Results

I. In vivo experiments:

1. Effect of pretreatment with AG and Silymarin on average serum liver enzymes in acetaminophen administered rats:

Induction of hepatotoxicity by a single injection of acetaminophen (500 mg / kg body weight) resulted in significant deterioration of hepatic function evidenced by elevation of liver enzymes SGOT, SGPT, and ALP from 45.83 ±.95 U/ml, 34.00 ±.58 U/ml and 11.66 ±.33 KAU respectively in control group to 90.33 ± 2.46 U/ml, 89.16 ± 3.08 U/ml and 36.00 ± .97 KAU in acetaminophen administered group. Table (3) & Fig. (4.5.6).

Treatment of acetaminophen administered rats with AG 7.6 gm / kg before giving them a single injection of acetaminophen (500 mg / kg body weight) reduced the liver enzymes SGOT, SGPT, and ALP significantly ($P< 0.05$) to 55.66 ± 2.39 U/ml 48.00 ± 2.65 U/ml and 16.33 ± .95 KAU respectively compared to acetaminophen administered rat group but there is still significant elevation in liver enzymes in the treated group in comparison with the control normal rats this indicats that the treatment caused improvement in the condition not complete recovery. Table (4) . Fig (4.5.6).

Treatment of acetaminophen administered rats with silymarin (200 mg / kg body weight) before giving a single injection of acetaminophen (500 mg / kg body weight) reduced the liver enzymes SGOT, SGPT, and ALP significantly ($P< 0.05$) to 70.33 ± 2.86U/ml 55.16 ± 1.78U/ml and 21.83 ± 1.30KAU respectively compared to acetaminophen administered rat group but there is still significant elevation in liver enzymes in the treated group in

comparison with the control normal rats this indicats that the treatment caused improvement in the condition not complete recovery. Table (5) & Fig. (4.5.6).

Comparing the result of Silymarin treated group to that of AG treated group, AG was significantly ($P < 0.05$) more effective than Silymarin in reducing the liver enzymes SGOT, SGPT and ALP. Table(7) & Fig (1.2.3).

Treatment of acetaminophen administered rats with both AG 7.6 gm / kg and silymarin (200 mg / kg body weight) before giving a single injection of acetaminophen (500 mg / kg body weight) reduced the liver enzymes SGOT, SGPT, and ALP significantly ($P< 0.05$) to $\pm$ 53.50 $\pm$ 4.28 U/ ml, 45.66 $\pm$ 2.08 U/ ml, and 16.00 $\pm$ 1.26 KAU respectively compared to acetaminophen administered rats but there is still significant elevation in liver enzymes in the treated group in comparison with the control normal rats this indicats that the treatment caused improvement in the condition not complete recovery. Table (6) & Fig. (4-5-6).

Comparing the result of AG&Silymarin treated group to that of AG treated group, adding AG to Silymarin was not significantly ($P > 0.05$) more effective than AG alone in reducing the liver enzymes SGOT, SGPT, and ALP Table (7) . Fig (4.5.6).

Comparing the result of AG&Silymarin treated group to that of Silymarin treated group, adding AG to Silymarin was significantly ($P < 0.05$) more effective than Silymarin alone in reducing the liver enzymes SGOT, SGPT but not for ALP which was not significantly affected Table (7) . Fig (4.5.6).

Table (3): Effect of acetaminophen administration (500 mg / kg) on average serum liver enzymes:

Parameter / Group	SGOT(U/ml)	SGPT(U/ml)	ALP(KAU)
Control	45.83 ± .95	34.00 ± 57735	11.66 ± .33
Acetaminophin adminstered	90.33 ± 2.46 *	89.16 ± 3.08 *	36.00 ± .97 *

Data represented as Mean ± SEM (n = 6)
* Significant compared with control rats

Table (4): Effect of pretreatment with AG (7.6 gm/kg) on average serum liver enzymes in acetaminophen administrated (500 mg / kg) rats:

Parameter / Group	SGOT(U/ml)	SGPT(U/ml)	ALP(KAU)
Control	45.83 ± .95	34.00 ± 57735	11.66 ± .33
Acetaminophin adminstered	90.33 ± 2.46 *	89.16 ± 3.08 *	36.00 ± .97 *
AG treated	* 55.66 ± 2.39 **	* 48.00 ± 2.65 **	* 16.33 ± .95 **

Data represented as Mean ± SEM (n = 6)

* Significant compared with control rats.
** Significant compared with Acetaminophin adminstered rats.

Table (5): Effects of pretreatment with Silymarin (200 mg / kg) on average serum liver enzymes in acetaminophin administered (500 mg / kg) rats:

Parameter / Group	SGOT(U/ml)	SGPT(U/ml)	ALP(KAU)
Control	45.83 ± .95	34.00 ± 57735	11.66 ± .33
Acetaminophin adminstered	90.33 ± 2.46 *	89.16 ± 3.08 *	36.00 ± .97 *
Silymarin treaeted	* 70.33 ± 2.86 **	* 55.16 ± 1.78 **	* 21.83 ± 1.30 **

Table (6): Effects of combined pretreatment with AG(7.6 gm/kg) and Silymarin(200 mg / kg) treatment on average serum liver enzymes in acetaminophen administrated (500 mg / kg) rats:

Parameter / Group	SGOT(U/ml)	SGPT(U/ml)	ALP(KAU)
Control	45.83 ± .95	34.00 ± 57735	11.66 ± .33
Acetaminophin adminstered	90.33 ± 2.46 *	89.16 ± 3.08 *	36.00 ± .97 *
AG&S treated	* 53.50 ± 4.28 **	* 45.66 ± 2.08 **	* 16.00 ± 1.26 **

Data represented as Mean ± SEM (n = 6)

* Significant compared with control rats.

** Significant compared with Acetaminophin adminstered rats.

Table (7): Effects of combined pretreatment with AG (7.6 gm/kg) and Silymarin(200 mg / kg) treatment on average serum liver enzymes in acetaminophen administrated (500 mg / kg) rats in all studed groups:

Parameter / Group	SGOT(U/ml)	SGPT(U/ml)	ALP(KAU)
Control	45.83 ± .95	34.00 ± 57735	11.66 ± .33
Acetaminophin adminstered	90.33 ± 2.46 *	89.16 ± 3.08 *	36.00 ± .97 *
AG treated	* 55.66 ± 2.39 **	* 48.00 ± 2.65 **	* 16.33 ± .95 **
Silmarin treated	* 70.33 ± 2.86 **	* 55.16 ± 1.78 **	* 21.83 ± 1.30 **
AG&S treated	* 53.50 ± 4.28 **	* 45.66 ± 2.08 **	* 16.00 ± 1.26 **

-Data represented as Mean ± SEM (n = 6)

* Significant compared with control rats.

** Significant compared with Acetaminophin adminstered rats.

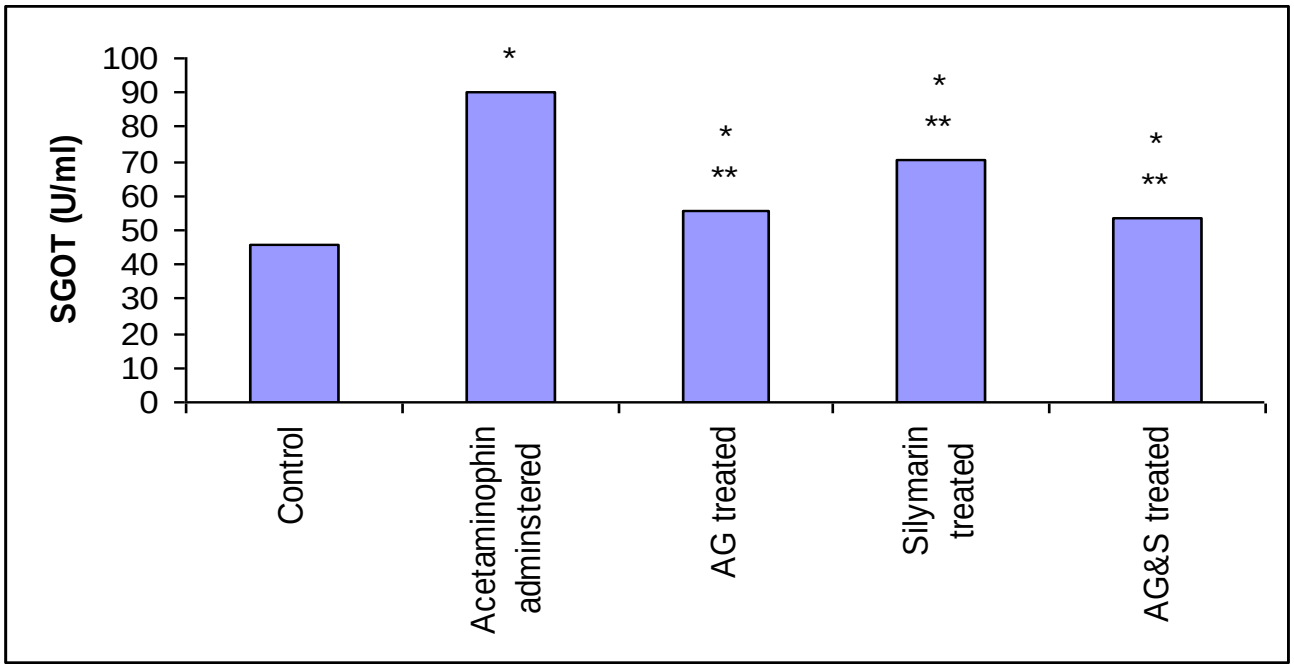

Figure (4): Histogram showing SGOT in various groups.

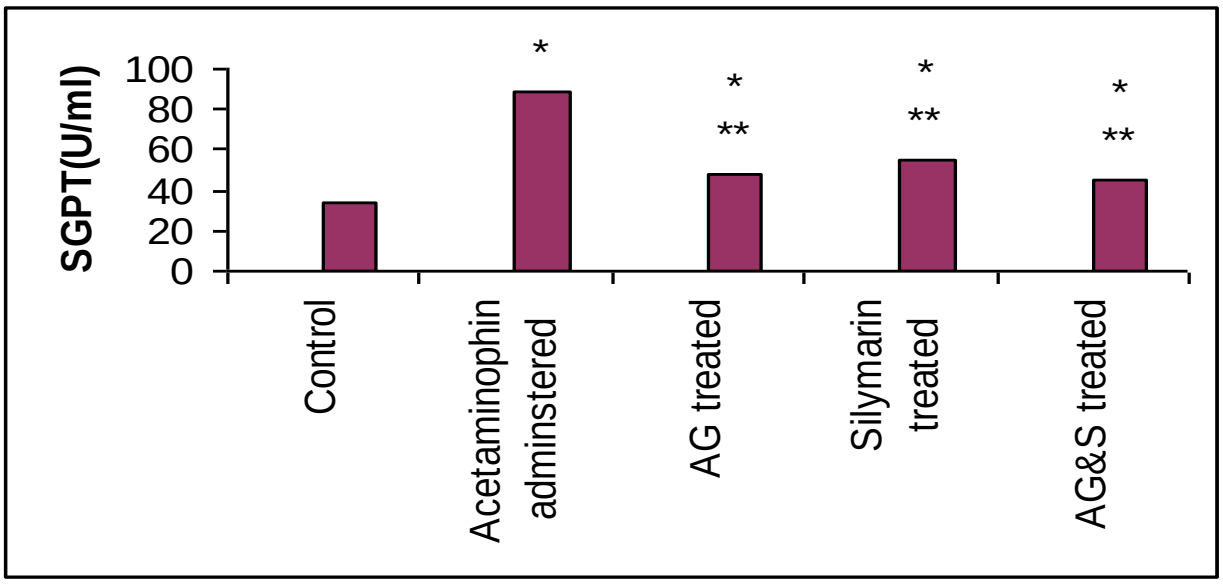

Figure (5): Histogram showing SGPT in various groups.

* Significant compared with control normal rats.

** Significant compared with Acetaminophin adminstered rats.

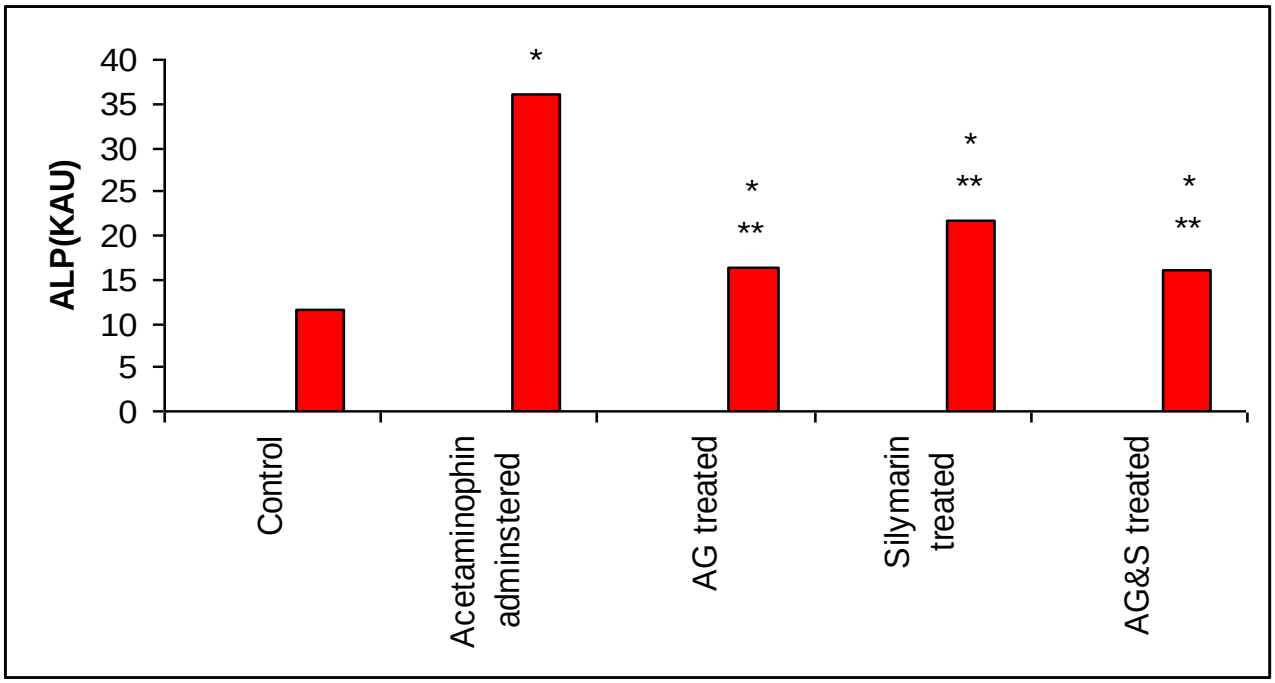

Figure (6): Histogram showing ALP in various groups.

* Significant compared with control normal rats.

** Significant compared with Acetaminophin adminstered rats.

2. Effect of pretreatment with AG and/or Silymarin on oxidative activity in acetaminophen administered rats:

Induction of hepatotoxicity by a single injection of acetaminophen (500 mg /kg body weight) resulted in a significant rise of oxidative activity eviedenced by elevation in malondialdehyde (MDA) from 3.25 $\pm$ .21µmol /ml in control group to 7.92 $\pm$ .44 µmol/ml in acetaminophen administered group (Table (8)& Fig (7)).

Pretreatment of acetaminophen administered group with AG (7.6 gm / kg) before giving them a single injection of acetaminophen (500 mg / kg body weight) reduced the oxidative activity evedenced by statistically significant reduction in MDA ($P < 0.05$) to 4.25 $\pm$ .21µmol/ml compared to acetaminophen administered group but there is still significant elevation in MDA in the treated group in comparison with the control normal rats this indicats that the treatment caused improvement in the condition not complete recovery. (Table (8) & Fig (7)).

Pretreatment of acetaminophen administered group with Silymarin(200 mg / kg) before giving them a single injection of acetaminophen (500 mg / kg body weight) reduced the oxidative activity evedenced by statistically significant reduction in MDA ($P < 0.05$) to 5.75 $\pm$ .21µmol/ml compared to acetaminophen administered group but there is still significant elevation in MDA in the treated group in comparison with the control normal rats this indicats that the treatment caused improvement in the condition not complete recovery. (Table (8) & Fig (7)).

Comparing the result of Silymarin treated group to that of AG treated group, AG was significantly ($P < 0.05$) more effective than Silymarin in reducing oxidative activity (Table (8) - Fig (7)).

Pretreatment of acetaminophen administered group with both AG (7.6 gm / kg) and Silymarin (200 mg / kg) before giving them a single injection of acetaminophen (500 mg / kg body weight) reduced MDA significantly ($P < 0.05$) to $4.33 \pm .28$ µmol/ml compared to acetaminophen administered group but there is still significant elevation in MDA in the treated group in comparison with the control normal rats this indicats that the treatment caused improvement in the condition not complete recovery. (Table (8) & Fig (7)).

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Comparing the result of AG&S treated group to that of AG treated group , adding Silymarin to AG was not significantly ($P > 0.05$) more effective than AG alone in reducing the oxidative activity (Table (8) - Fig (7)).

Comparing the result of AG&S treated group to that of Silymarin treated group, adding AG to Silymarin was significantly ($P < 0.05$) more effective than Silymarin alone in reducing the oxidative activity (Table (8) - Fig (7)).

Table (8): Effects of both AG and Silymarin pretreatment on oxidative activity in acetaminophin adminsterated in rats:

Parameter / Group	MDA(µmol/ml)
Control	3.25 ± .21
Acetaminophin adminstered	7.92 ± .44 *
AG treated	4.25 ± .21 ** *
Silymarin treated	5.75 ± .21 ** *
AG&S treated	4.33 ± .28 ** *

Data represented as Mean ± SEM (n = 6)

* Significant compared with control normal rats.

** Significant compared with Acetaminophin adminstered rats.

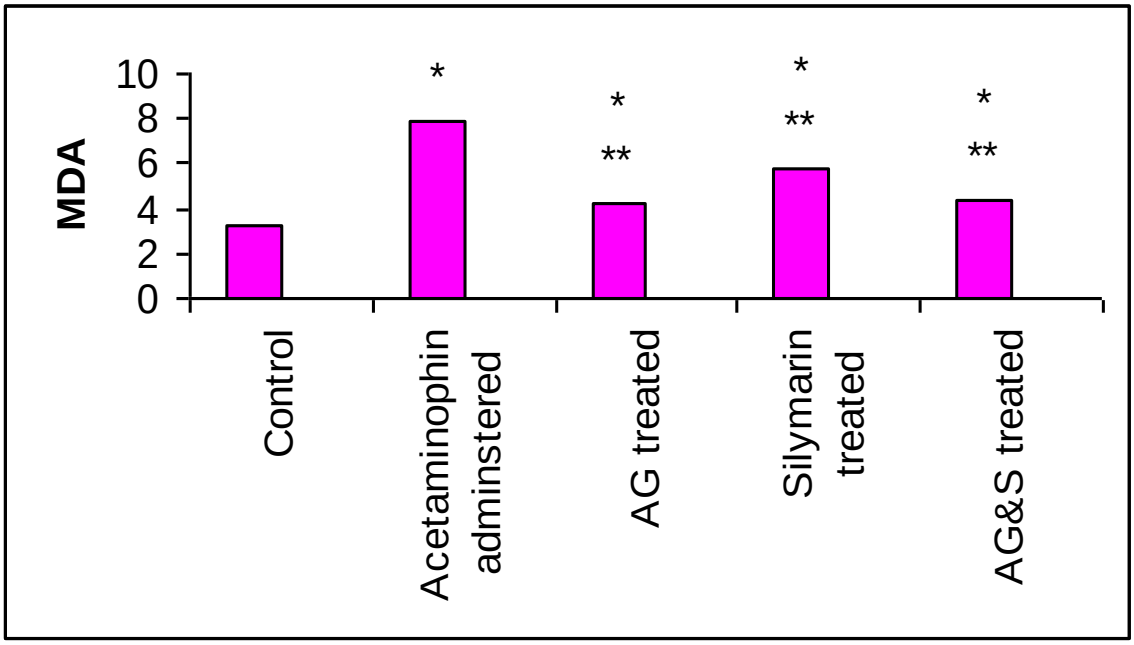

Figure (7): Histogram showing MDA (µmol/ml) in various groups.

* Significant compared with control normal rats.

** Significant compared with Acetaminophin adminstered rats.

3. Effect of both AG and/ or Silymarin pretreatment on Portal pressure in acetaminophin adminsterated rats:

Induction of hepatotoxicity by a single injection of acetaminophen (500 mg / kg body weight) resulted in a significant rise of portal pressure from 12.13 ± .13 mmHg in control group to 13.5 ± .20 mmHg in acetaminophin adminsterated group (Table (9)).

Pretreatment of acetaminophin adminsterated group with AG (7.6 gm / kg) before giving them a single intra-peritoneal injection of acetaminophen (500 mg / kg body weight) reduced the portal pressure significantly ($P < 0.05$) to 12.7± .14mmHg compared to acetaminophin adminsterated group but there is still significant elevation in portal pressure in the treated group in comparison with the control normal rats this indicats that the treatment caused improvement in the condition not complete recovery. (Table (9)).

Pretreatment of acetaminophin adminsterated group with Silymarin (200 mg / kg body weight) before giving them a single injection of acetaminophen (500 mg / kg body weight) reduced portal pressure significantly ($P < 0.05$) to 12.4 ± .13 mmHg compared to acetaminophin adminsterated group but there is still significant elevation in portal pressure in the treated group in comparison with the control normal rats this indicats that the treatment caused improvement in the condition not complete recovery. (Table (9)).

Comparing the result of Silymarin treated group to that of AG treated group , there was no statistically significant (P >0.05) between AG and Silymarin in reducing portal pressure (Table (9) - Fig (8)).

Pretreatment of acetaminophin adminsterated group with both AG (7.6 gm / kg) and Silymarin (200 mg / kg) before giving them a single injection of acetaminophen (500 mg / kg body weight) reduced portal pressure significantly (P < 0.05) to 12.13 $\pm$ .14 mmHg compared to acetaminophin adminsterated group comparing theseresults with control normal rats there is no statistically significant difference between this group and the control group this in dicates complete recovery with the compiened treatment (Table (9) - Fig (8)).

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Comparing the result of AG&S treated group to that of AG treated group , adding Silymarin to AG was not significantly (P > 0.05) more effective than AG alone in reducing the portal pressure (Table (9) - Fig (8)).

Comparing the result of AG&S treated group to that of Silymarin treated group , adding AG to Silymarin was significantly (P <0.05) more effective than Silymarin alone in reducing the portal pressure (Table (9) - Fig (8)).

Table (9): Effects of pretreatment with AG (6.7gm / kg) and Silymarin (200 mg / kg) treatment on portal pressure in acetaminophen administered (500 mg / kg) rats:

Parameter / Group	Portal pressure (mmHg)
Control	**12.13 ± .13**
Acetaminophen adminstered	**13.5 ± .20 ***
AG treated	**12.7± .14 ** ***
Silymarin treated	**12.4 ± .13 ** ***
AG&S treated	**12.13 ± .14 ****

Data represented as Mean ± SEM (n = 6)

* Significant compared with control normal rats.

** Significant compared with Acetaminophin adminstered rats.

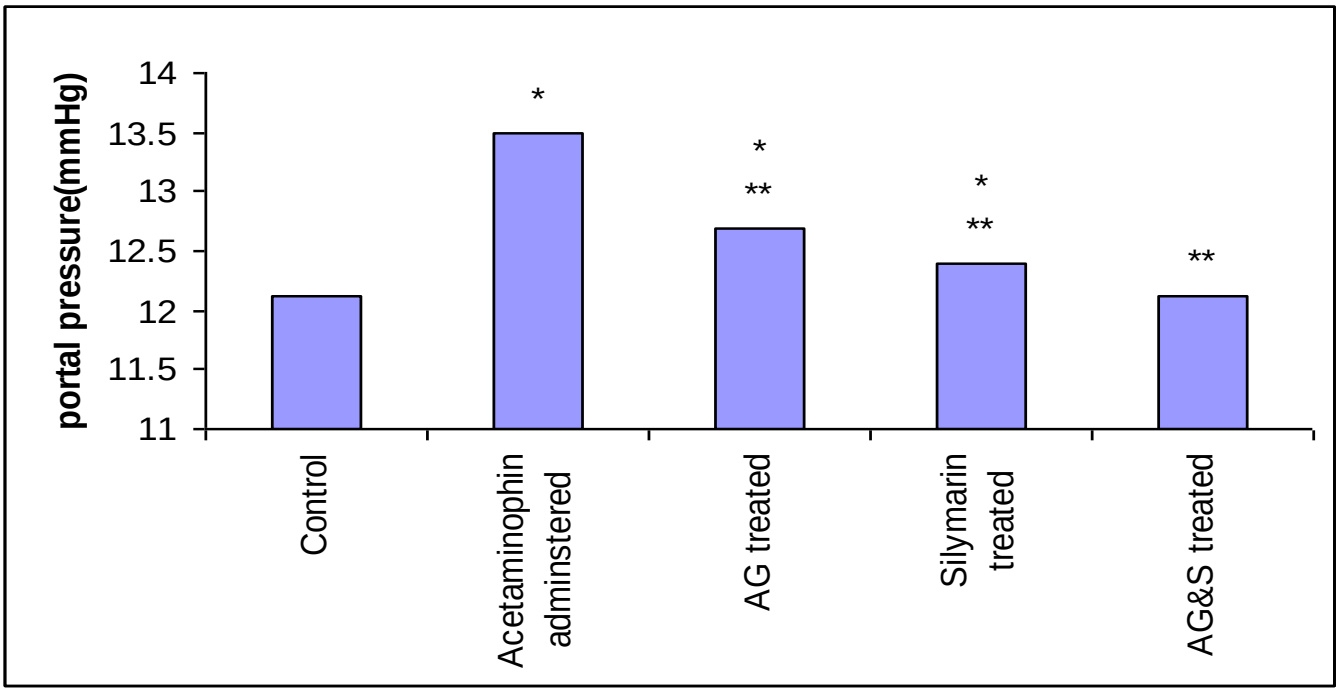


Figure (8): Histogram showing Portal pressure in various groups.

* Significant compared with control normal rats.

** Significant compared with Acetaminophin adminstered rats.

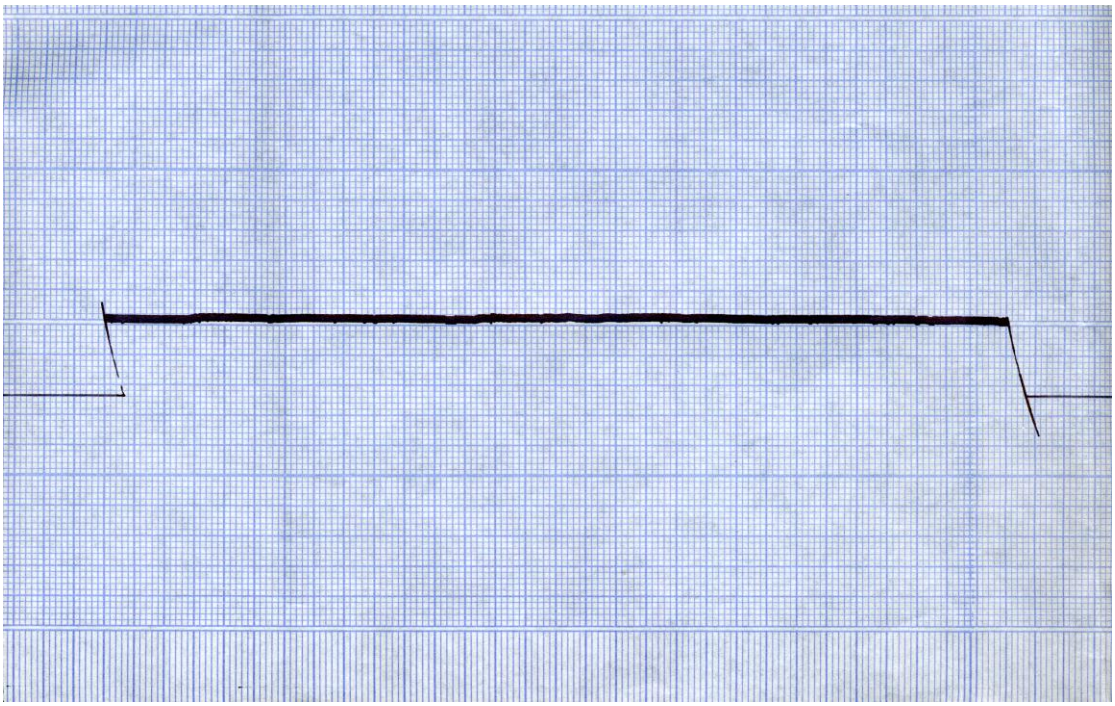


Fig. (9): Portal blood pressure measurement of control rats.

(A typical trace of 6 seprate experiments)

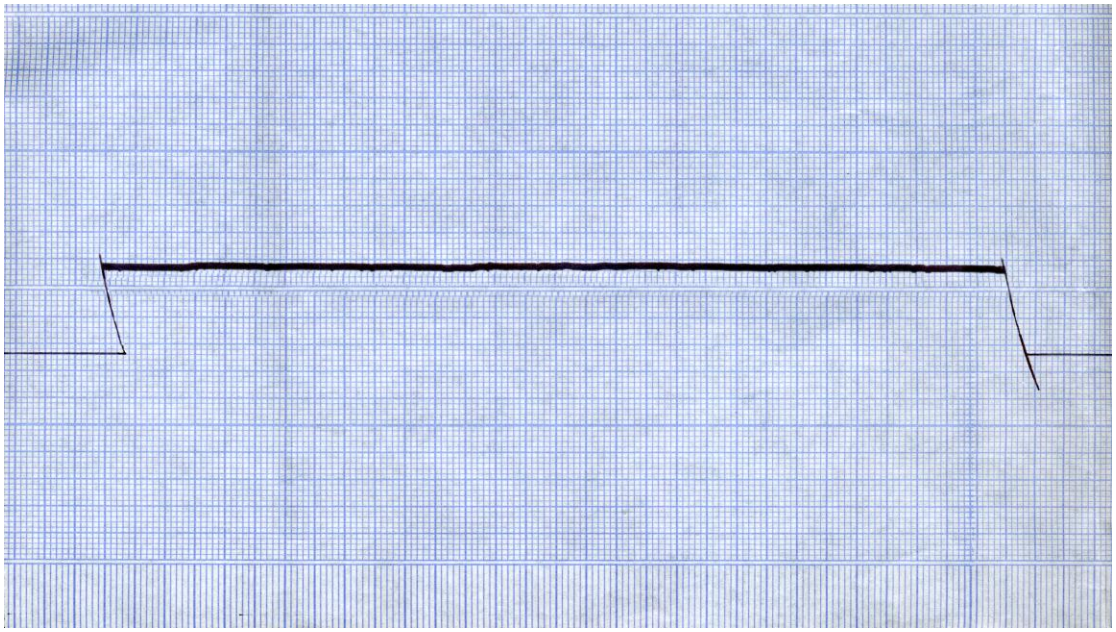


Fig. (10): Portal blood pressure measurement of acetaminophin adminstered rats. **(A typical trace of 6 seprate experiments)**

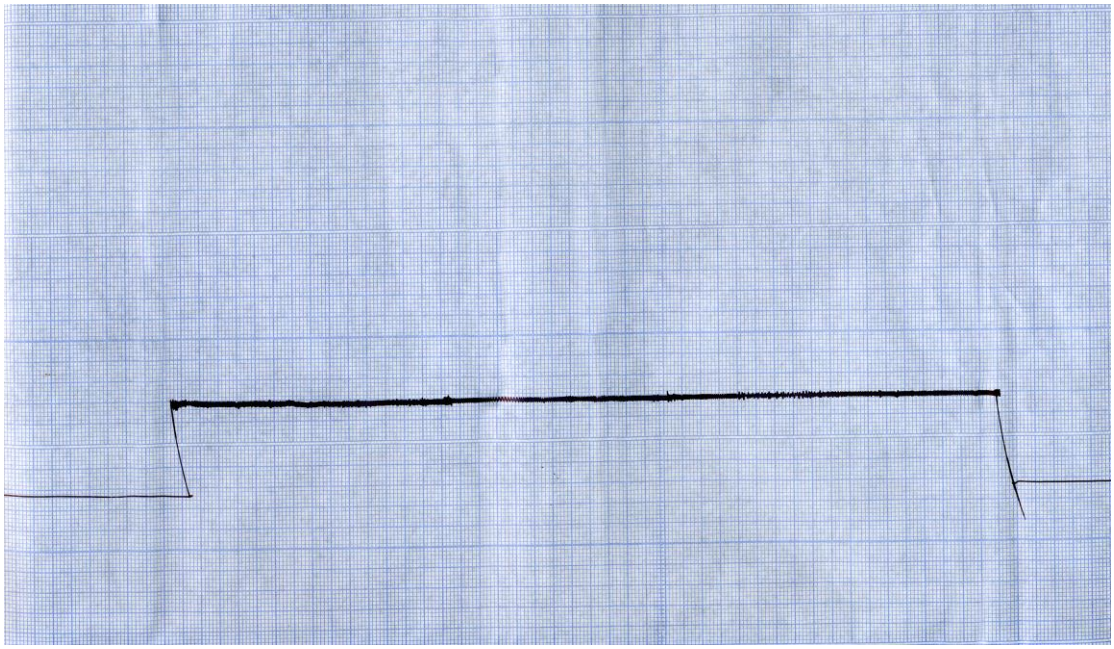


Fig. (11): Portal blood pressure measurement of AG treated rats.

(A typical trace of 6 seprate experiments)

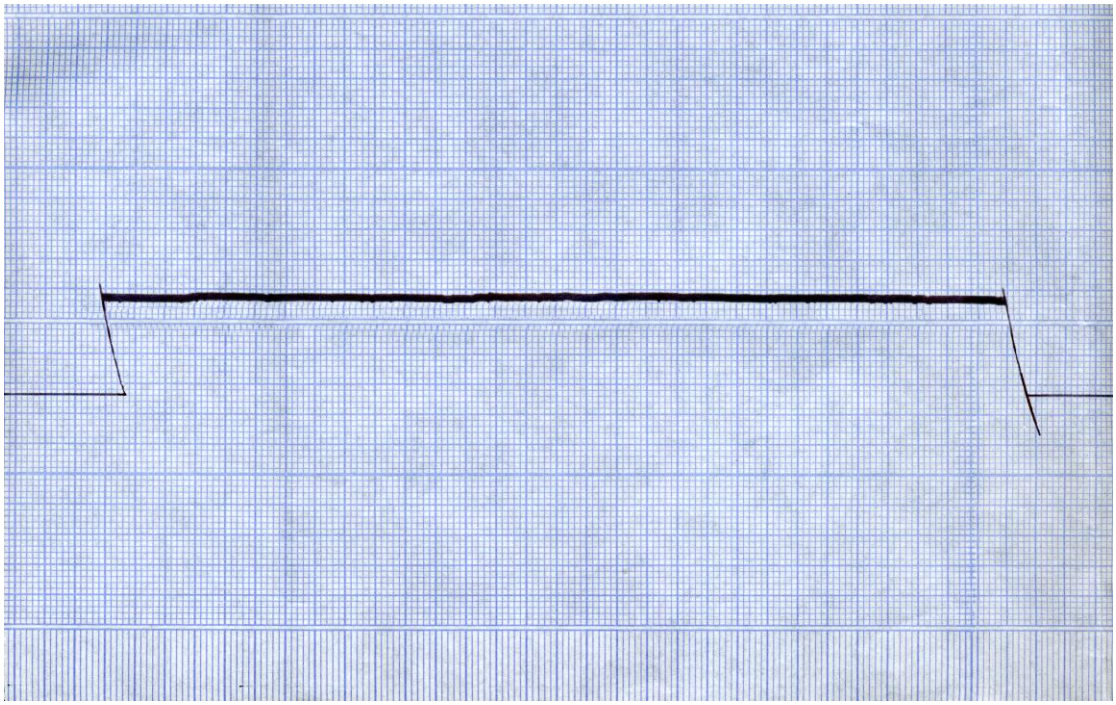


Fig. (12): Portal blood pressure measurement of silymarin treated rats.

(A typical trace of 6 seprate experiments)

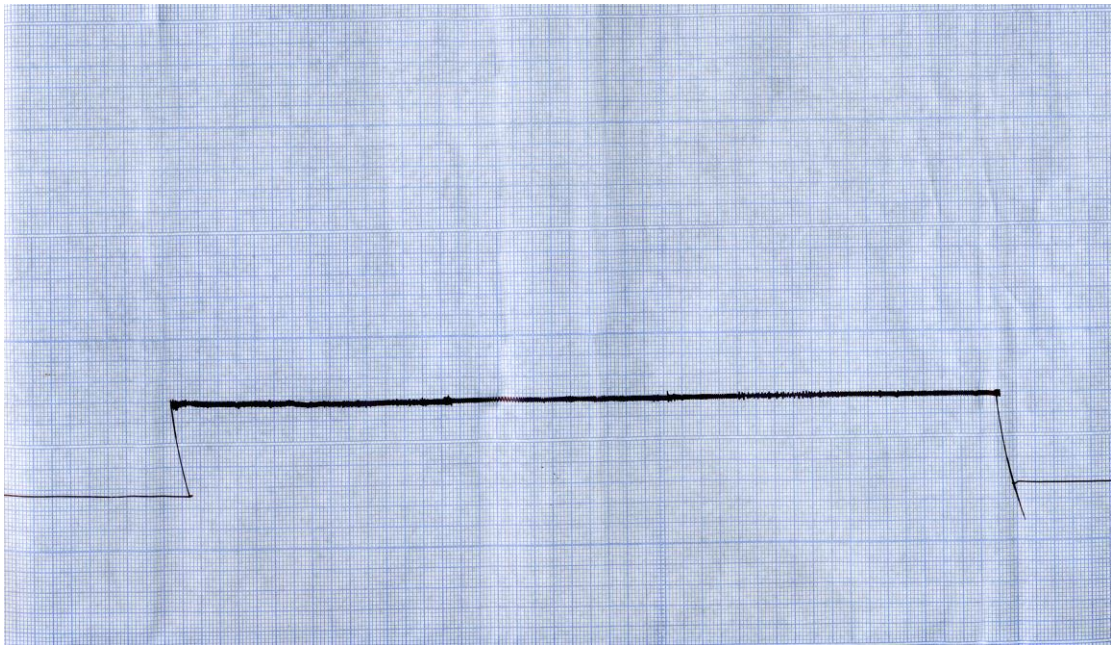


Fig. (13): Portal blood pressure measurement of AG&Silymarin treated rats. **(A typical trace of 6 seprate experiments)**

4- Histopathological evaluation of the liver:

Histological examination of cut sections of the liver of control group (group I) showed that there was normal liver architecture composed of hexagonadal or pentagonadal lobules with central veins . Hepatocytes are arranged in trabecules running radiantly from the central vein and are separated by sinusoids containing Kupffer cells (Figure 14).

In acetaminophin adminstered (Group II), there was preserved liver architecture, hepatocytes show marked hydropic changes, some necro-infilammmatory foci , dilated congested central veins and portal tract infilammation (Figure15).

In AG treated group (group III), there was preserved liver architecture, hepatocytes show no hydropic changes , no necro-infilammmatory foci , congested central veins and portal tract infilammation (Figure 16).

In Silymarin treated group (group IV), there was preserved liver architecture, hepatocytes show moderate hydropic changes , few necro-infilammmatory foci , congested central veins and no portal tract infilammation (Figure 17).

In AG & Silymarin treated (Group V), there was preserved liver architecture , hepatocytes show few hydropic changes , no necro-infilammmatory foci (Figure 18).

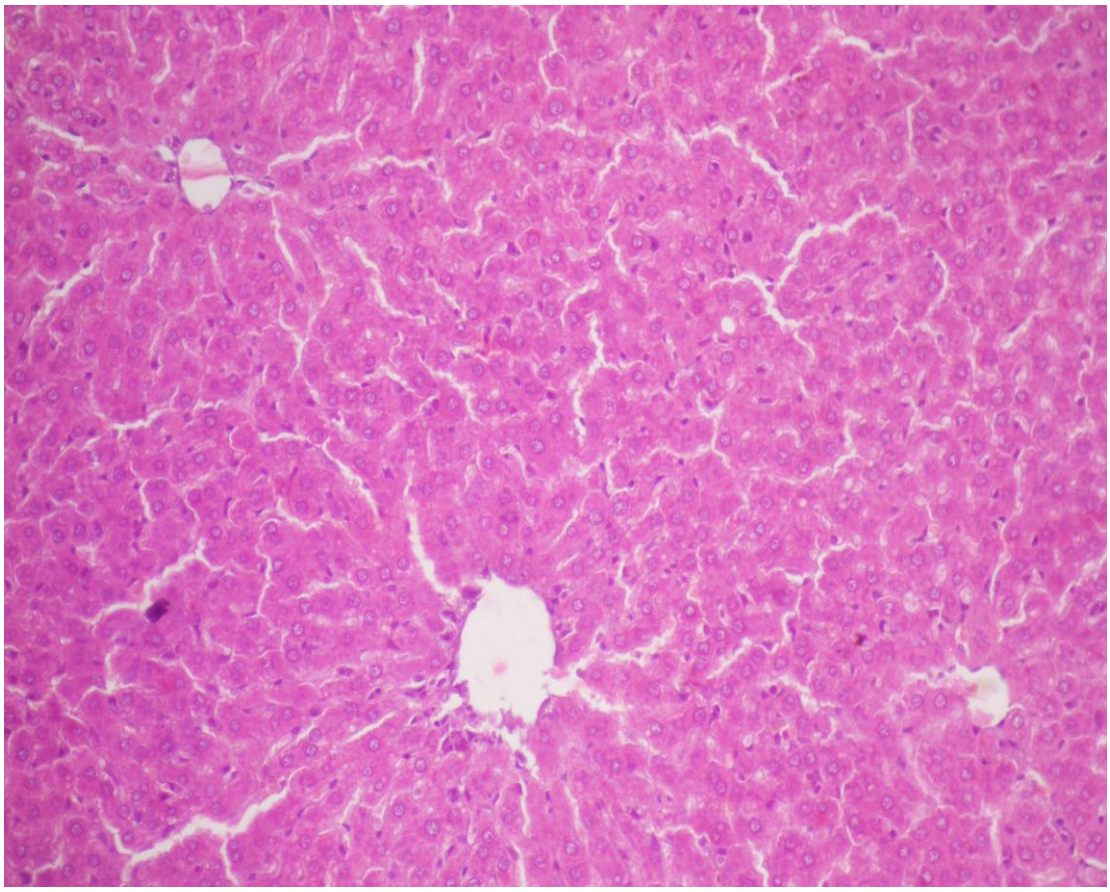


Figure (14): A photomicrograph of a cut section in the liver of a control rat (group I) showing normal liver architecture composed of hexagonadal or pentagonadal lobules with central veins and peripheral hepatic triads or tetrads embedded in connective tissue. Hepatocytes are arranged in trabecules running radiantly from the central vein and are separated by sinusoids containing Kupffer cells. (H x & E x 400).

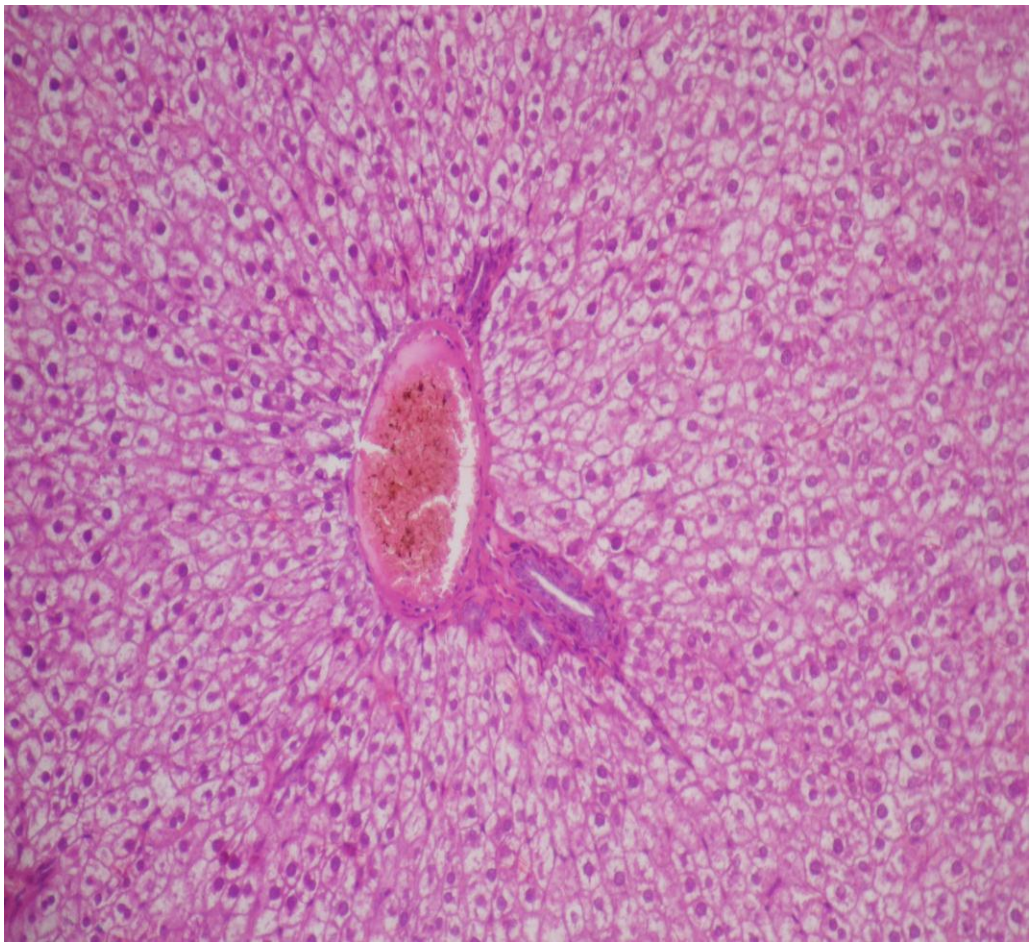


Figure (15): A photomicrograph of a cut section in the liver of acetaminophen administered group showing preserved liver architecture, hepatocytes show marked hydropic changes, some necro-infilammmatory foci and portal tract infilammation .

(H x & E x 400).

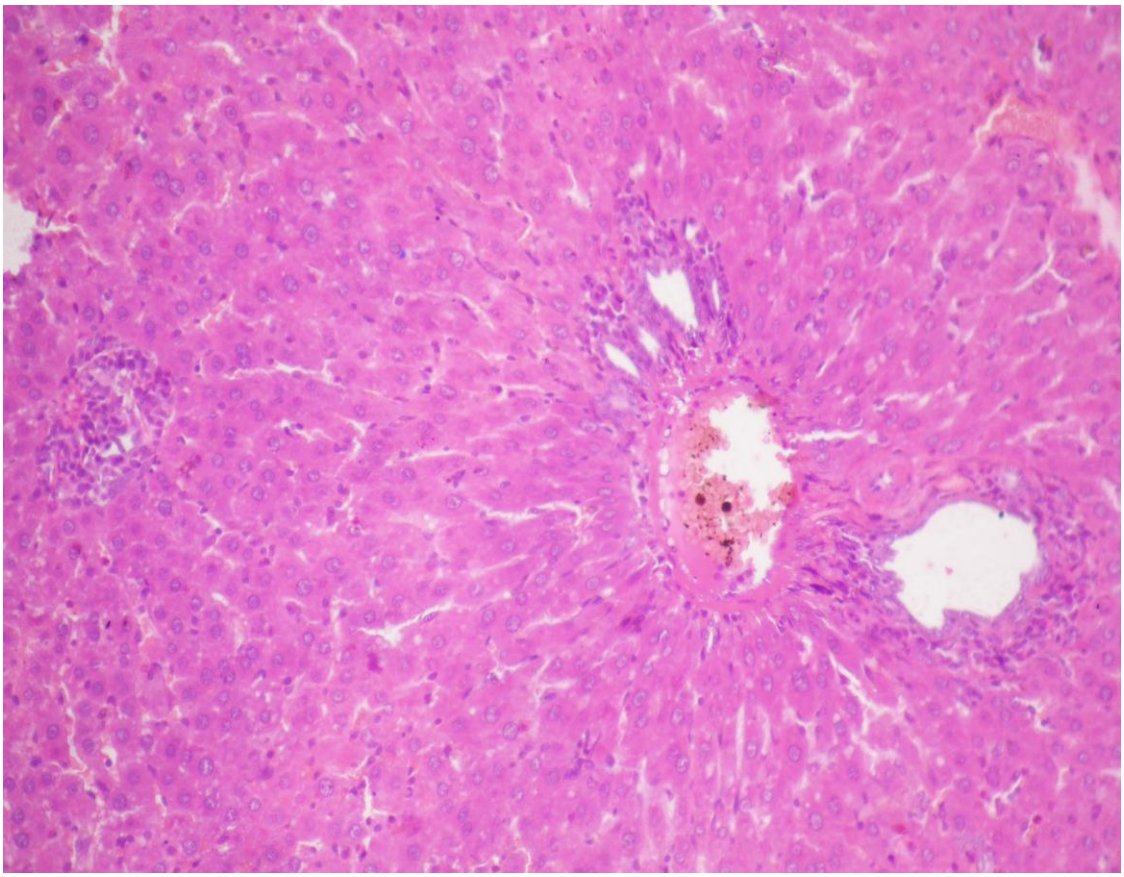


Figure (16): A photomicrograph of a cut section in the liver in AG treated group (group III), showing preserved liver architecture, hepatocytes show few hydropic changes , no necro-infilammmatory foci , congested central veins and no portal tract infilammation .

(H x & E x 400).

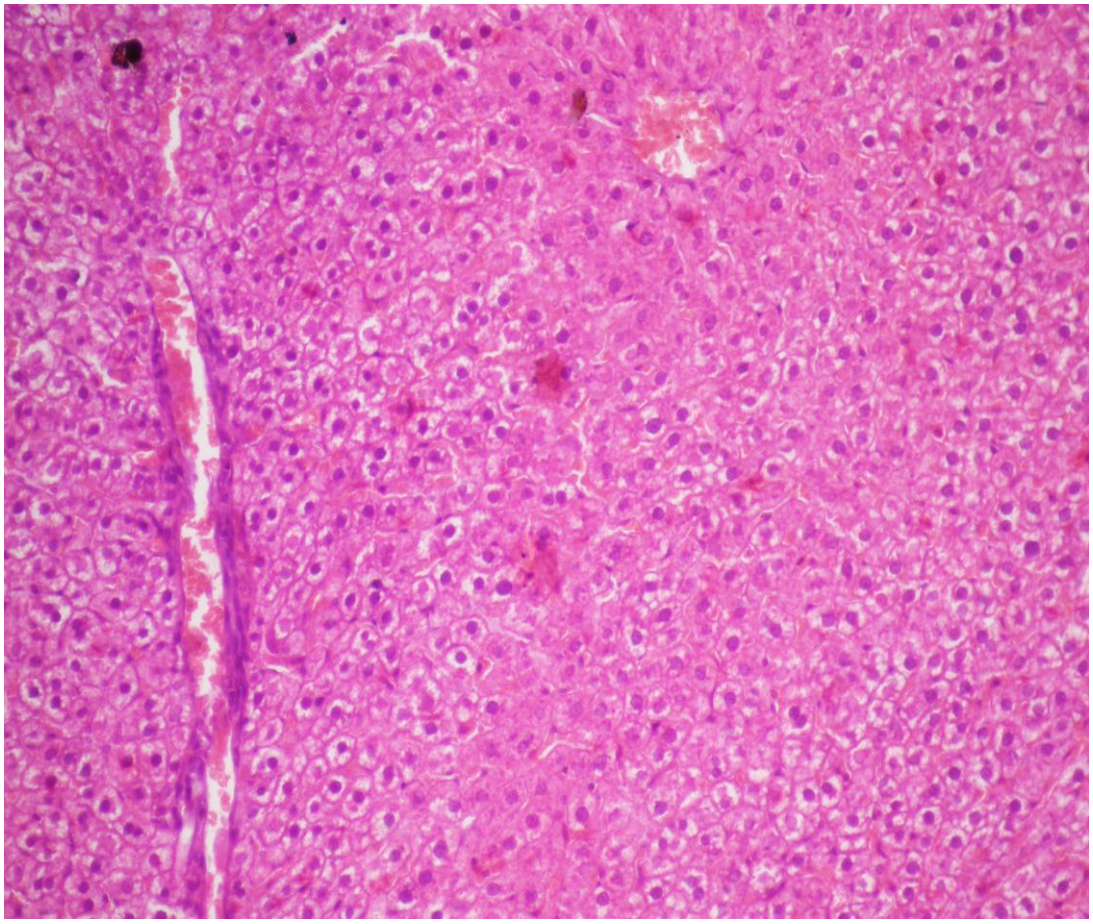


Figure (17): A photomicrograph of a cut section in the liver in Silymarin treated group (group IV), showing preserved liver architecture, hepatocytes show moderate hydropic changes , few necro-infilammmatory foci , congested central veins and no portal tract infilammation. (H x & E x 400).

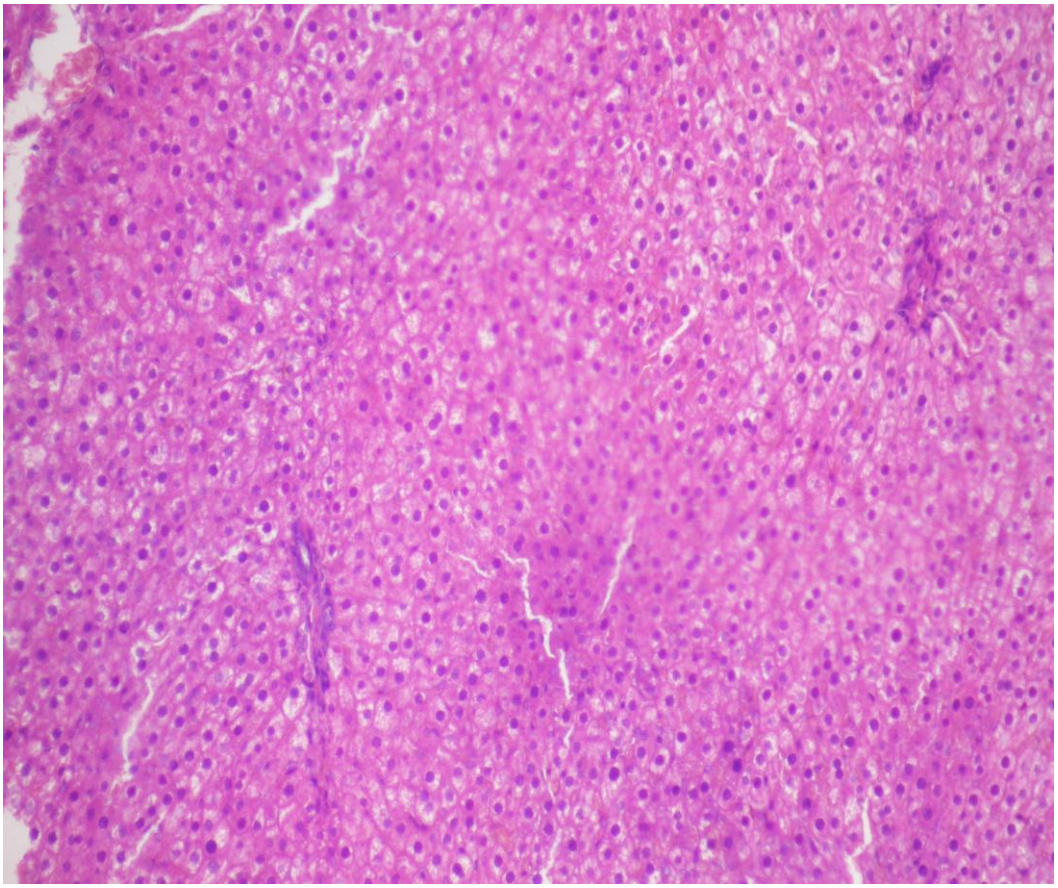


Figure (18): A photomicrograph of a cut section in the liver in AG & Silymarin treated (Group V), showing preserved liver architecture , hepatocytes show few hydropic changes , no necro-infilammmatory foci .

(H x & E x 400).

3. Effects on isolated perfused rabbit's jejunum:

(A) Effect of AG on isolated perfused rabbit's jejunum:

It was observed that addition of AG in different dose levels (30, 100, 300 and 1000 µg/ml bath) produced dose related stimulation of rhythmic contraction of rabbit's jejunum (Table (10), Figures (19-20)).

- **Site of action of AG on isolated perfused rabbit's jejunum:**

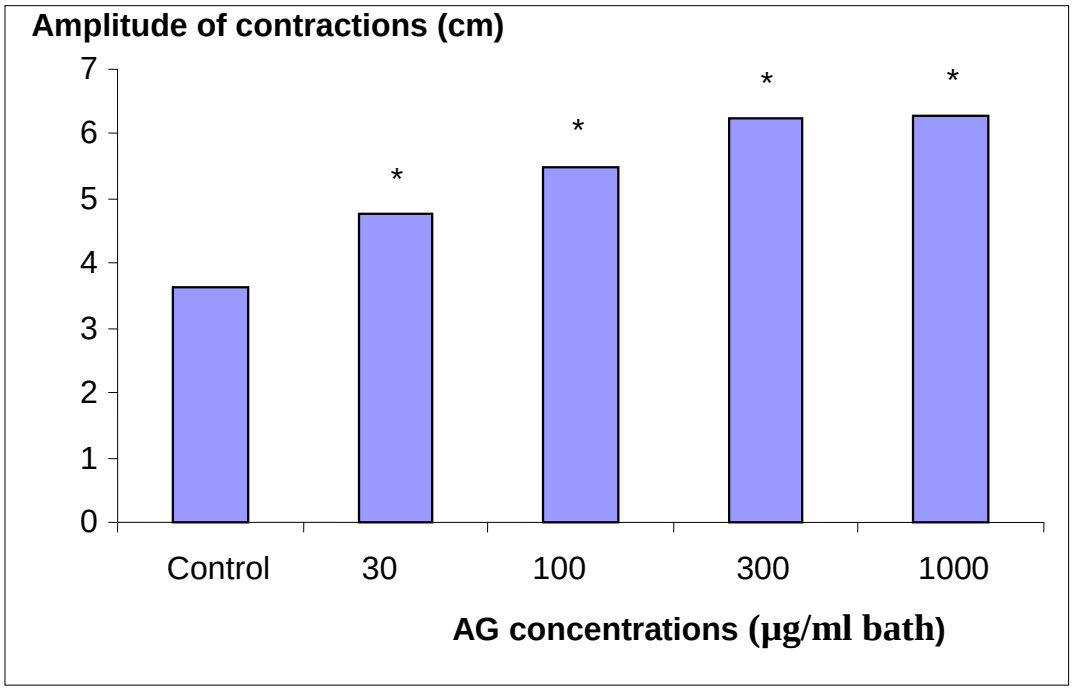
It was observed that blocking of muscarinic, nicotinic, histaminic and seretonergic receptors did not affect the stimulatory action of AG (100 ug/ml bath). This indicates that AG did not act through the muscarinic, nicotinic, histaminic or seretonergic receptors (Figures (21-22-23))

Table (10): Effect of AG on the amplitude of spontaneous rhythmic contraction of isolated perfused rabbit's jejunum.

Dose of AG µg/ml bath	Level of contraction before (cm)	Level of contraction after (cm)
30	3.63+.2	4.75+ ,3 *
100		5.50 + .4 *
300		6.25 + .5 *
1000		6.30+.5 *

Data represented as mean ± SEM of six experiments

***Significant level compared to control.**



* Significant compared with control group.

Figure (19): Histogram showing the stimulatory effect of AG on the amplitude of spontaneous contraction of isolated perfused rabbit's jejunum.

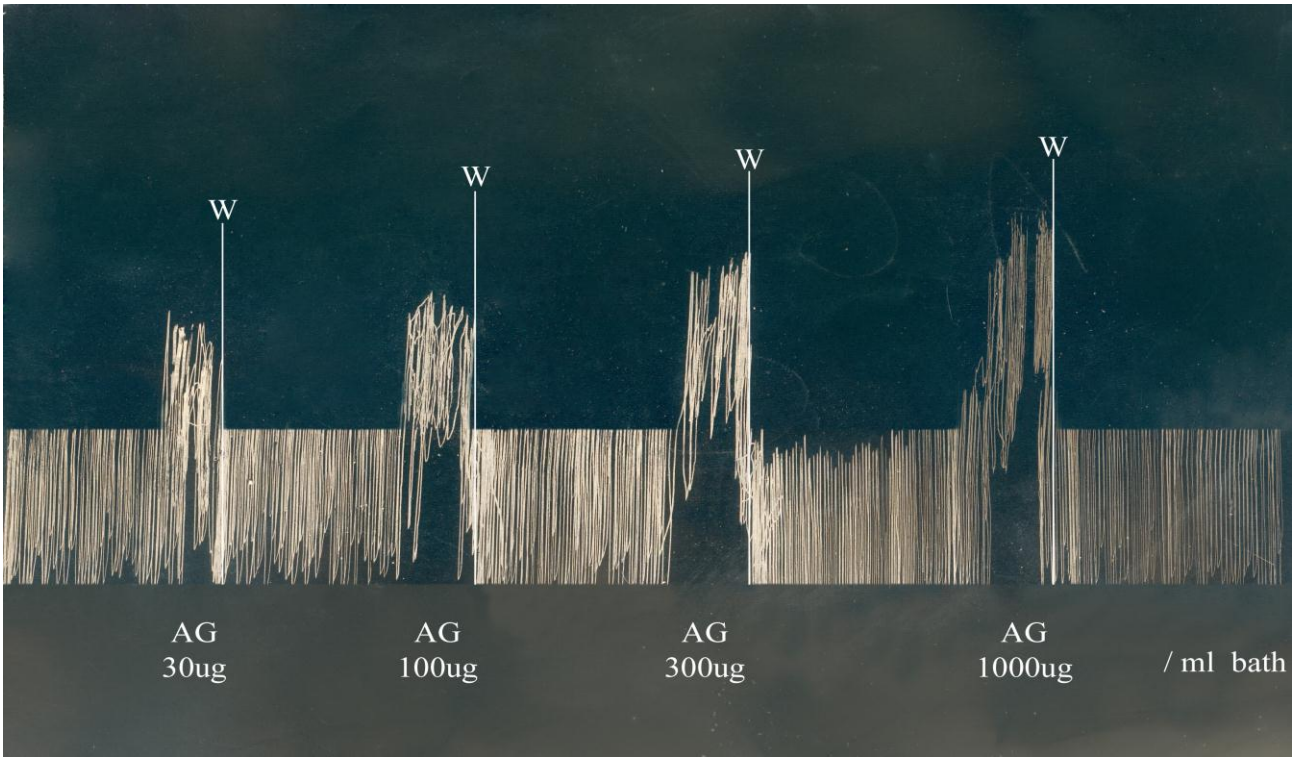


Figure (20): A record demonstrating the effect of AG on isolated perfused rabbit's jejunum.

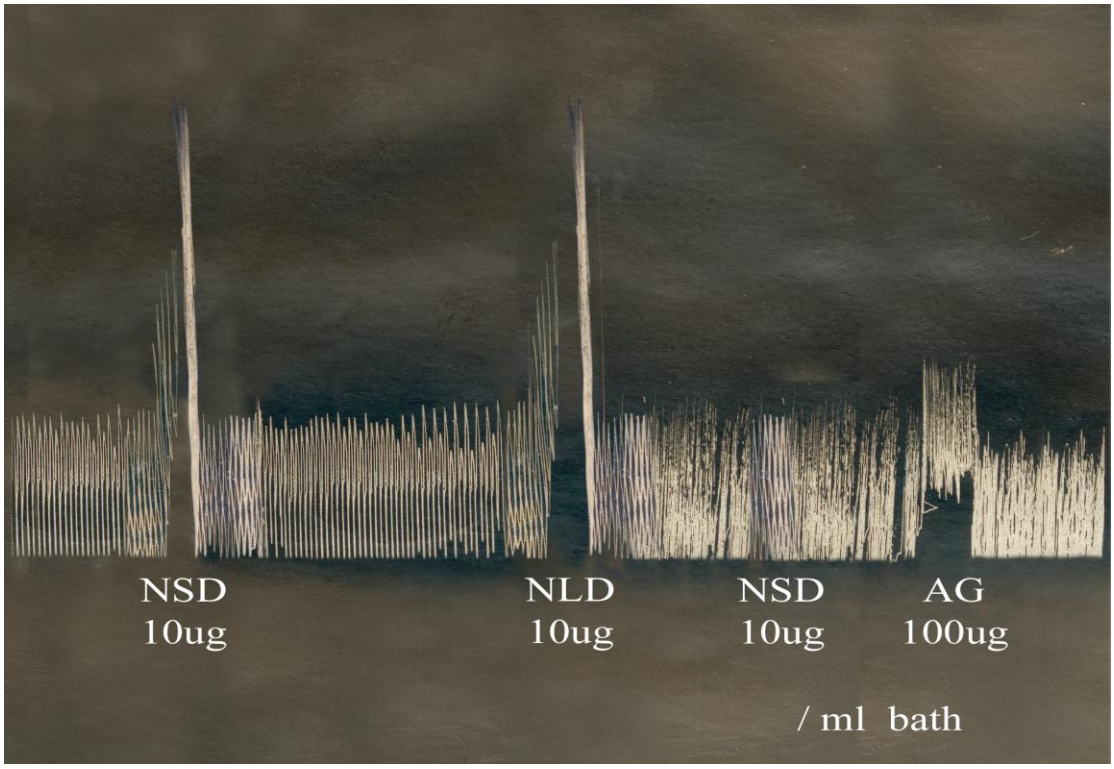


Figure (21): A record demonstrating the site of action of AG on isolated perfused rabbit's jejunum. (Nicotinic receptors).

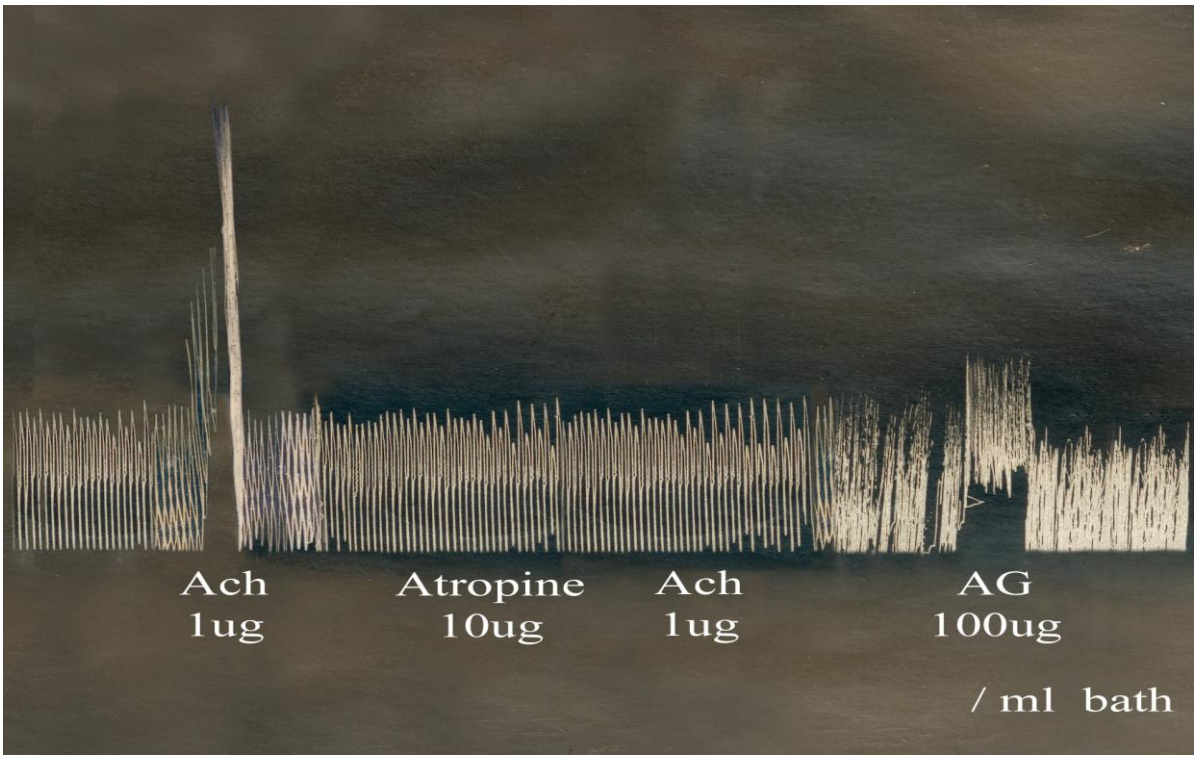


Figure (22): A record demonstrating the site of action of AG on isolated perfused rabbit's jejunum. (Muscarinic receptors).

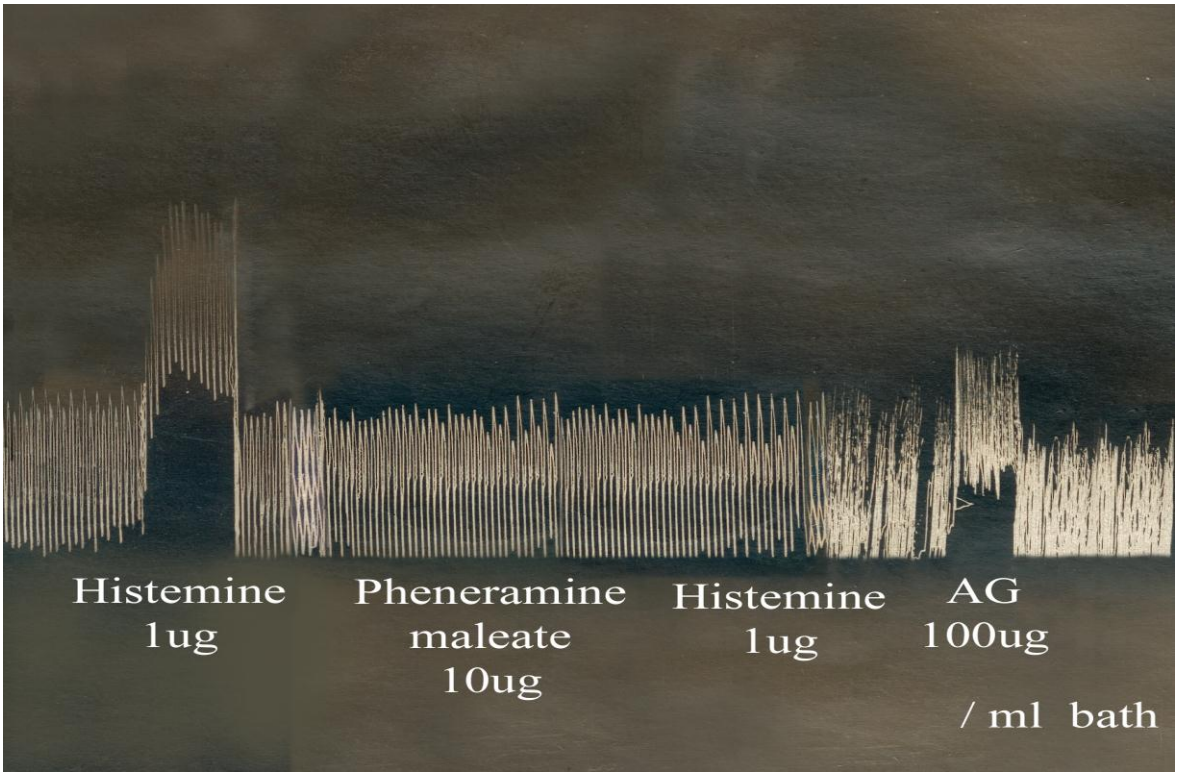


Figure (23): A record demonstrating the site of action of AG on isolated perfused rabbit's jejunum (Histaminic receptors).

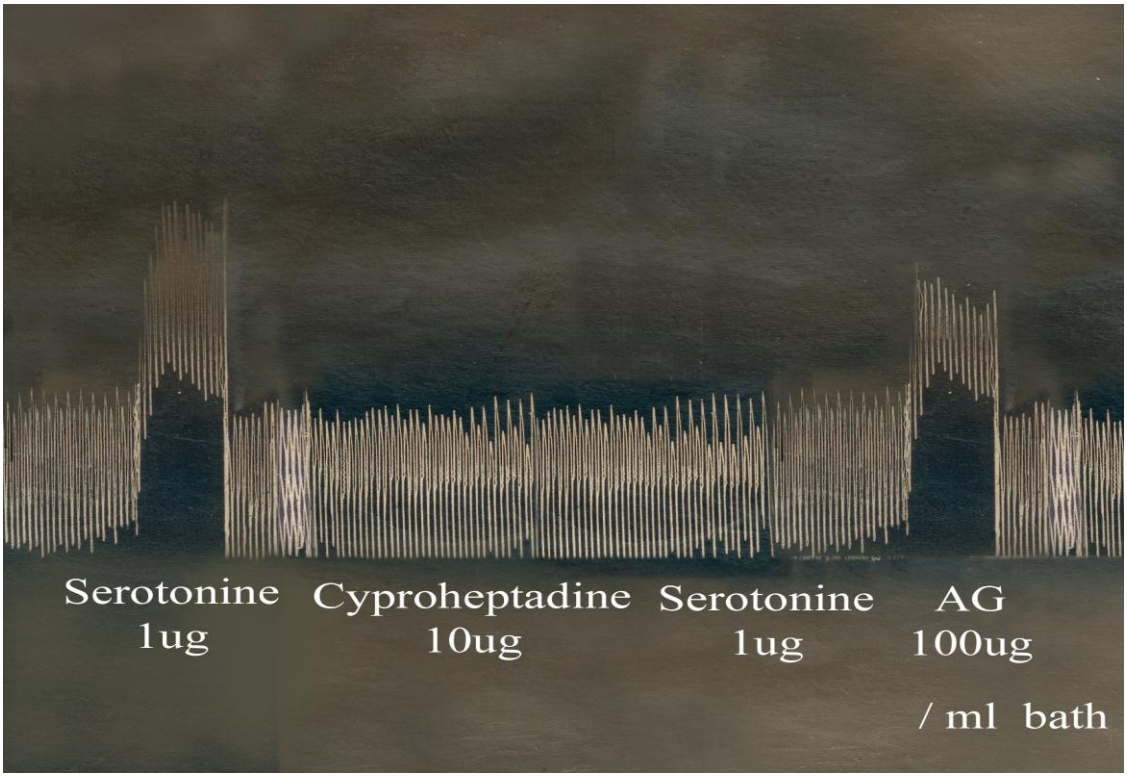


Figure (24): A record demonstrating the site of action of AG on isolated perfused rabbit's jejunum (Serotonergic receptors).

(B) Effect of Silymarin on isolated perfused rabbit's jejunum:

It was observed that addition of Silymarin in different dose levels (10, 30, 100 and 300µg/ml bath) produced dose related stimulation of rhythmic contraction of rabbit's jejunum (Table (11), Figures (24-25)).

- **Site of action of Silymarin on isolated perfused rabbit's jejunum:**

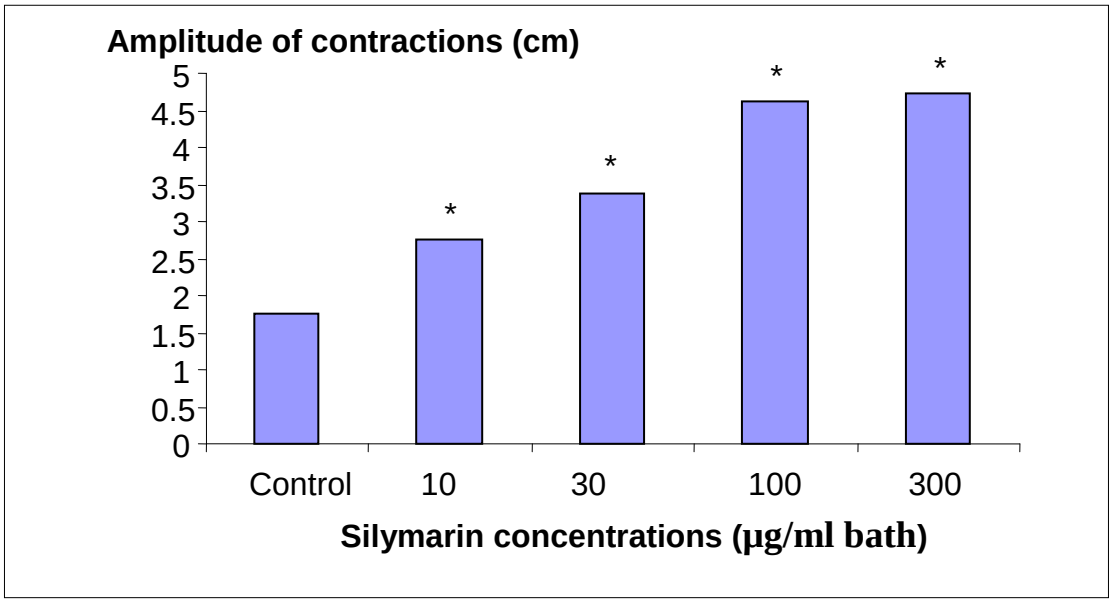
It was observed that blocking of muscarinic, nicotinic, histaminic and seretonergic receptors did not affect the stimulatory action of Silymarin (30 ug/ml bath).This indicates that Silymarin did not act through the muscarinic, nicotinic, or histaminic and seretonergic receptors (Figure (26-27-28)).

Table (11): Effect of Silymarin on the amplitude of spontaneous rhythmic contraction of isolated perfused rabbit's jejunum.

Dose of Silymarin µg/ml bath	Level of contraction before (cm)	Level of contraction after (cm)
10	1.75 +.1	2.75 + ,2 *
30		3.38 +.3 *
100		4.63+ .4 *
300		4.73 + .3 *

Data represented as mean ± SEM of six experiments

***Significant level compared to control group.**



* Significant compared with control group.

Figure (25): Histogram showing the stimulatory effect of Silymarin on the amplitude of spontaneous contraction of isolated perfused rabbit's jejunum.

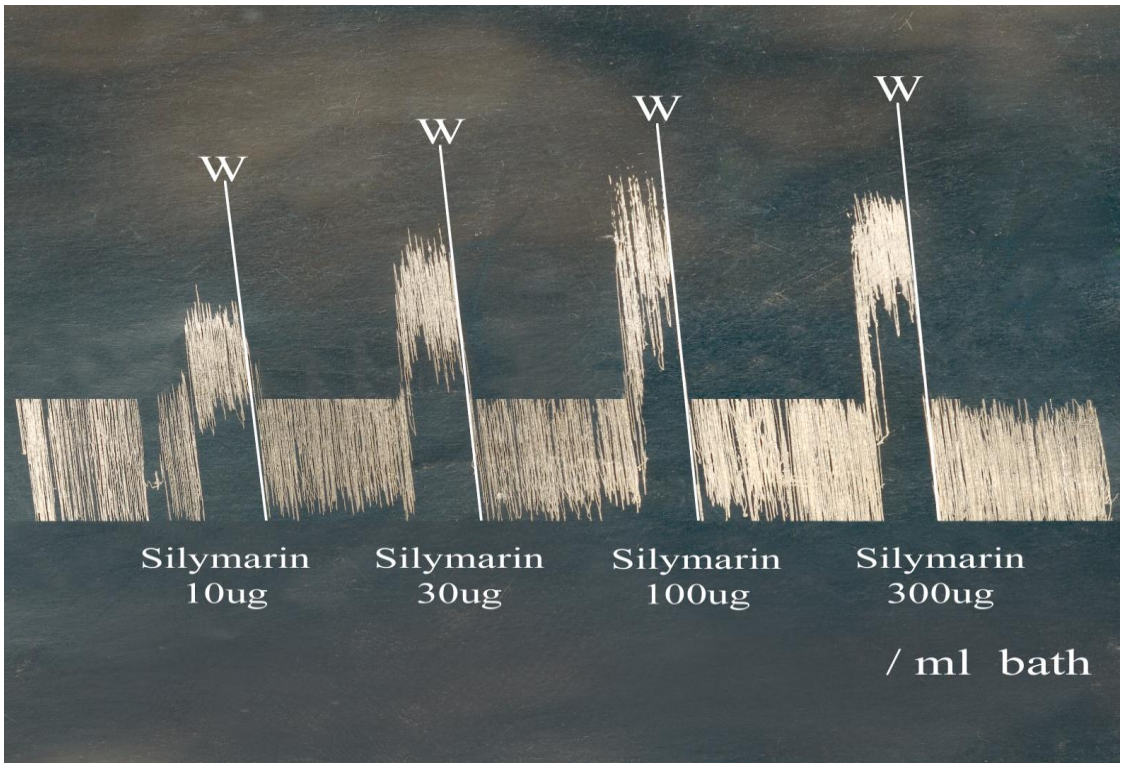


Figure (26): A record demonstrating the effect of Silymarin on isolated perfused rabbit's jejunum.

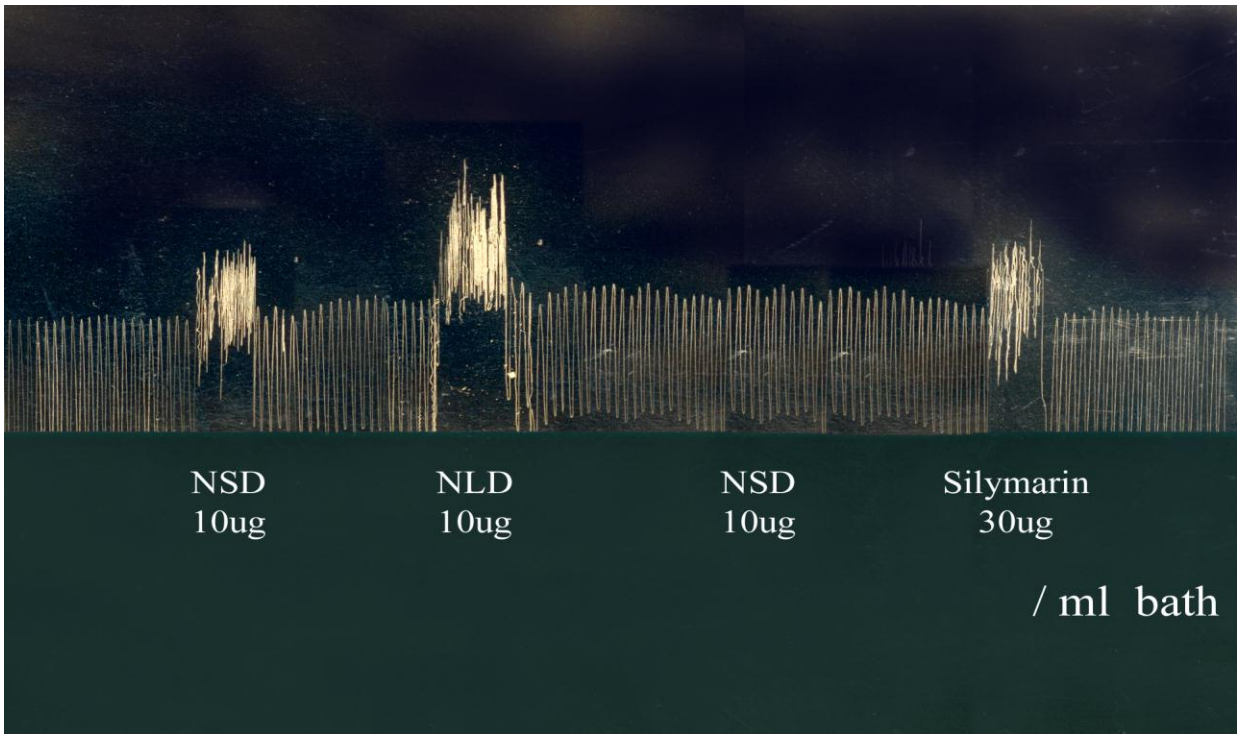


Figure (27): A record demonstrating the site of action of Silymarin on isolated perfused rabbit's jejunum. (Nicotinic receptors).

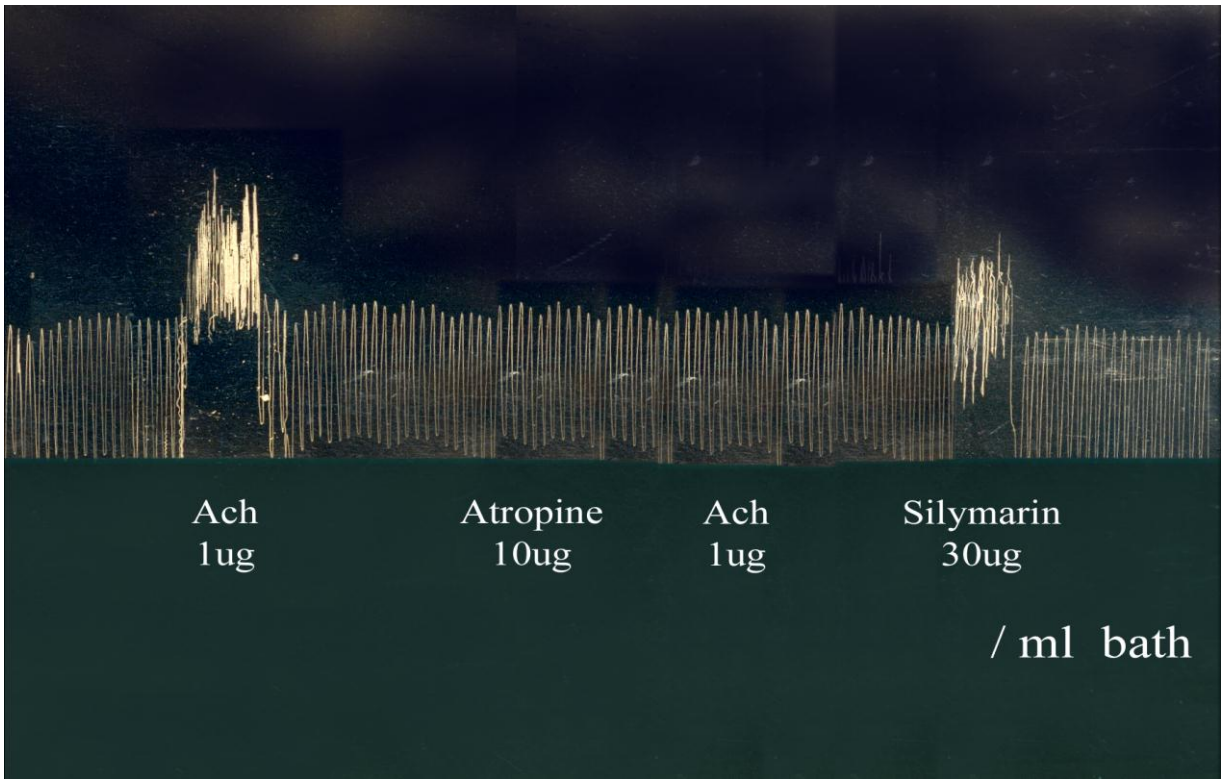


Figure (28): A record demonstrating the site of action of Silymarin on isolated perfused rabbit's jejunum. (Muscarinic receptors).

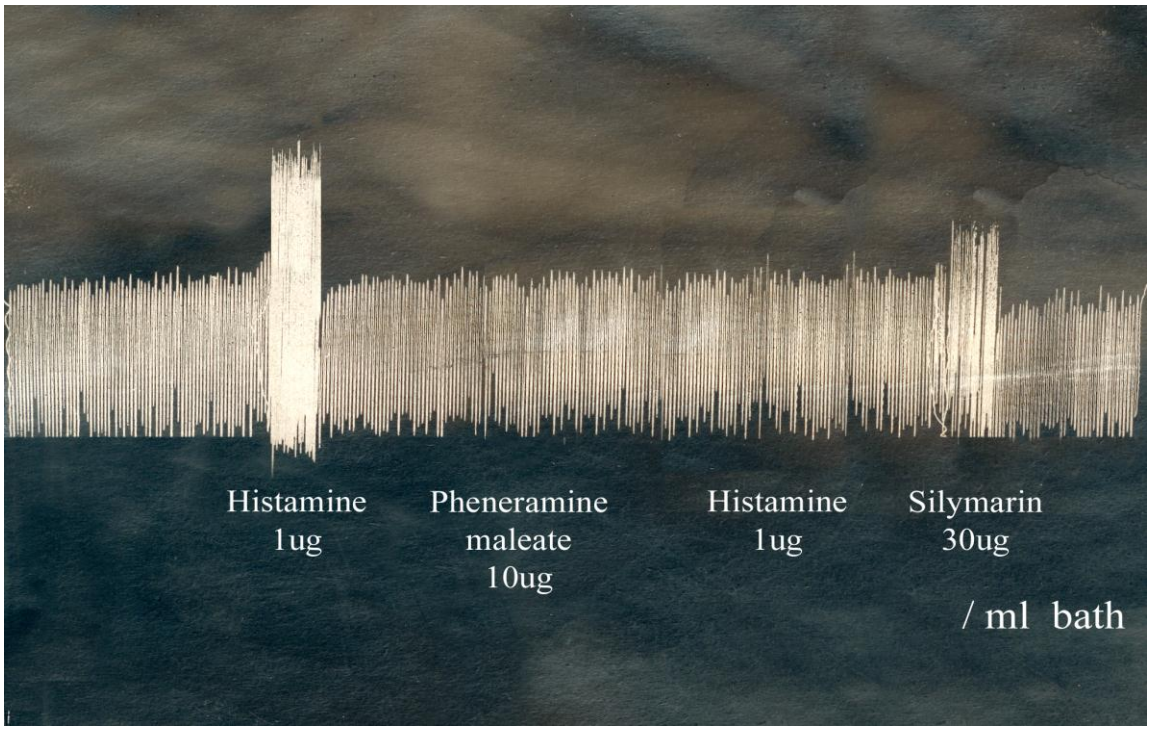


Figure (29): A record demonstrating the site of action of Silymarin on isolated perfused rabbit's jejunum (Histaminergic receptors).

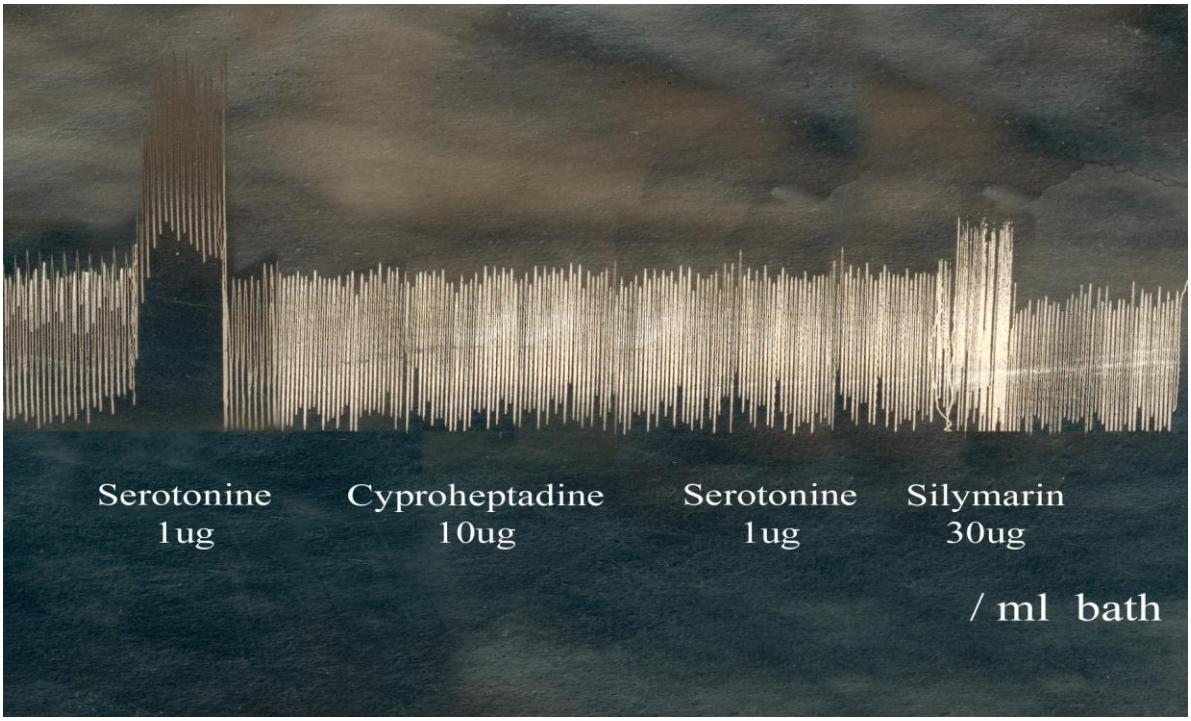


Figure (30): A record demonstrating the site of action of Silymarin on isolated perfused rabbit's jejunum (Seretonergic receptors).