HEPATOPROTECTIVE EFFECTS OF PUNICA GRANATUM BARK AND CISSUS QUADRANGULARIS ROOT USING ETHANOLIC EXTRACTS AGAINST CCL4 INDUCED LIVER DAMAGE

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***Abstract*: Liver is susceptible for a few metabolic workouts despite the fact that the ethics of its proximity may expose additional proteins as well as a large number of damaging toxicants, chemicals and medicinal products that could damage it. In our thinking about hepatotoxicity, therapeutic medicines are used to treat hepatic harm as they are used for therapeutic purposes by individuals. Medicinal medication mechanism caused hepatotoxicity: In this hazardous state, lipoperoxides, conjugated dienes are improved. The enhanced AST, ALT, mounting and wilting SOD levels are indicators of liver injury. Oxidative push is one of the main reasons behind liver harm, since the activity of a dangerous chemical known as poison, in Kupffer's cells, is released without question by the blessing of the negative bacteria inside the bowel. Currently it is unveiled that the plant extract has also been used to increase the chemical protein inside the body which indicates that the plant is also susceptible to producing the inhibitor property. On the basis of our results, it may be worth suggesting that Punica granatum and Cissus quadrangularis are antioxidant actively affected by the CCL4-induced oxidative stress of rats by reducing the serum AST oxidant stress indicators, serum ALT in liver. Superoxide dismutase is increasing in liver tissue in CCL4 caused by oxidative stress in rats.**

***Keywords*: hepatoprotective effects, Punica granatum and Cissus quadrangularis**

INRTODUCTION

HEPATOPROTECTIVE

The property of the chemical to avoid liver harm is called hepatoprotective activity or the Hepatotoxicity or the capacity to prevent HeteroDensitivity. Whereas the drug hepatotoxicity damages the liver, these do not.

HEPATIC TOXICITY

Specifically, the release of bile from the liver is an essential action of the human body. The growth that, development, and composition of life in every human cell is totally unknown to the outside world. The semen is contained inside the pelvic cavity. Protein foods play a crucial role in improving assimilation and kidney function, as well as on articulation. Ceraments or other drugs such as antacids or Rifaximinophyl were formerly accepted in conventional settings including the main digestive tract; however, they are tested in a wider range of different methods and processes.[1]

PUNICA GRANATUM:-

In most cases, the pomegranate is alluded to by the logical title punica granatum. The Punicoideae are individuals of the Lythraceae family, whereas the Cedrela family is comprised of deciduous trees. Numerous individuals all over the globe endure from liver maladies counting jaundice, cirrhosis, and greasy liver infection as a result of this. As an antibacterial and indeed as an antioxidant, it plays an imperative part. This natural product is by and by found within the Center East, the Caribbean, and the Indian Sea, in spite of the fact that its roots may be followed back to old times within the Center East, as well as the Chinese and Mediterranean oceans within the past. It may be a tropical natural product.

When it comes to accessibility, the natural product is generally found within the southern regions from March to May within the northern parts, due to the reality that it is in season at the time. It may reach a tallness of between 5 and 10 meters. Punica granatum may be isolated into a few compartments, each of which has pharmacological and harmful characteristics. These compartments incorporate seeds, peels, petals, clears out, blooms, and roots, all of which are utilized in different exercises. The berry ranges in measure from 5 to 12 cm in distance across and includes a ruddy bark. It contains almost 600 oval-shaped seeds that are coated by a mash that ranges in color from white to ruddy.[2]

Punica granatum has a wealth of health benefits:

Ciccus quadrangularis

In Jewish convention, it is alluded to as "abstrew." Cis-grass is classified as a perpetual of its capacity to outlive for a long period of time. Antibacterial capabilities have late been found in plants having a place to the Vitaceae family, whose clears out has been utilized in conventional Chinese pharmaceutical.[3]

Distinctive fossil orzoideosic sorts of wild grass have been appeared to be supportive within the treatment of bone and ligament wounds and sicknesses. For their capacity to successfully treat issues and repairs for broken and sprained tendons, as well as to protect wellbeing and typical joint work, they are alluded to as "yoga medications."

In expansion to being utilized as a clean, pain reliever, and to treat scurvy, it is additionally utilized as a medication for an assortment of distinctive afflictions, counting as an anti-scurvy agent. The Cissus plant is inborn to the landmass of Asia, to be specific to the countries of the Center East and Africa, as well as to certain regions inside those countries. Due to the reality that it has its beginnings in India, the celebrated therapeutic herb known as Ashoka (which is utilized to make stride bone thickness) is vital in this context. In expansion to asthisankhara, hadjodh (one of its equivalent words is borkeliocha, and another is bunderbuss), and veld vine, it is additionally known by the names ciceraad or dagoes spine, depending on where you're within the world: in Africa, Australia, China, or elsewhere.[4]

AIM AND OBJECTIVES

Pomegranate and Cissus quadrangularis:-

Aim and objective of the study is to carry out the extractions of Punica granatum (pomegranate) and *Cissus quadrangualris* (hadjod) by using maceration technique.

The present study is taken for objective following:-

a) Collection and drying of Punica granatum material and *Cissus quadrangualris.*
b) Authentication of the Punica granatum material and *Cissus quadrangularis.*
c) Extraction of dried bark of Punica granatum and *Cissus quadrangularis.*
d) Carrying out preliminary test for phytochemical constituents.

Evaluation of hepatoprotective activity using different experimental models.

MATERIALS AND METHODOLOGY:-

When rats were given benfotamp-serum, the glutamic transaminase activities in their muscles (hepatic and muscular) and heart muscle were seen to be enhanced; however, following dosage repletion, the activities remained constant. The enzymes are present in the circulation and are distributed extravascularly via the skin in order to cope with the injury.

SGPT and ALT are generated when tissue damage occurs in the liver and the heart, and these enzymes leak into the circulation and are detected in the organs responsible for the damage, the liver and the heart.

COLLECTION OF PLANT MATERIALS:-

Punica granatum (pomegranate) and Cissus quadrangularis (Hadjod):-

I finally visited various botanical gardens in Hyderabad in order to collect the necessary specimens, among them the various plants and trees at the greenhouses I finally found one of the kind of pomegranate bark.

AUTHENTICATION OF PLANT MATERIAL:-

The characters are mostly of greater interest to botanists, horticulturists, and staff members of the Unani and Ayurvedic Centers than to whom library users of the centre of research. It was okay with me if I had to make a few additional enquiries. The concerned testing center has given me proof that is true and up to date.

EXTRACTION OF PLANT MATERIAL:-

Punica granatum:

To be able to obtain the necessary substance, I've peeled the bark from the tree branches for almost ten to fifteen days, and after that I crushed it into tiny pieces in our own equipment.

To dry plant the pre-out day 1 , I applied 200ml of de-atmospheric (ethyl 99%) alcohol and held it stirring in a glass rod for around 24 hours in an airtight jar

If the concentrate has been diluted to this way (two times its original concentration), denatured, let it sit for 5 minutes to allow the solution to settle, then slowly apply 100 mg of the ground bark powder, starting with 100% ethyl alcohol spirit and mixing it for another 5 minutes to obtain the necessary consistency.

To collect data from this container, the samples were left unstirred for around a week before this phase of stirring was started.

After the sample filtration, the residue was allowed to dry, the filtrate was filtered via muslin. Polyethylene neutralized sieves at a temperature and pressure below 0ºC to avoid contamination.

Cissus quadrangularis:

For the extraction of needed stuff, I normally gathered wild vines, plucked them out of the ground, dried them in the shade for 10 to 15 days, and grinded them.

To expand the sample in airtight container, I attached 50 mg of the dried root powder to 100 ml of denatured (ethyl-99%) alcohol and stirred it using a glass rod for around 24 hours.

To achieve the same levels of denaturation, I've applied a batch of the root powder on day 2 and mixed it with an ethyl 99% solution for an additional 5 minutes, creating a solution that's as thick as the previous one that was held away (slightly thinner)

This was carried on for a week longer than that, to ensure the consistency of the mixture, and then it was left to settle for a fortnight before filtering.

After that, the muslin was pressed and the remaining extract was dried at standard temperature, the sieve filtered. Polyethylene neutralized sieves at a temperature and pressure below 0ºC to avoid contamination.

EXPERIMENTAL DESIGN:-

Sixty rats are divided into six groups of six rats each (n=06) and treated orally as follows

Group-1: (Normal): it was used as normal saline rats seven days.

Group-2: (ccl4 1ml/kg): rats received distilled water orally daily for seven days, on the fifth day rats received oral dose of ccl4.

Group – 3: (ccl4 1ml/kg + Silymerin 100mg/kg): rats received silymerin orally daily for seven days, on the fifth day rats received oral dose of ccl4.

Group-4: (ccl4 1ml/kg + Punica granatum 200mg/kg): rats received extract orally for seven days; on the fifth day rats received oral dose of ccl4

Group -5 : (ccl4 1ml/kg +Punica granatum 500mg/kg) :rats received extract orally for seven days ; on the fifth day received oral dose of ccl4 .

Group -6 : (ccl4 1ml/kg +Cissus quadrangularis 100mg/kg) :rats received extract orally for seven days ; on the fifth day received oral dose of ccl4 .

Group -7 : (ccl4 1ml/kg +Cissus quadrangularis 200mg/kg) :rats received extract orally for seven days ; on the fifth day received oral dose of ccl4 .

Group -8 :(ccl4 1ml/kg +Punica granatum 200mg/kg + Cissus quadrangularis 100mg/kg) : rats received extract orally for seven days ; on the fifth day received oral dose of ccl4.

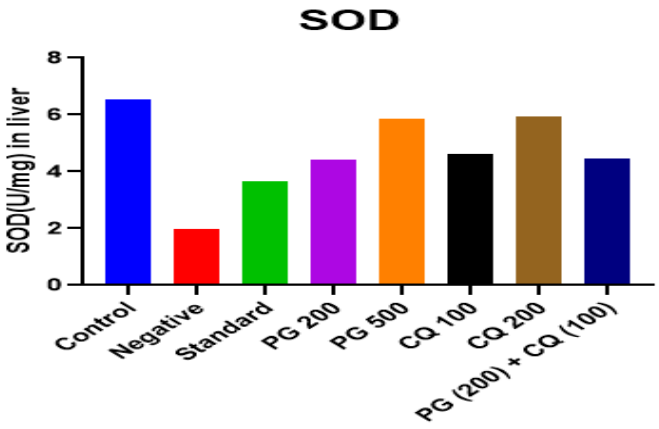
RESULTS AND DISCUSSION

Chemical constituent	Test	PGExtract	CQExtract
Tannins	Ferric chloride test	+	+
	Lead acetate test	+	+
	Acetic acid sol.	+	+
	Dil. Iodine sol.	+	+
Alkaloids	Mayer's test	+	+
	Dragendroff's test	+	+
	Hager's test	+	+
	Wagner's test	+	+
Glycoside			
A. Cardiac glycosides	Baljet's test	+	+
	Legal's test	+	+
	Keller-killiani test	+	+
	Liebermann's test	+	+
B. Steroids	Salkowski test	+	+
	Liebermann-burchard test	+	+
	Liebermann's test	+	+
C.Saponins	Foam test	+	+
D. Flavonoids	Schinoda test	+	+
	Lead acetate test	+	+
	NaOH test	+	+
E. Anthraquinones	Borntrager's test	+	+
	Modified-borntrager's test	+	+
Carbohydrates	Molisch test	+	+
	Fehling's test	+	+
	Benedict's test	+	+
Proteins	Biuret's test	+	+
	Millon's test	+	+

Results of Phytochemical Analysis Extract of *Plants*

Superoxide dismutase levels in liver tissue homogenate

Group	SOD(U/mg) in liver
Group 1 (Control)	6.56 ±0.002
Group 2 (Negative)	1.98 ±0.003
Group 3 (Standard)	3.67 ±0.001
Group 4 (PG 200)	4.42 ±0.003
Group 5 (PG 500)	5.85 ±0.004
Group 6 (CQ100)	4.62 ±0.005
Group 7 (CQ200)	5.96 ±0.002
Group 8 (PG (200) + CQ (100))	4.44 ±0.001
SD	1.462
SEM	0.517



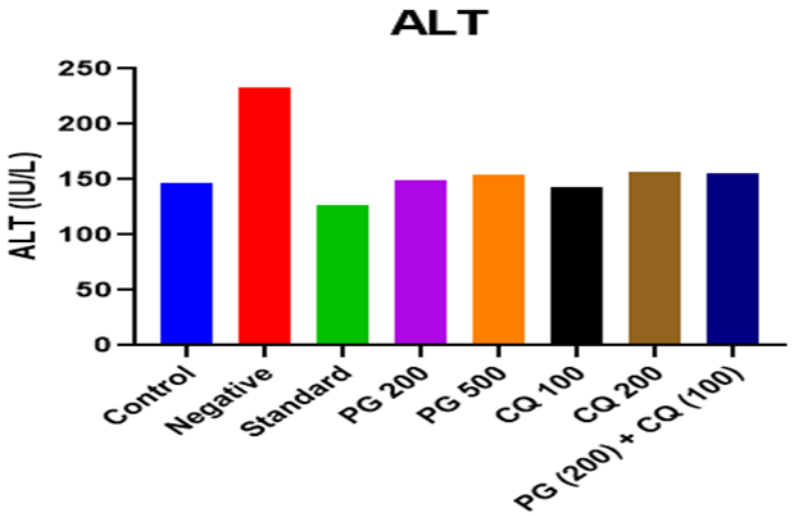
Effect on superoxide dismutase levels in liver tissue homogenate in rats treated with CCL4 .
Effect on Liver weight in rats treated with CCL4 .

Group name	Liver weight
Group 1 (Control)	7.64 ±0.004
Group 2 (Negative)	7.53 ±0.002
Group 3 (Standard)	6.55 ±0.002
Group 4 (PG 200)	7.82 ±0.003
Group 5 (PG 500)	6.35 ±0.005
Group 6 (CQ100)	7.32 ±0.001
Group 7 (CQ200)	6.41 ±0.004
Group 8 (PG (200) + CQ (100))	7.25 ±0.005
SD	0.586
SEM	0.207

SERUM ALANINE AMINOTRANSFERASE (ALT) :

Effects of test compound on serum ALT levels in rats treated with CCl4

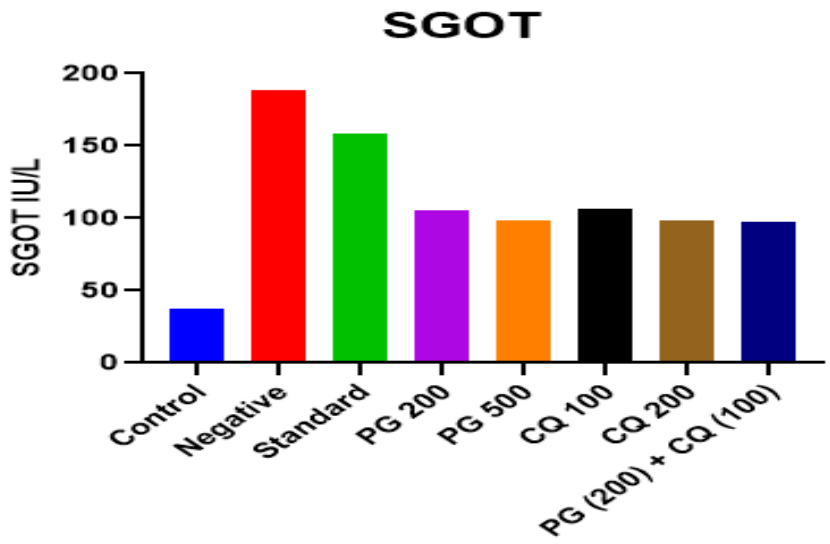
Group name	ALT (IU/L)
Group 1 (Control)	147.35 ±0.005
Group 2 (Negative)	232.58 ±0.003
Group 3 (Standard)	126.53 ±0.003
Group 4 (PG 200)	149.43 ±0.002
Group 5 (PG 500)	154.57 ±0.004
Group 6 (CQ100)	143.73 ±0.005
Group 7 (CQ200)	156.52 ±0.001
Group 8 (PG (200) + CQ (100))	155.23 ±0.001
SD	31.541
SEM	11.151

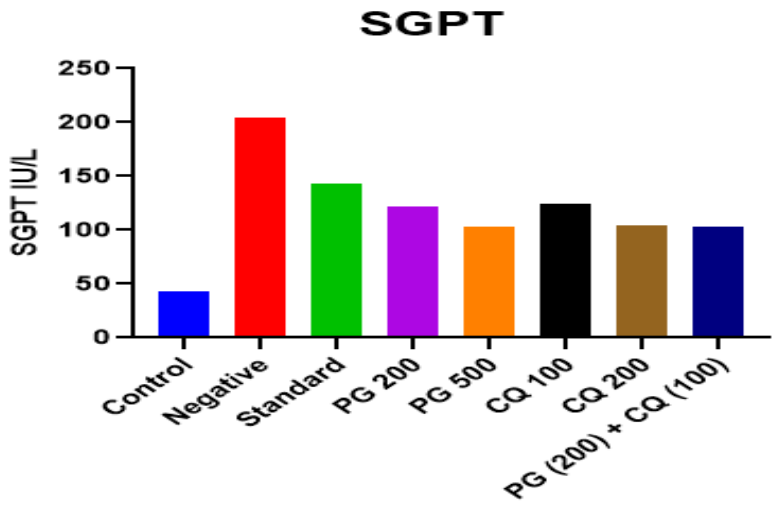


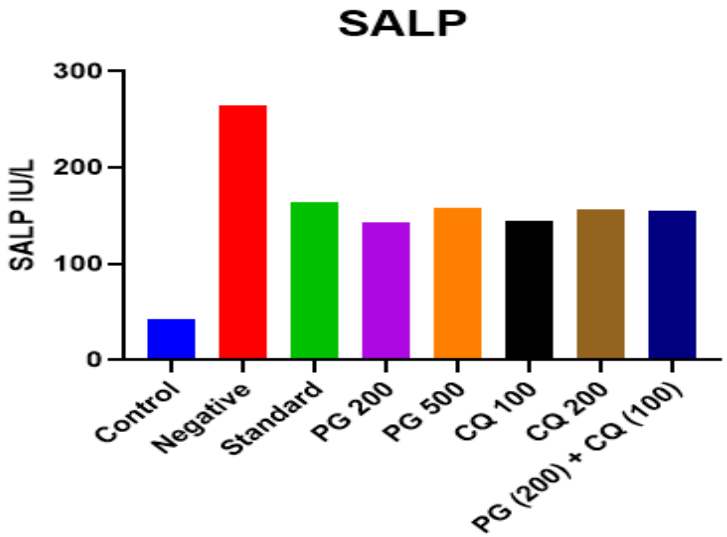
Serum ALT levels in rats treated with CCl4

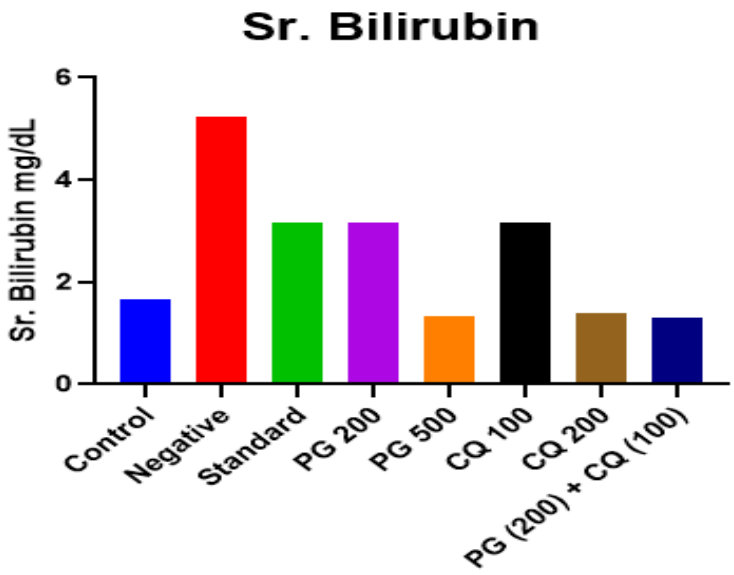
Serum Biochemical Parameters Punica granatum

Group	SGOT IU/L	SGPT IU/L	SALP IU/L	Sr. Bilirubin mg/dL
Group 1 (Control)	37.25±0.005	42.94±0.005	42.43±0.005	1.679±0.002
Group 2 (Negative)	188.43±0.005	204.21±0.005	264.56±0.005	5.248±0.003
Group 3 (Standard)	158.36±0.005	142.64±0.005	164.36±0.005	3.153±0.005
Group 4 (PG 200)	105.35±0.005	122.25±0.005	142.64±0.005	3.174±0.003
Group 5 (PG 500)	98.32±0.005	102.52±0.005	158.43±0.005	1.342±0.002
Group 6 (CQ100)	106.55±0.005	124.65±0.005	145.34±0.005	3.175±0.004
Group 7 (CQ200)	98.72±0.005	104.32±0.005	157.33±0.005	1.387±0.001
Group 8 (PG (200) + CQ (100))	97.82±0.005	103.55±0.005	155.33±0.005	1.312±0.004
SD	45.061	45.308	59.779	1.390
SEM	15.931	16.018	21.135	0.491

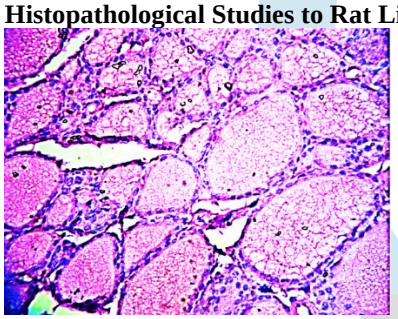

SGOT
SGOT IU/L
200
150
100
50
0
Control
Negative
Standard
PG 200
PG 500
CQ 100
CQ 200
PG (200) + CQ (100)


SGPT
SGPT IU/L
250
200
150
100
50
0
Control
Negative
Standard
PG 200
PG 500
CQ 100
CQ 200
PG (200) + CQ (100)

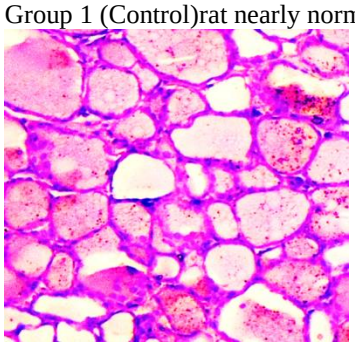

SALP
SALP IU/L
300
200
100
0
Control
Negative
Standard
PG 200
PG 500
CQ 100
CQ 200
PG (200) + CQ (100)

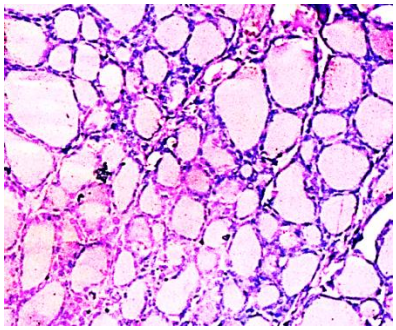


Histopathological Studies to Rat Liver Tissue:

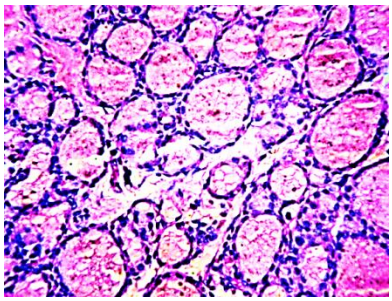


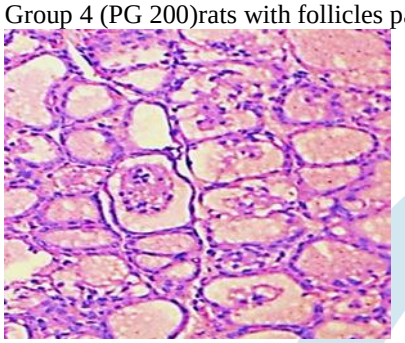
Group 1 (Control)rat nearly normal liver cells observed

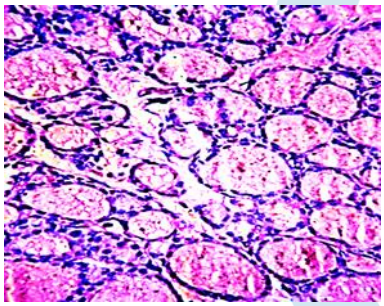


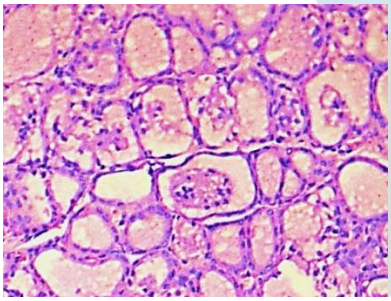


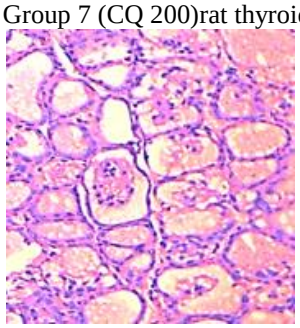
Group 2 (Negative)rats N-Focal Necrosis, PTI-Extensive portal triad inflammation
Group 3 (Standard)CVC-Central vein congestion.


Group 4 (PG 200)rats with follicles packed with a 60% colloid lined by cubidal epithel cells.


Group 5 (PG 500)rat thyroid gland segment of follicles lined with 80 percent colloid-filled cuboidal epithelial cells.


Group 6 (CQ 100)rats with follicles packed with a 50% colloid lined by cubidal epithel cells.


Group 7 (CQ 200)rat thyroid gland segment of follicles lined with 70 percent colloid-filled cuboidal epithelial cells.


Group 8 (PG (200) + CQ (100))CVC-Central vein congestion, RH-Regenerating hepatocytes.

DISCUSSION

Liver is liable for a few metabolic exercises in spite of the fact that numerous destructive toxicants, chemicals, and medications which may harm the liver are present. In our hepatotoxicity think about, therapeutic medication was utilized as a poison to actuate liver damage, since it's utilized by people for therapeutic reasons.

Mechanism of medicinal drug induced hepatotoxicity:

In this harmfulness there's upgraded arrangement of lipoperoxides, conjugated dienes. The improved levels of AST, ALT, mount and wilted levels of SOD are markers of liver damage.

Oxidative push is one major issue for the reason behind liver damage, within the fundamental of Kupffer cells by the activity of a harmful substance known as poison, that's released by beyond any doubt gram -negative microorganism blessing inside the bowel.

Biochemical parameters:

Amid viscus damage, cellular chemicals like AST, height and mount blessing inside the liver cells burst and spill into the body liquid, driving to improved concentrations. restorative sedate managed for twenty one days impressively improved of these body liquid proteins. inside the current think about treatment of rats with ethanolic extricate of vegetable seed significantly withered the degree of height and AST that's characteristic property of hepatoprotective action. As we expected any uncovered unremitting admissions of therapeutic medicate withered the exercises of the ROS rummaging proteins, viz. Sod. Current think about uncovered that the plant extricate has too been utilized in expanding the chemical protein inside the body that moreover shows that the plant to boot liable for fabricating inhibitor property.

CONCLUSION

On the basis of our findings, it may be worthy to suggest that

- Punica granatum and Cissus quadrangularis has antioxidant activity against CCL4 induced oxidative stress in rats by decreasing the oxidative stress biomarkers serum AST, serum ALT in liver.
- Punica granatum and Cissus quadrangularis has antioxidant effect, elevated by measuring antioxidant enzymes. There is increase in superoxide dismutase in liver tissue in CCL4 induced oxidative stress in rats.

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