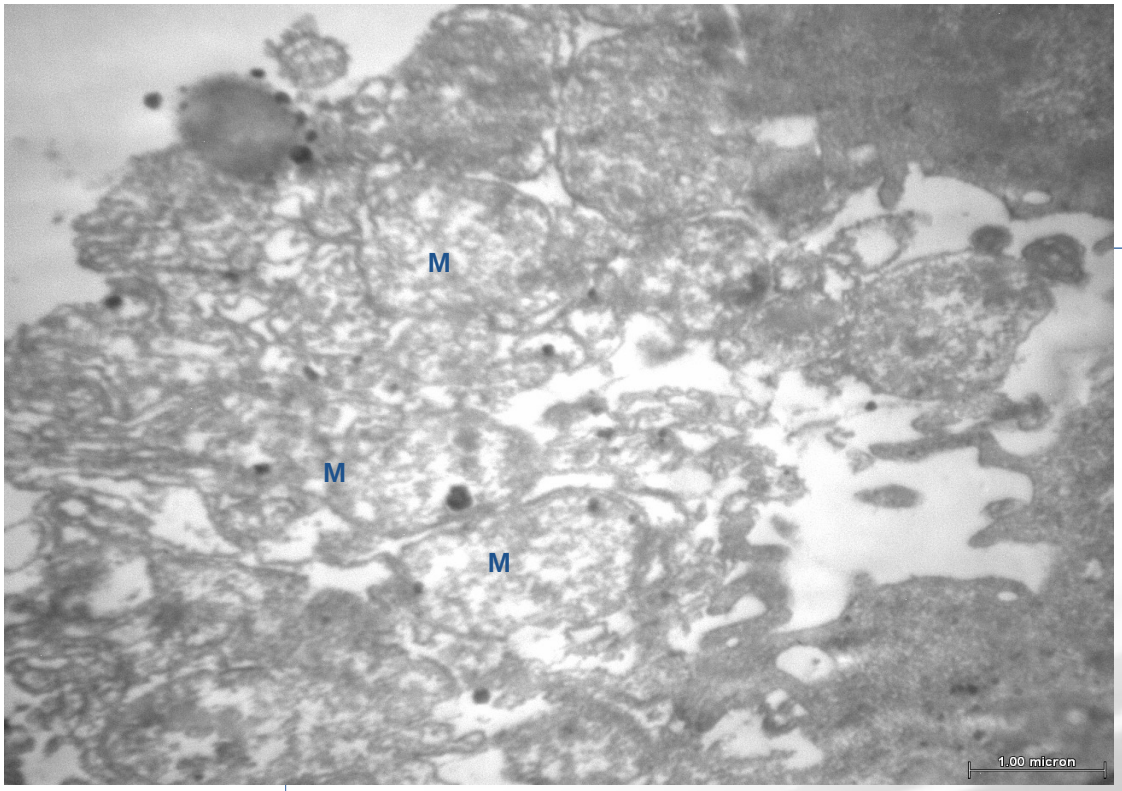
2µg/ml AgNPs



High numbers of cytoplasmic vacuoles, degeneration of large number of mitochondria (M) and nuclear (N) karyorrhexis (nuclear destruction)

Effects on cell morphology

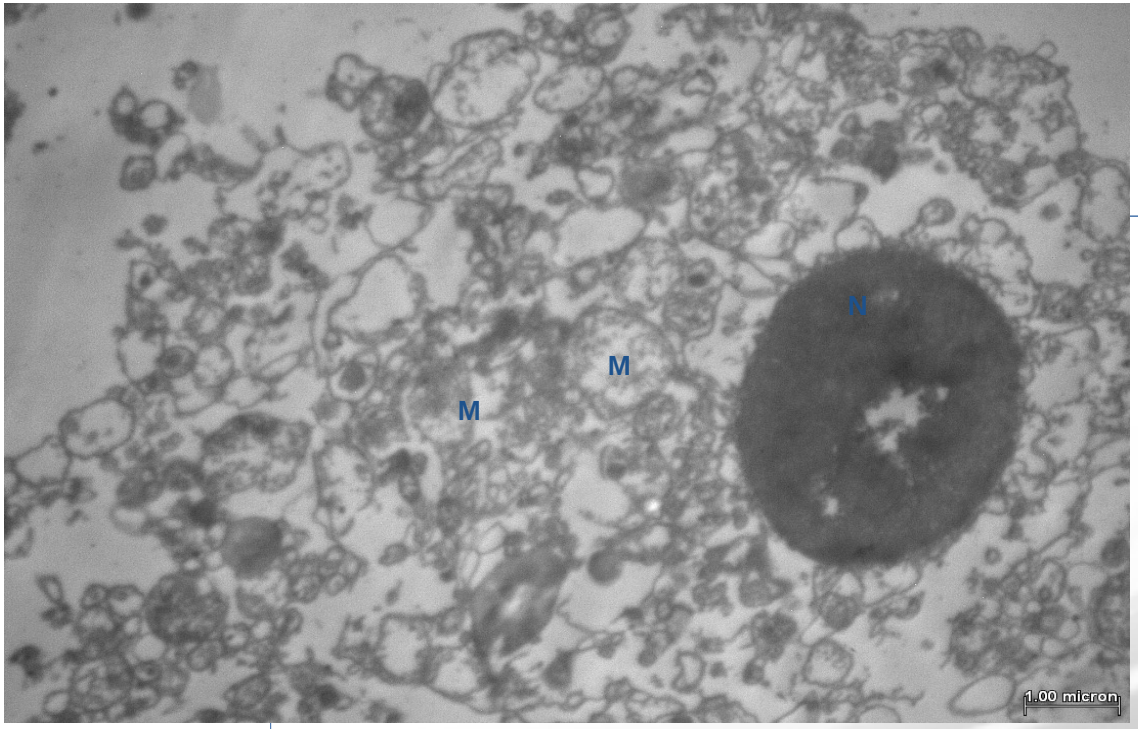
1µg/ml $AgNO_3$



high numbers of cytoplasmic vacuoles, severe degeneration of large number of mitochondria(M)

Effects on cell morphology

2µg/ml $AgNO_3$

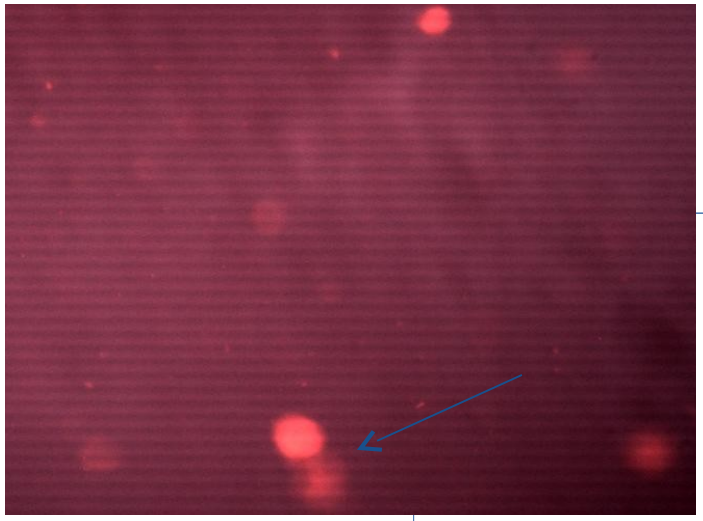


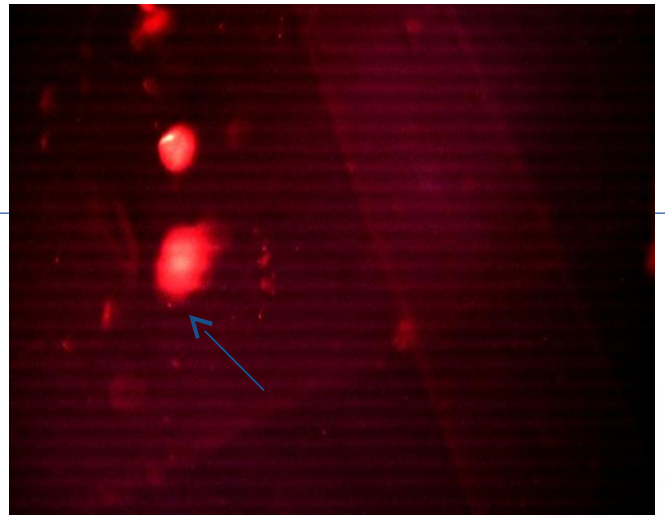
High numbers of cytoplasmic vacuoles, severe degeneration of large number of mitochondria (M) and nuclear (N) pyknosis

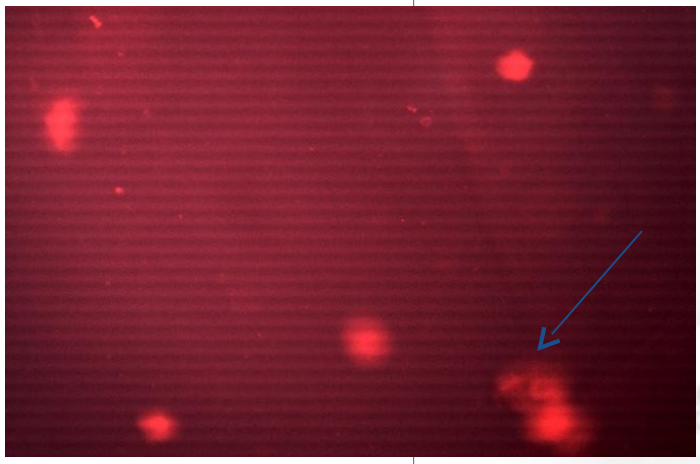
Tail Moments of COMET Assay

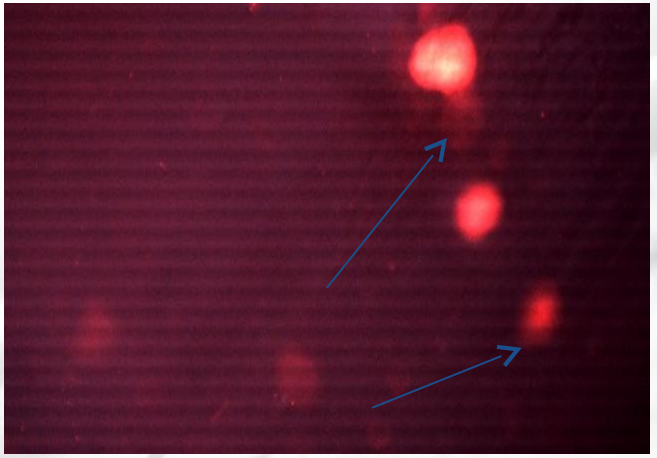
Groups	0 min.	120 min.
Control	0.08	0.09
AgNPs (1 µg/ml)	0.43	0.64
AgNPs (2 µg/ml)	0.531	1.56
$AgNO_3$(1 µg/ml)	0.651	1.68
$AgNO_3$(2 µg/ml)	0.682	1.97

Examples of COMET picture









Role of different chelating agents on hepatotoxicity induced by aluminumchloride

- Aluminum(Al) is a very abundant and widely distributed element in the environment. It is found in most rocks, soils, waters, air, antacid drugs and foods.
- This study was designed to investigate the hepatotoxic effects of $AlCl_3$ on isolated rat hepatocytes and the ameliorative role of desferrioxamine(DFO) and/or deferiprone (L1) as chelating agents.
- AlCl exposure caused severe adverse effects on the liver and lipid peroxidation which can be alleviated by pre-treatment with DFO or L1 as aluminum chelators.
- Also, we noted that L1 is more effective than DFO

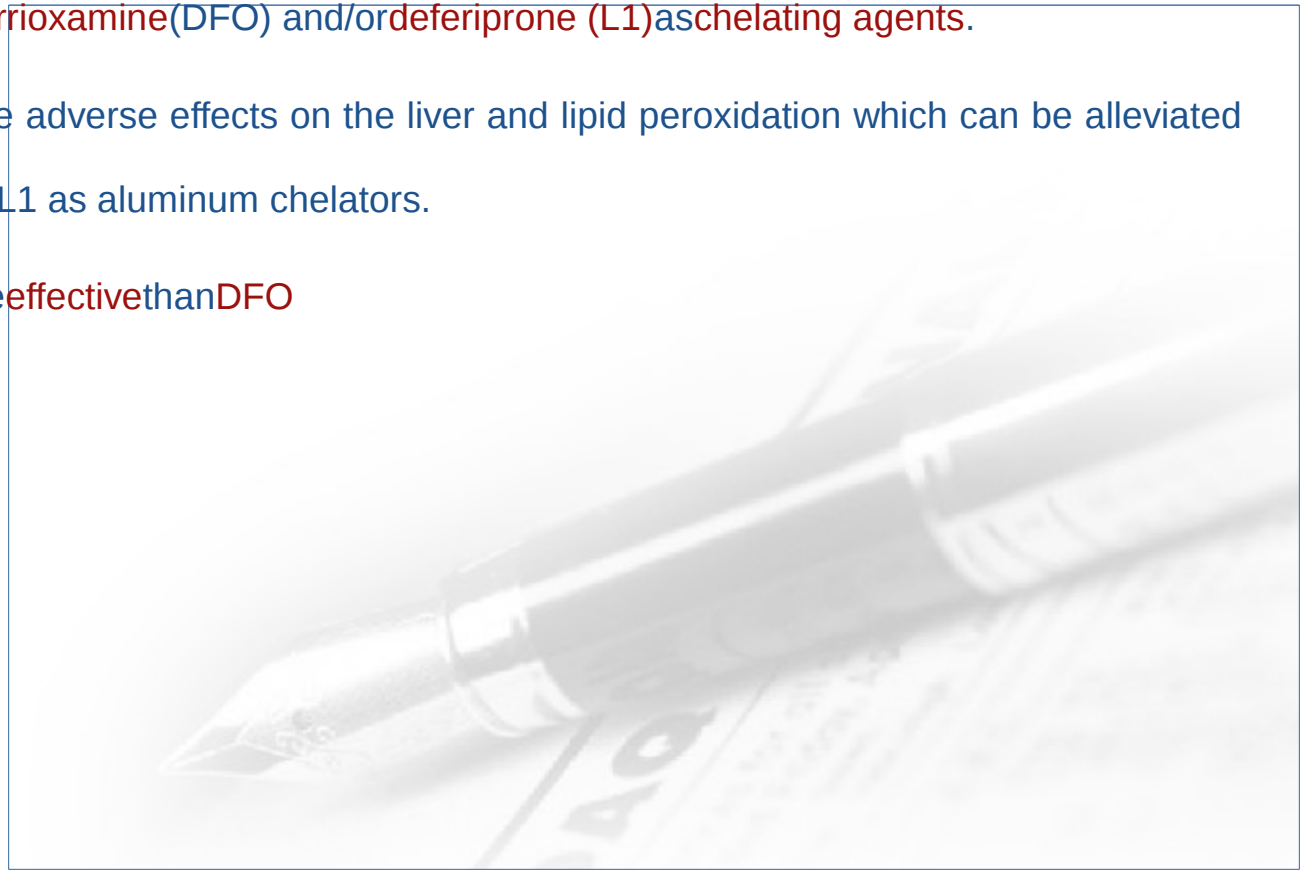
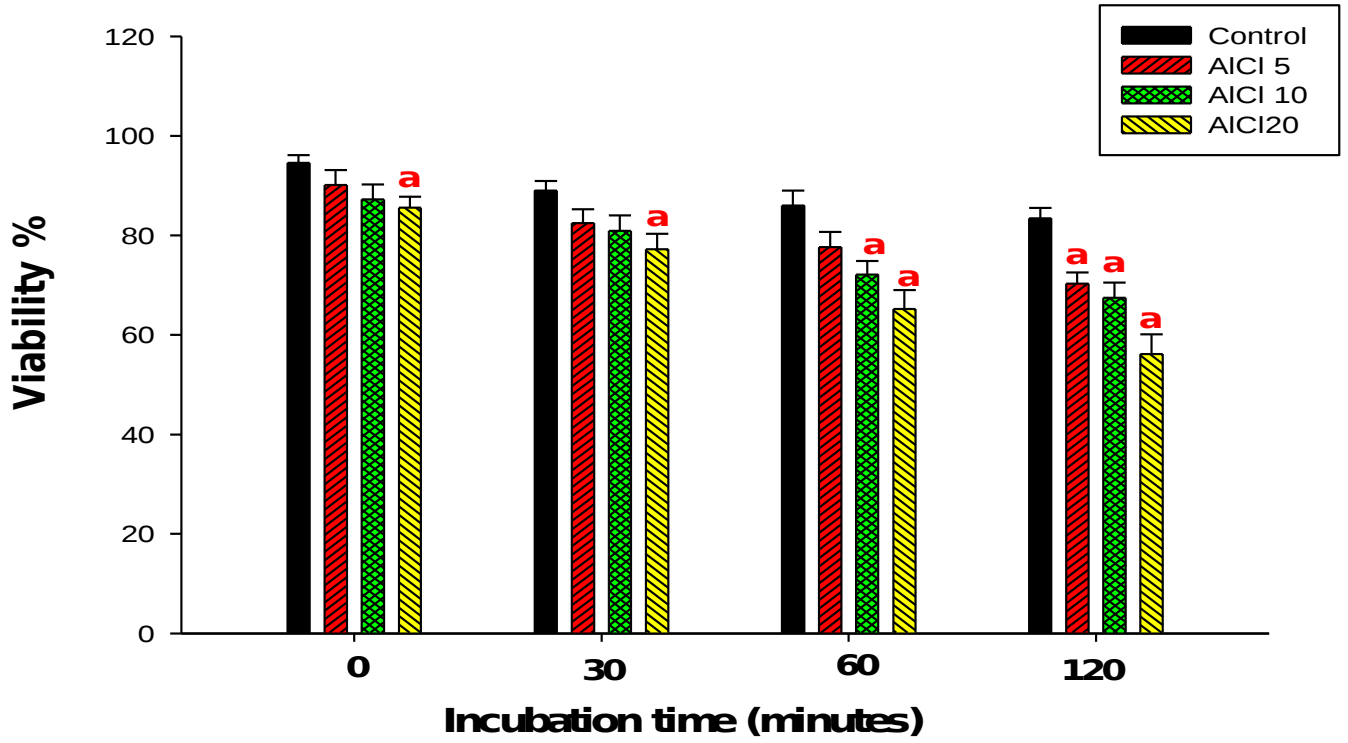


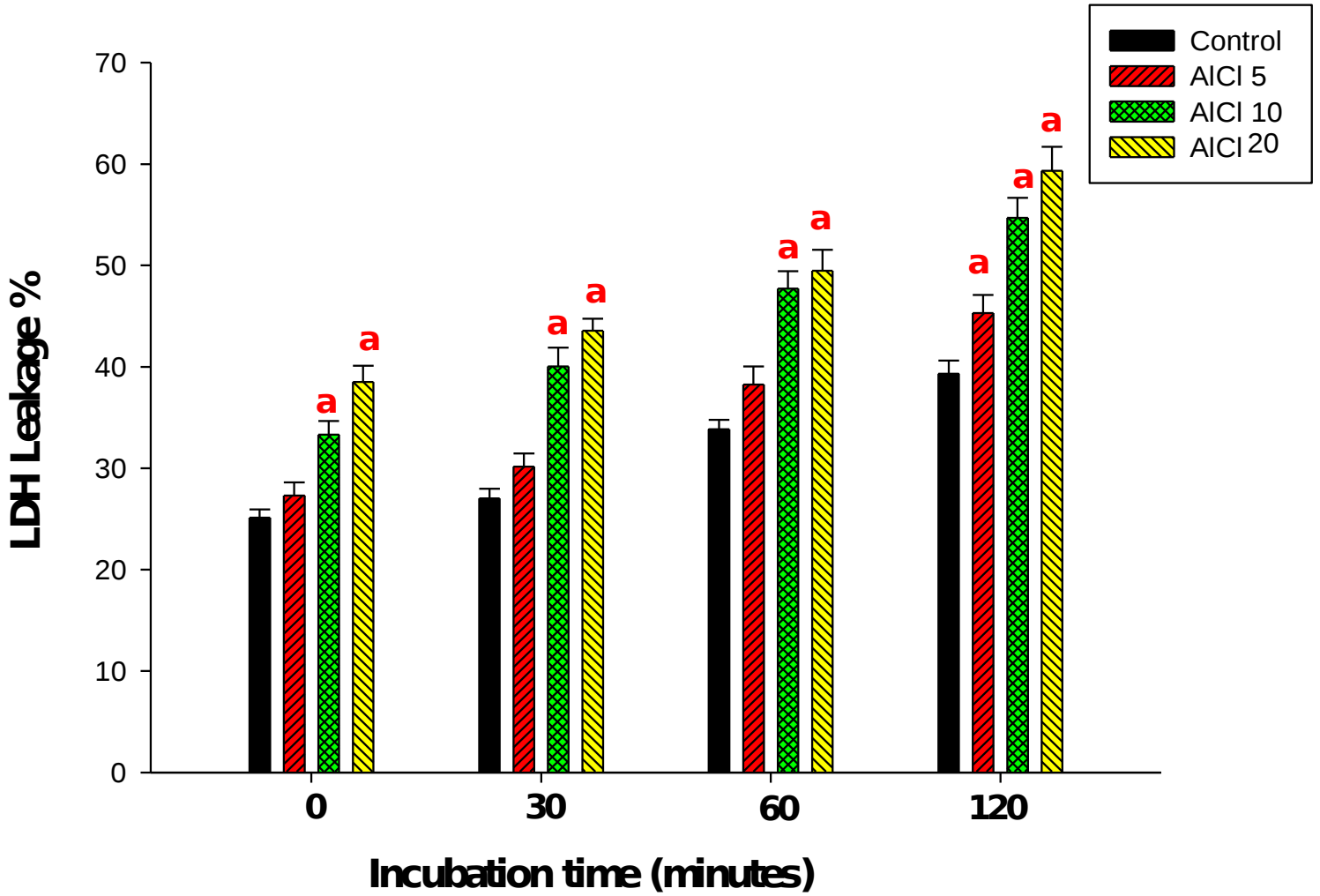
Fig.(3): Effects of different AlCl concentrations on viability%of isolated rat hepatocytes.



Data are expressed as mean + S.E. (n=5 replicates)

(a) Significant difference from corresponding control group by one- way ANOVA at P< 0.05.

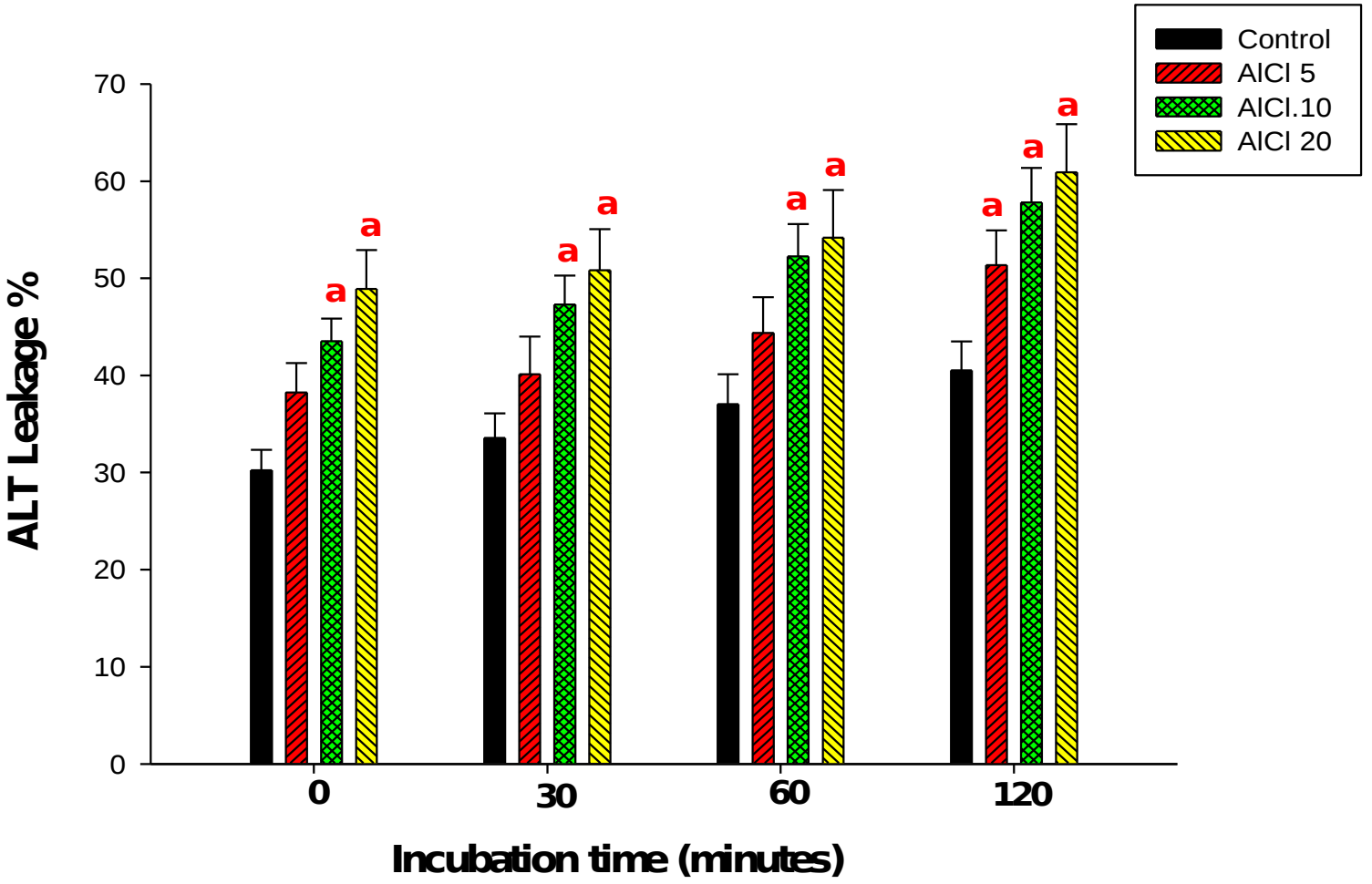
Fig.(5): Effects of different AlCl concentrations on LDH leakage%of isolated rat hepatocytes



Data are expressed as + S.E. (n=5 replicates).

(a) Significant difference from corresponding control group by one- way ANOVA at P< 0.05.

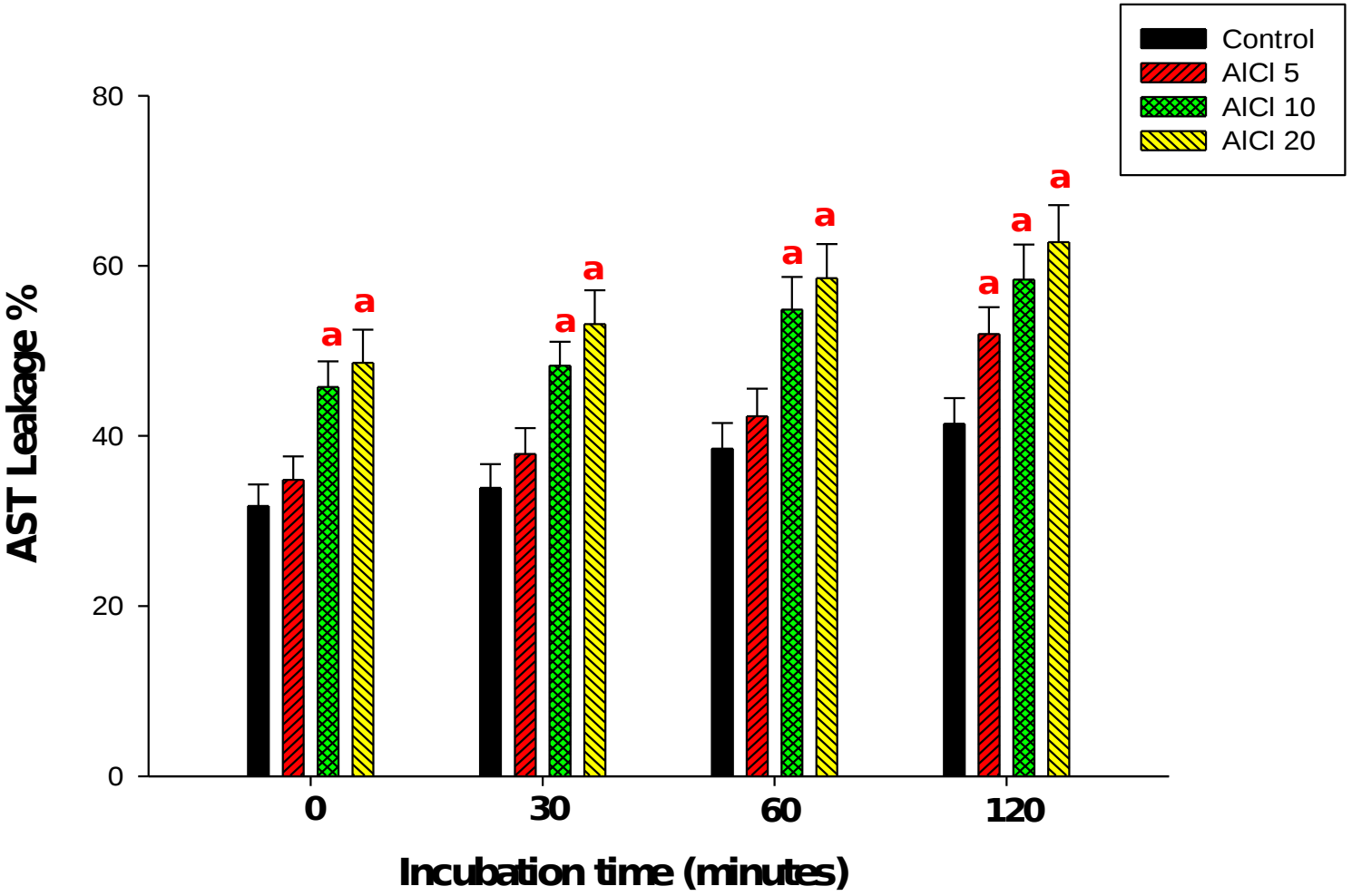
Fig.(7): Effects of different AlCl concentrations on ALT leakage % of isolated rat hepatocytes



Data are expressed as mean + S.E. (n=5 replicates).

(a) Significant difference from corresponding control group by one- way ANOVA at P< 0.05.

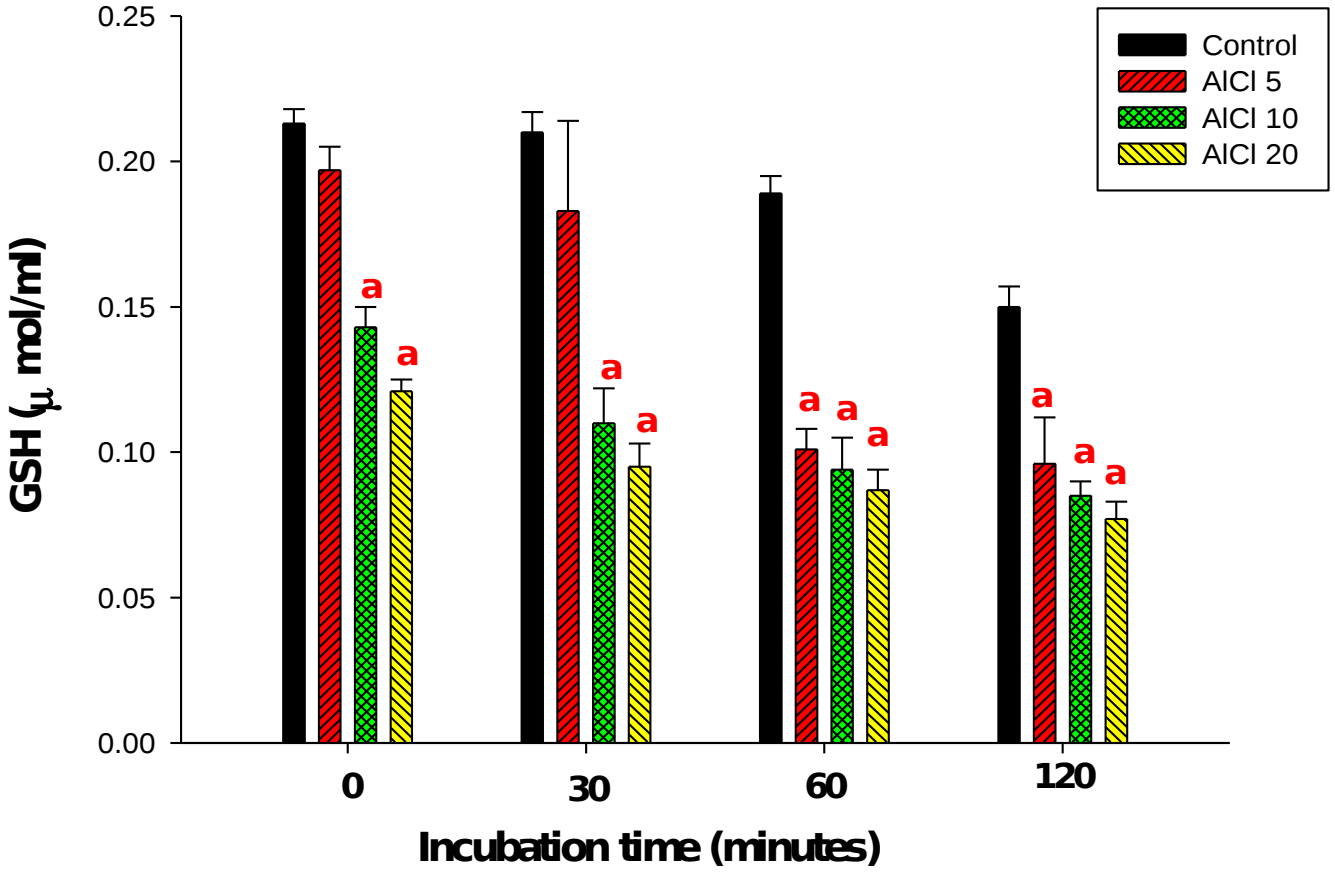
Fig.(9): Effects of different AlCl concentrations on AST leakage % of isolated rat hepatocytes



Data are expressed as mean + S.E. (n=5 replicates).

(a) Significant difference from corresponding control group by one- way ANOVA at P< 0.05.

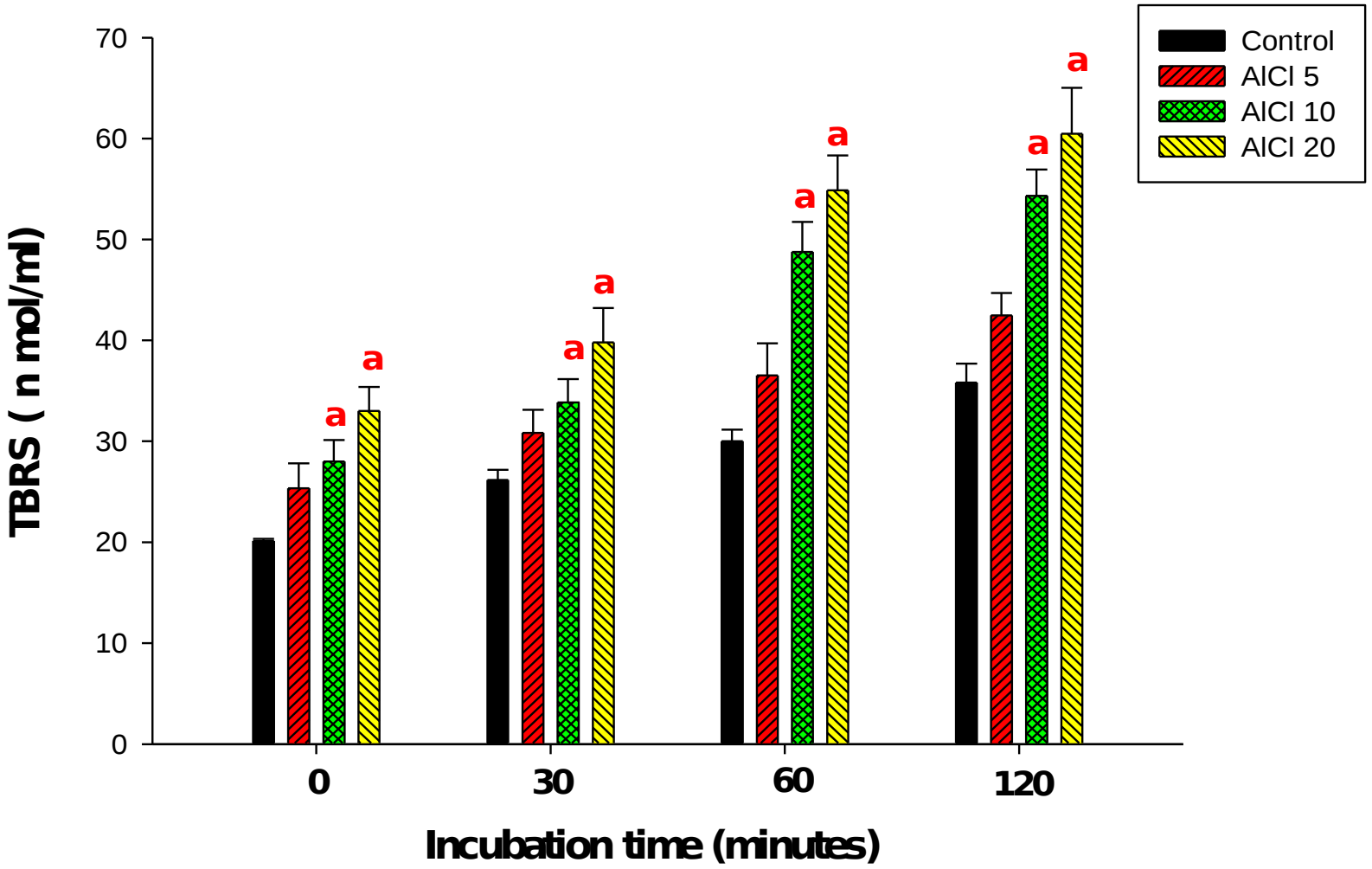
Fig.(11): Effects of different AlCl concentrations on reduced GSH content of isolated rat hepatocytes



Data are expressed as mean + S.E. (n=5 replicates).

(a) Significant difference from corresponding control group by one- way ANOVA at P< 0.05.

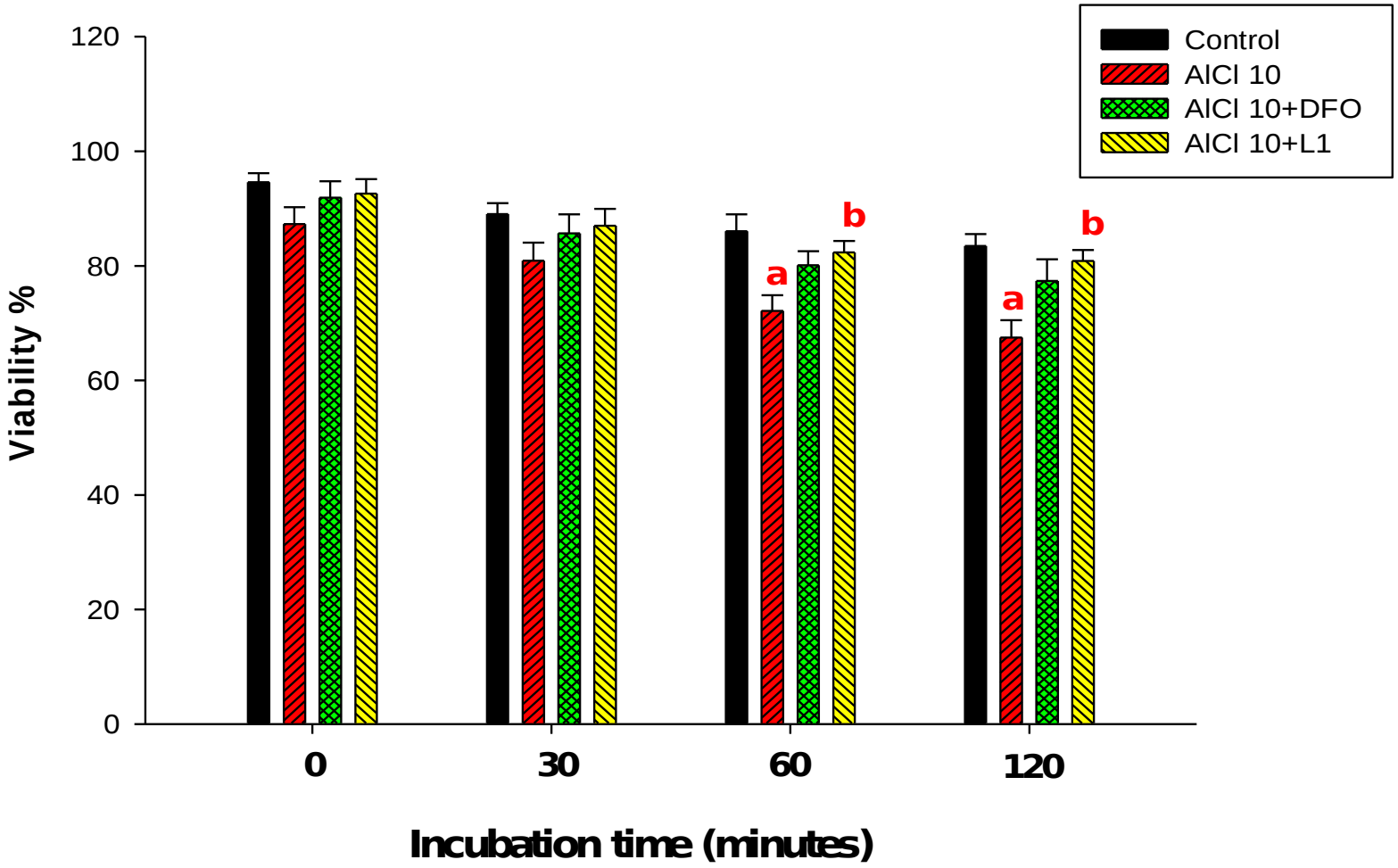
Fig.(13): Effects of different AlCl concentrations onTBARS formation of isolated rat hepatocytes



Data are expressed as mean + S.E.(n=5 replicates).

(a) Significant difference from corresponding control group by one- way ANOVA at P< 0.05

Fig.(4): Effects of DFO and/or L1on AlCl treated isolated rat hepatocytes viability.

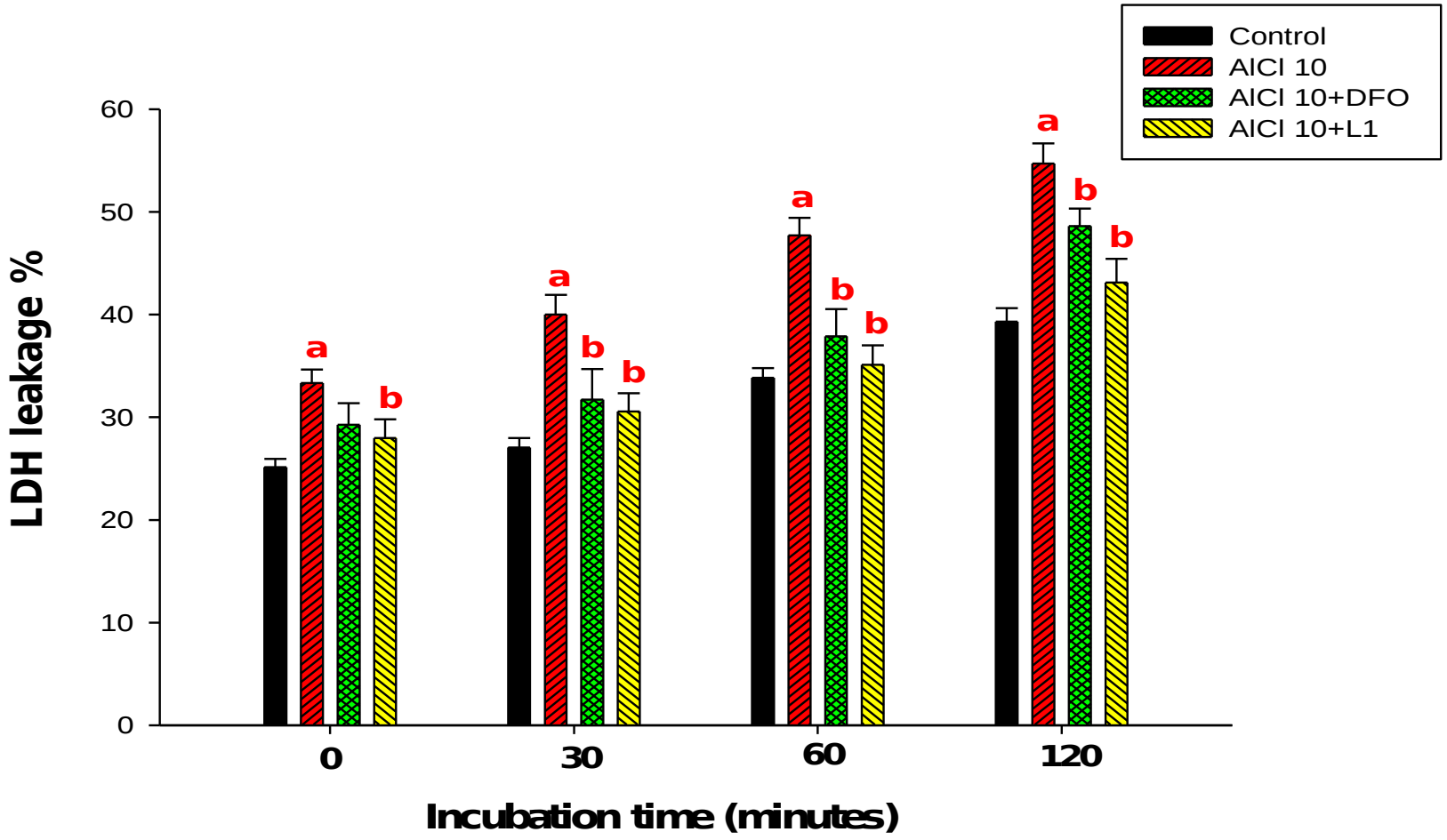


Data are expressed as mean + S.E. (n=5 replicates).

(a) Significant difference from corresponding control group by one- way ANOVA at P < 0.05.

(b) Significant difference from corresponding AlCl alone- treated groups by one- way ANOVA at P < 0.05

Fig.(6): Effects of DFO and/or L1on AlCl induced LDH leakage% in isolated rat hepatocytes

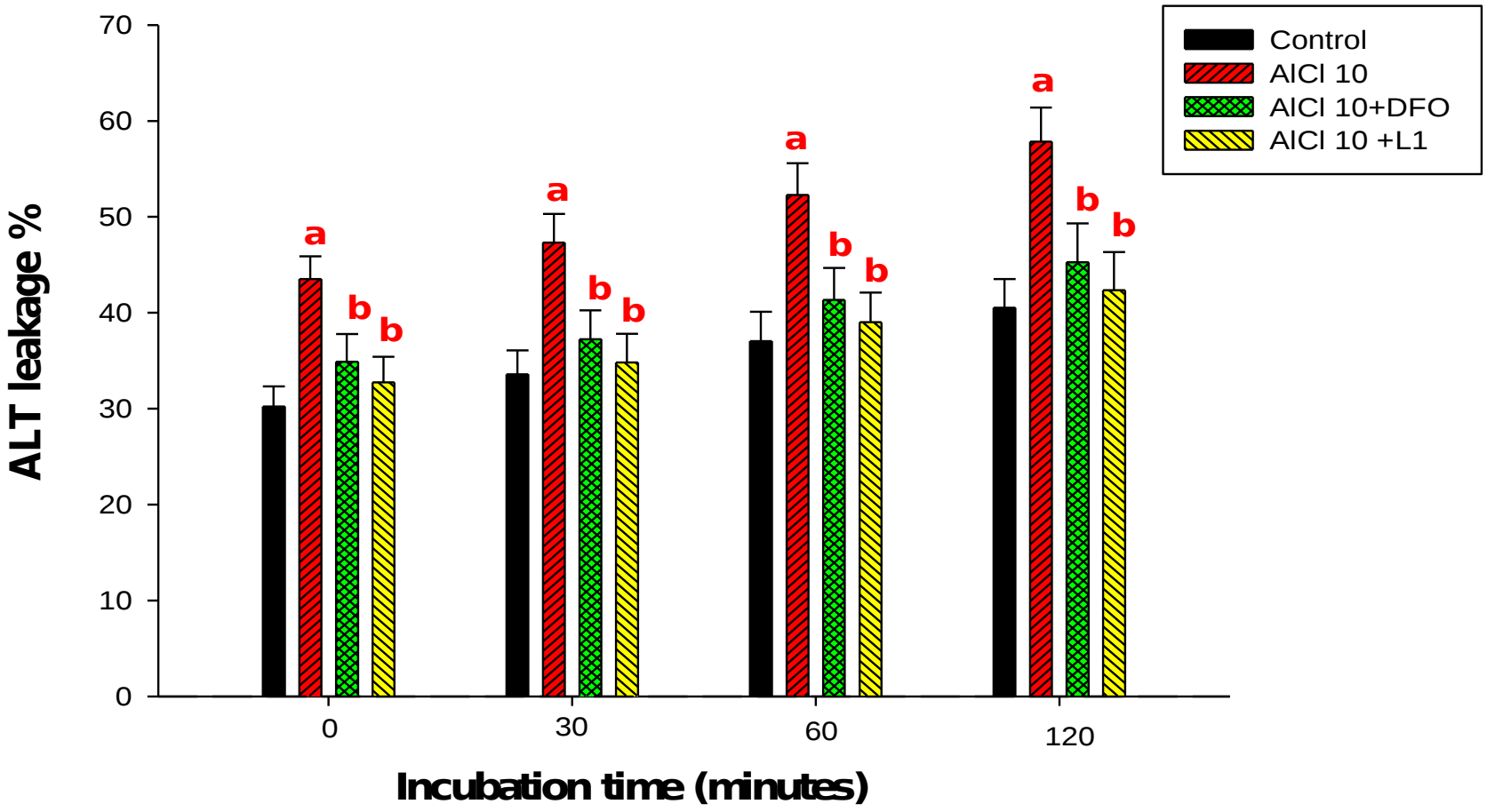


Data are expressed as mean + S.E. (n=5 replicates).

(a) Significant difference from corresponding control group by one- way ANOVA at P< 0.05 .

(b) Significant difference from corresponding AlClalone- treated groups by one- way ANOVA at P < 0.05.

Fig.(8): Effects of DFO and/orL1 on AlCl induced ALT leakage in isolated rat hepatocytes.

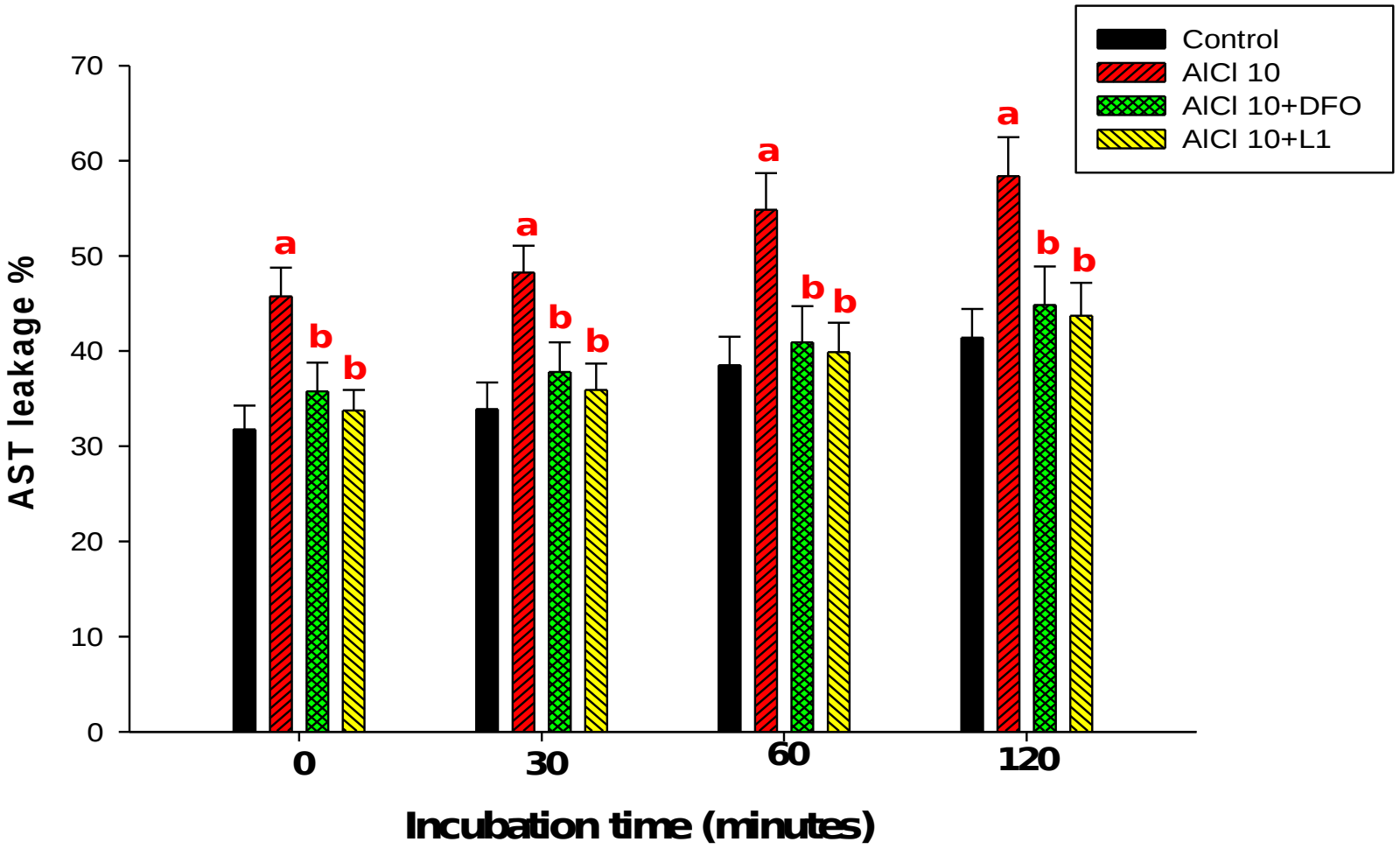


Data are expressed as mean + S.E. (n=5 replicates).

(a) Significant difference from corresponding control group by one- way ANOVA at P< 0.05.

(b) Significant diffremce from corresponding AlCl alone- treated groups by one- way ANOVA at P< 0.05.

Fig.(10): Effects of DFO and/or L1 on AlCl induced AST leakage in isolated rat hepatocytes

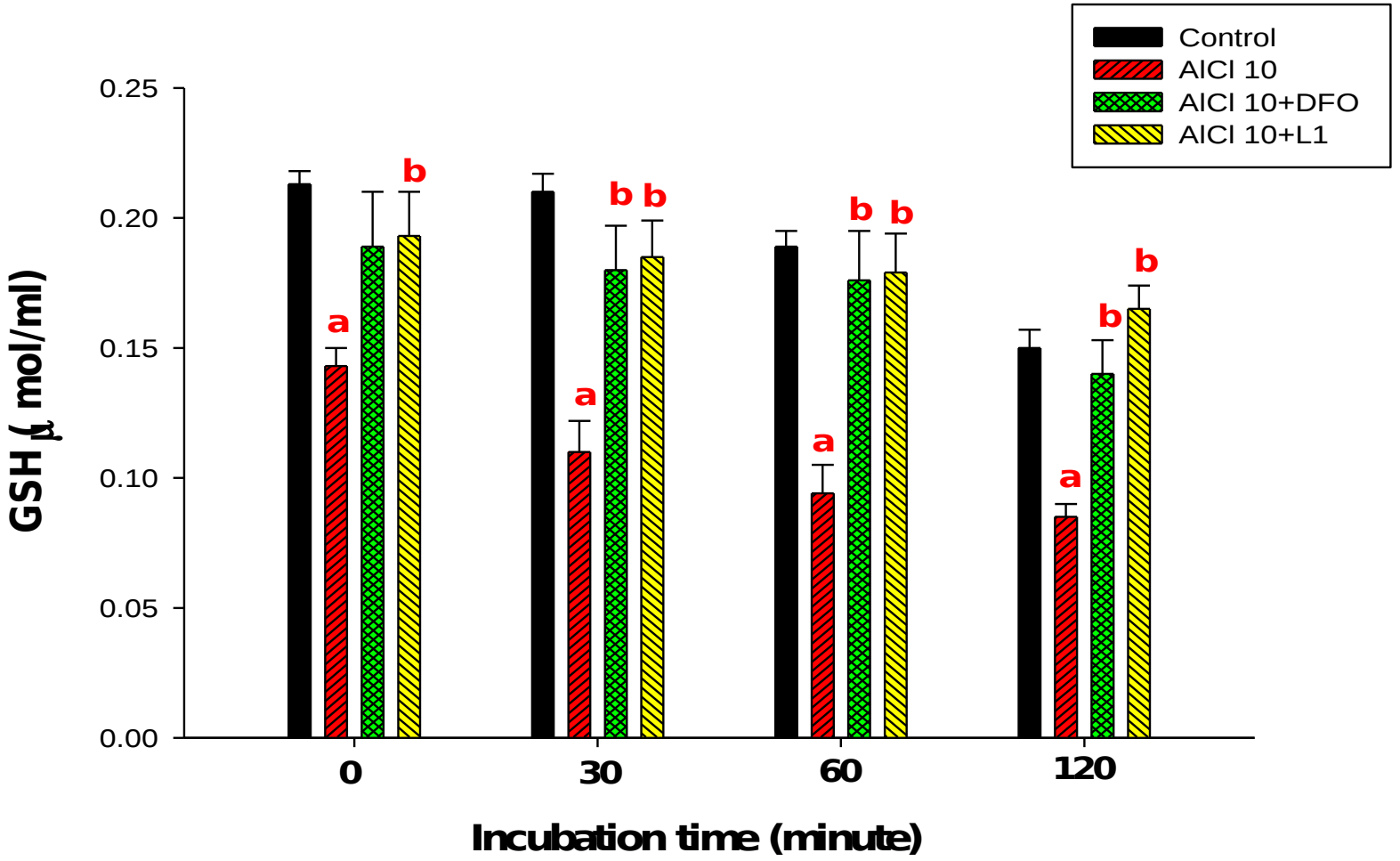


Data are expressed as mean + S.E. (n=5 replicates).

(a) Significant difference from corresponding control group by one- way ANOVA at P< 0.05.

(b) Significant difference from corresponding AlCl alone- treated groups by one- way ANOVA at P< 0.05.

Fig.(12): Effects of DFO and/or L1 on AlCl reduced GSH contents of isolated rat hepatocytes

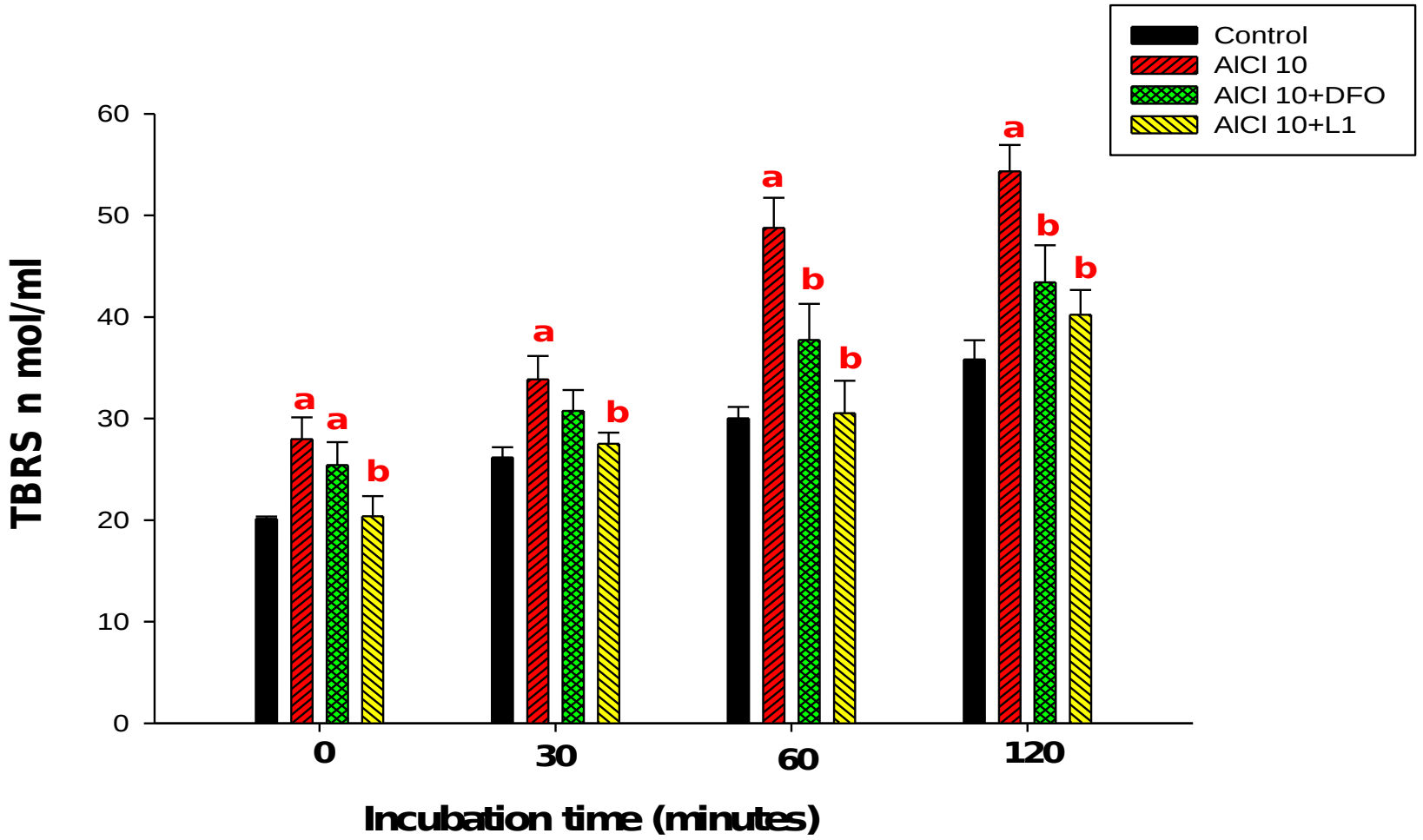


Data are expressed as mean + S.E.(n=5 replicats).

(a) Significant difference from corresponding control group by one- way ANOVA at P< 0.05.

(b) Significant difference from corresponding AlCl alone- treated groups by one- way ANOVA at p < 0.05.

Fig.(14): Effects of DFO and/or L1 on AlCl- induced TBARS of isolated rat hepatocytes

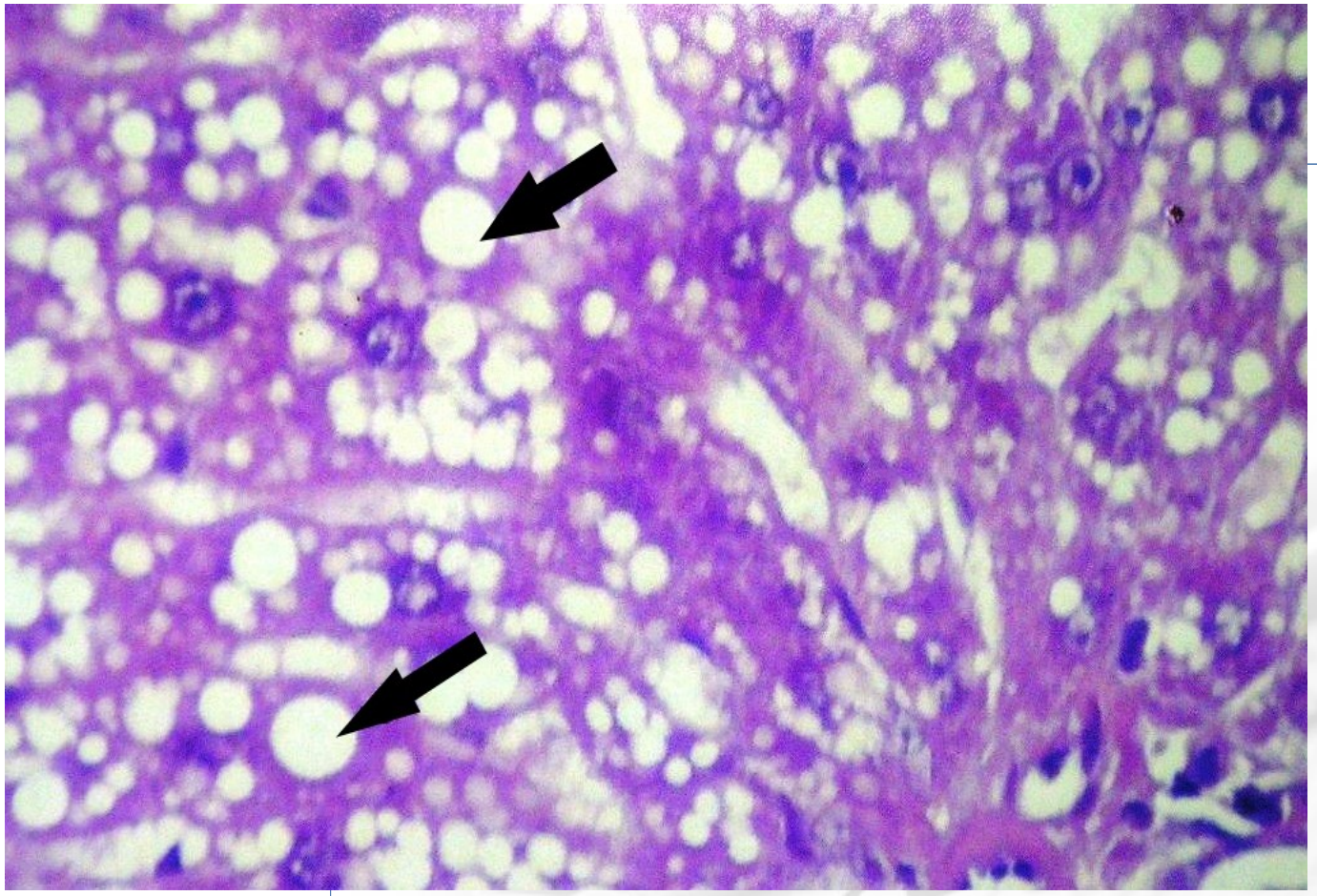


Data are expressed as mean + S.E.(n=5 replicates).

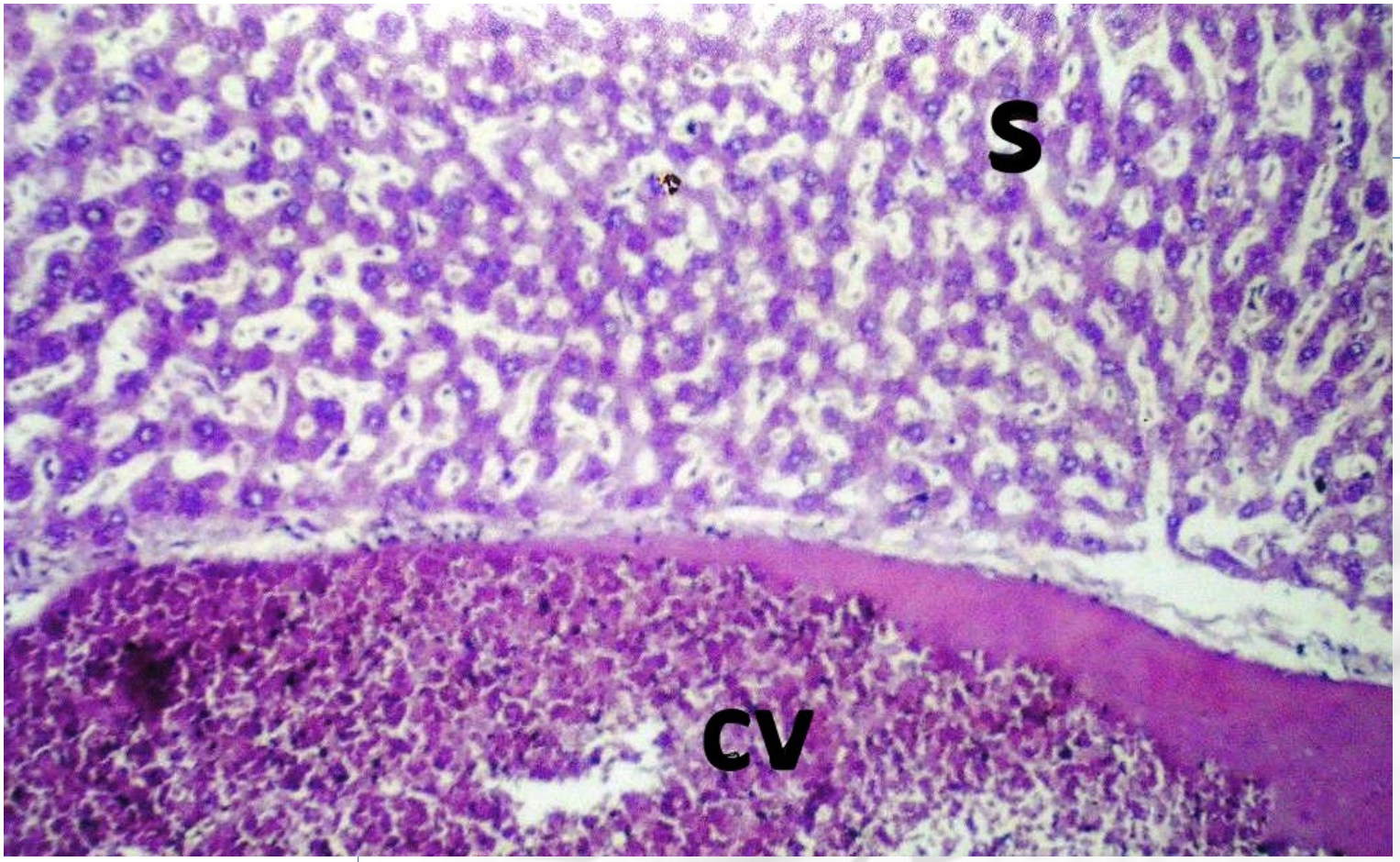
(a) Significant difference from corresponding control group by one- way ANOVA at $p < 0.05$.

(b) Significant difference from corresponding AlCl alone- treated groups by one- way ANOVA at $P < 0.05$.

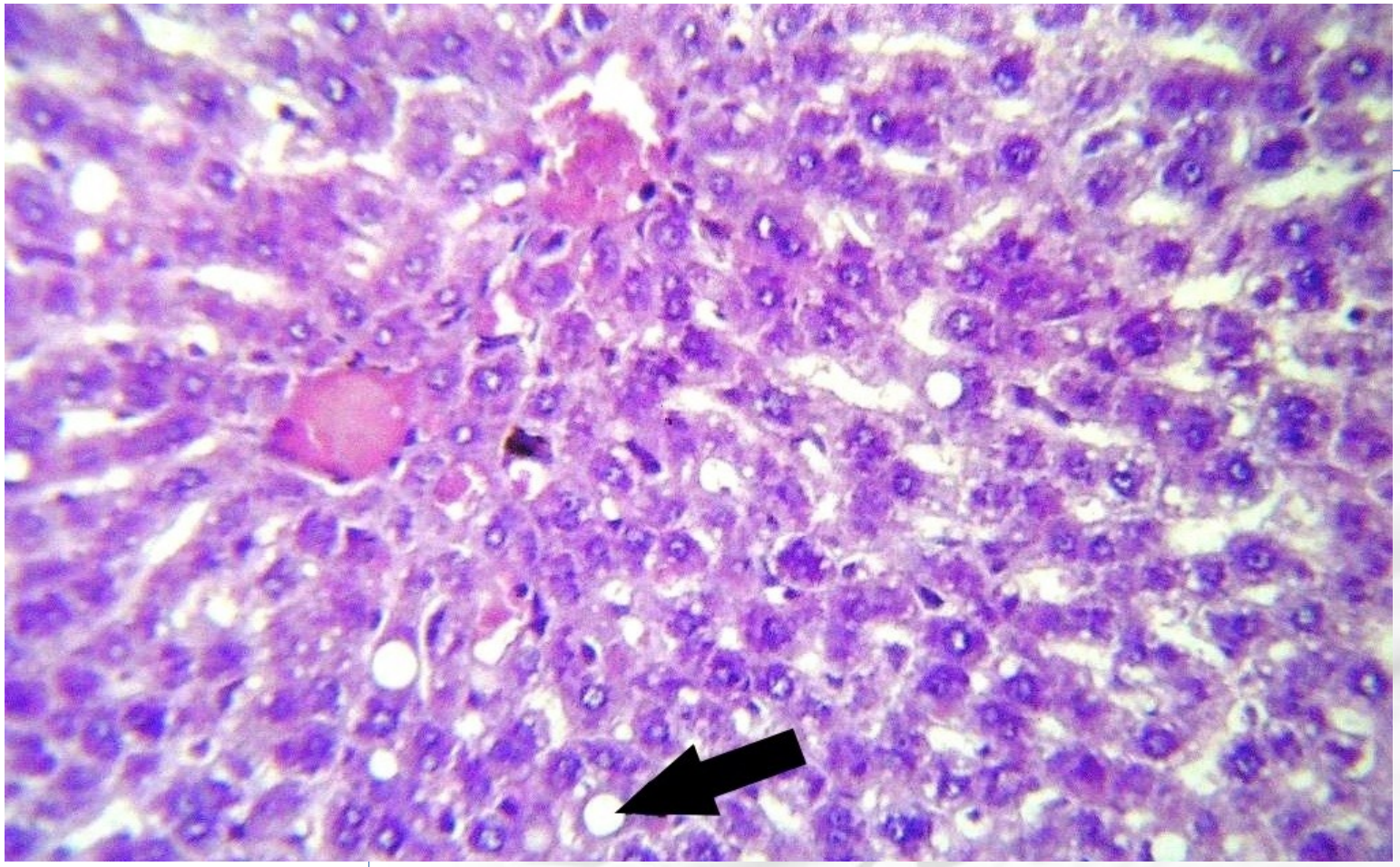
Liver of an AlCl- exposed rat at 200 mg/ kg, orally showing diffuse fatty changes all over the hepatocytes (arrows)



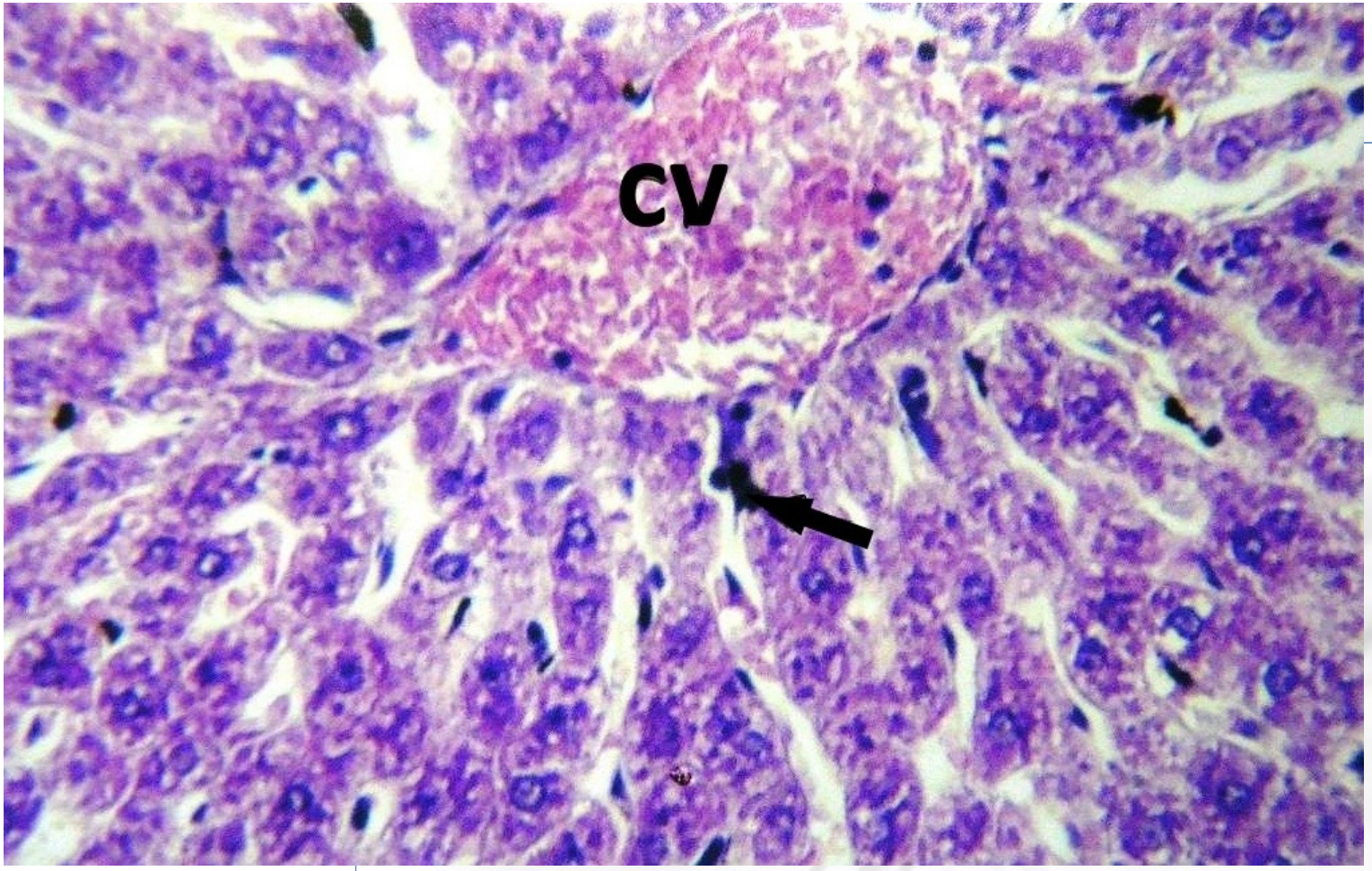
Liver of an AlCl- exposed rat at 200 mg/ kg, orally showing severe congestion and dilatation of CV and sinusoids (S)



Liver of a DFO- pretreated rat 1 hr before AlCl exposure showing fatty changes in a few hepatocytes.



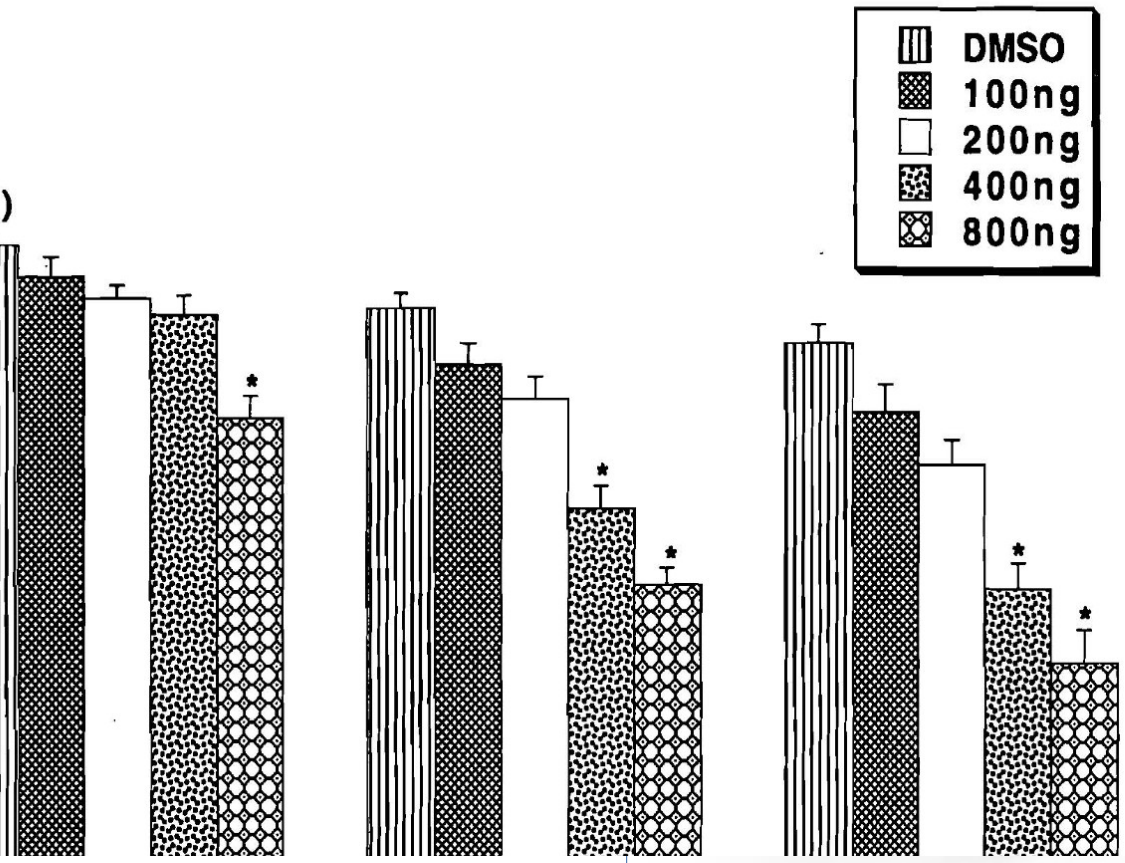
- **Liver of an L1- pretreated rat 1 hr before AlCl exposure showing diffuse kupffer cells proliferation with pigmented materials in between the degenerated hepatocytes (arrow) and slight congestion in the central vein (CV).**



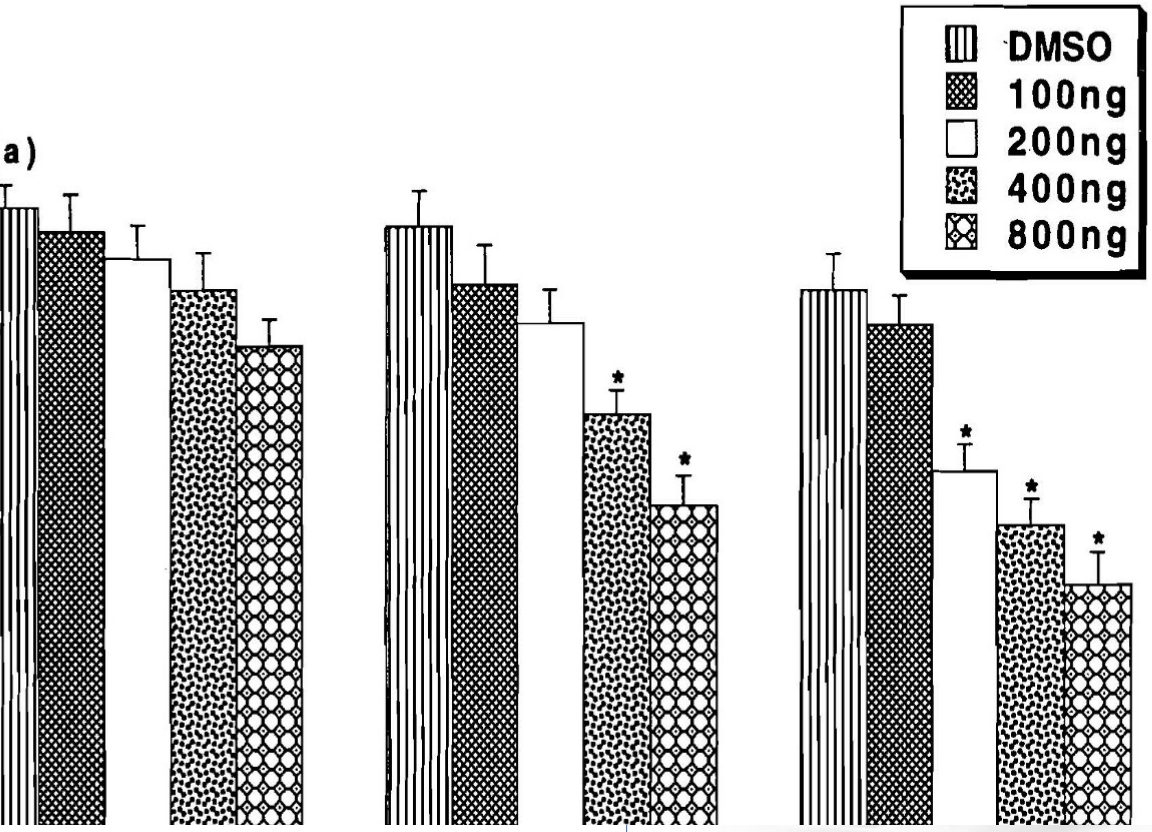
Effects ofcypermethrininsecticide on isolated male and female rat hepatocytes

- Cypermethrinis a newsyntheticpyrethroidwhich is currently gaining popularity as a potent insecticide.
- This studywas designed to investigate the toxicity ofcypermethrinon freshlyisolated maleandfemalerat hepatocytes.
- Cypermethrinhas toxiceffectson male and female rat hepatocytes withdose and time dependent.
- Thefemalerat hepatocytes weremore sensitiveto the toxic effect ofcypermethrinthan malecells.

Viability % Male



Viability % Female



ALT Leakage % Male

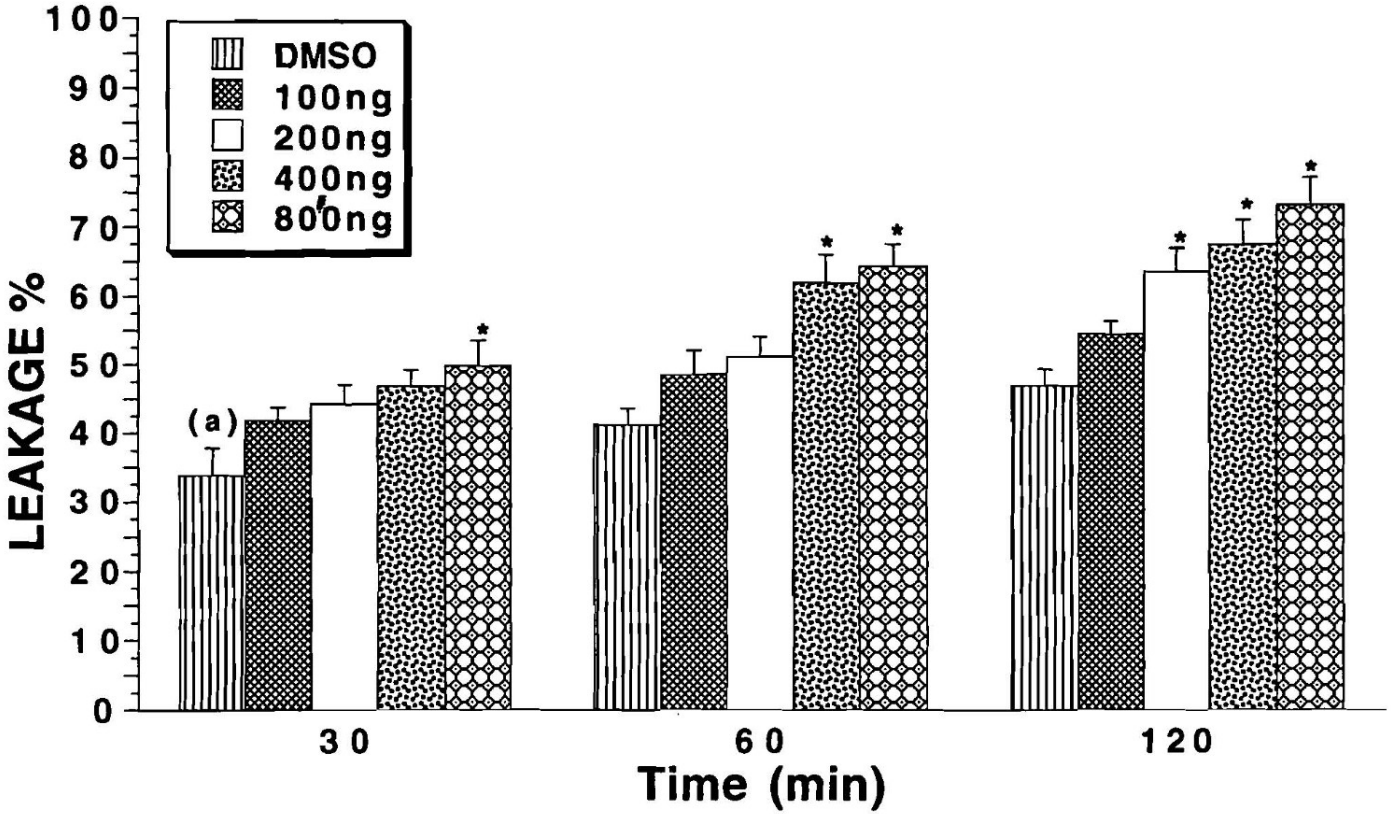
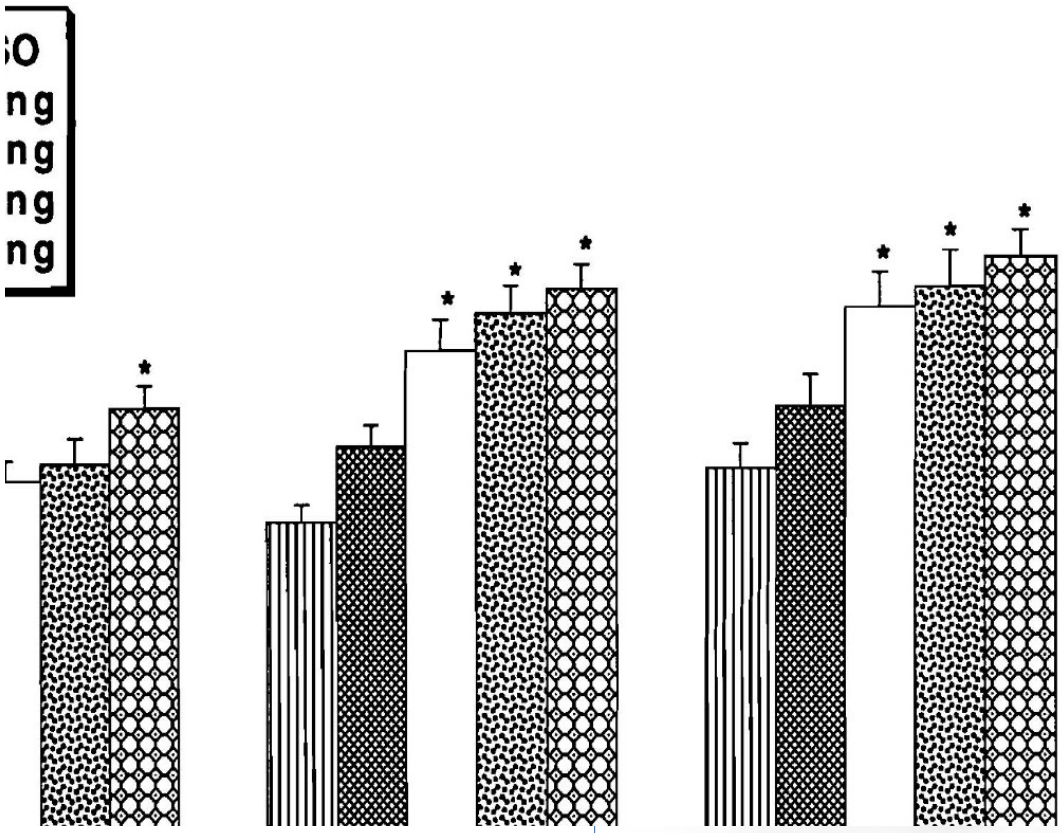
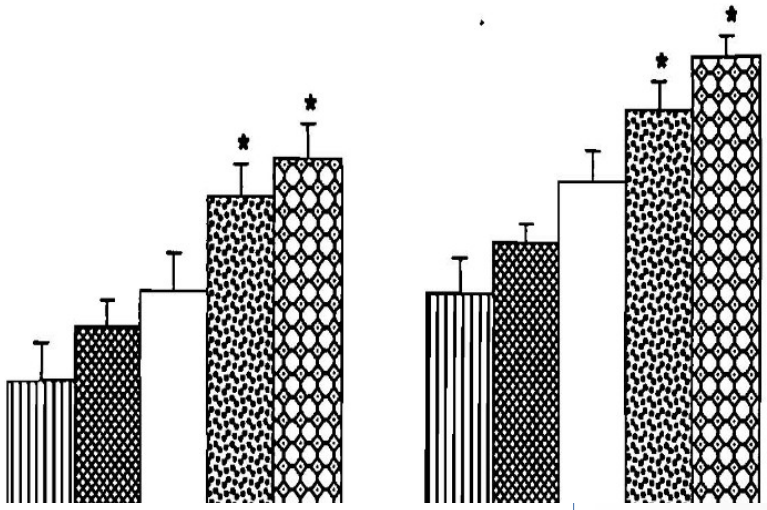


FIGURE 2. Effect of cypermethrin on ALT leakage in isolated male rat hepatocytes. Mean ± SE of the ALT leakage percentage from 12 hepatocyte replicates from 7 male rats shown by (a). Asterisk indicates significantly different from the respective control (DMSO) (one-way ANOVA) with Scheffe's test ($p < .05$).

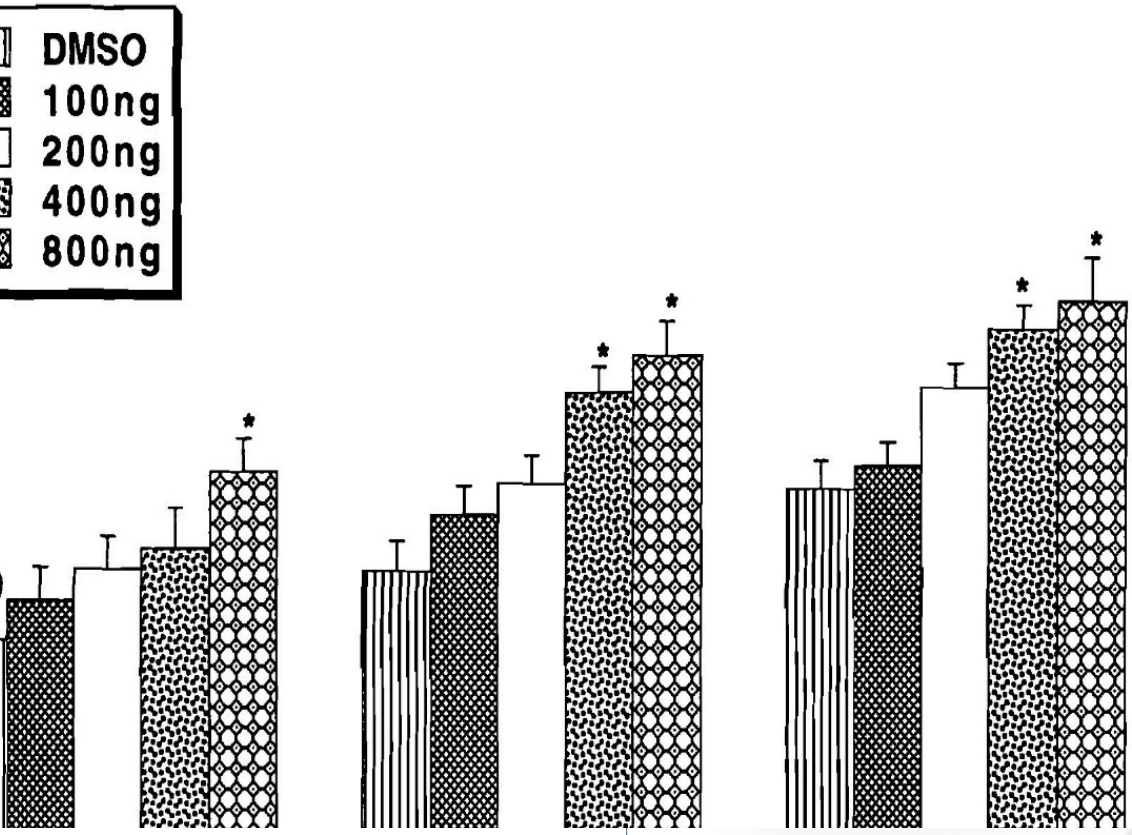
ALT Leakage % Female



AST Leakage % Male



AST Leakage % Female



My Take-Home Messages / Future Prospective

- **Establish**an*in vitro*toxicology unit for Primary Viable Hepatocytes isolation at different research institutes in order to minimize the number of experimental animals used.
- **Collaborate**with the researchers in innovation center and translational medicine units to standardize toxicity testing using different toxicological animal models.
- **Support**the clinical trials departments in the field and hospitals to assess the effectiveness of the new hepatoprotective drugs.
- **Survey**different Egyptian cities for its native plants which have potential hepatoprotective properties.
- **Develop**new and enhance the existing*in vitro*toxicology courses specially hepatotoxicology course.

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- Graduate StudentsfromPharmacology and ToxicologyDepartments of different Egyptian Universities:-
  - Cairo University
  - AinShams University
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  - Zagazig University
  - Sadat University


Thank You