Results

I. In vivo experiments:

1. Effects of both green tea extract (GTE) and atorvastatin treatment on lipid profile in hypercholesterolemic rats:

High fat diet supplementation (1% cholesterol and 10% coconut oil) for 7 weeks resulted in significant rise ($P < 0.001$) of total cholesterol from 69.25 ± 2.81 mg/dl in control group (group I) to 148.31 ± 2.23 mg/dl in hypercholesterolemic group (group II), significant rise ($P < 0.001$) of LDL from 30.33 ± 2.76 mg/dl to 123.36 ± 2.58 mg/dl, and significant decrease of HDL ($P < 0.001$) from 41.35 ± 2.89 mg/dl to 20.4 ± 1.77 mg/dl, compared to hypercholesterolemic group (group II). (Table 6, Figures 4, 5, 6, 7).

▪Total cholesterol:

Treatment of hypercholesterolemic rats of group III with GTE (each rat receiving 325mg daily) oral for 3 weeks reduced total cholesterol significantly ($P < 0.01$) to 109.85 ± 4.76 mg/dl compared to group II. Treatment of hypercholesterolemic rats in group IV with atorvastatin (30mg /kg/day) for 3 weeks reduced total cholesterol significantly ($P< 0.001$) to 88.35 ± 2.91 mg/dl compared to group II (Table 6, Figure 4, 7). Comparing the result of group IV to that of group III, atorvastatin was significantly ($P <0.05$) more effective than GTE in reducing total cholesterol (Table 6, Figures 4, 7).

▪Low density lipoprotein(LDL):

Treatment of group III with GTE reduced LDL significantly ($P < 0.05$) to 89.41 ± 2.74 mg/dl compared to group II. Treatment of hypercholesterolemic rats in group IV with atorvastatin reduced LDL significantly ($P< 0.001$) to 61.4 ± 3.96 mg/dl compared to group II (Table 6, Figure 5, 7). Comparing the result of group IV to that of group III, atorvastatin was significantly ($P < 0.001$) more effective than GTE in reducing LDL (Table 6, Figures 5, 7).

▪High density lipoprotein(HDL):

Treatment of group III with GTE increased HDL significantly ($P < 0.05$) to 26.75 ± 2.33 mg/dl compared to group II. Meanwhile, atorvastatin treatment of group IV increased HDL significantly ($P < 0.05$) to 28.06± 2.3 mg/dl compared to group II (Table 6, Figure 6, 7). Comparing the result of group IV to that of group III, there was no significant differences ($P > 0.05$) between GTE and atorvastatin in their effect on HDL (Table 6, Figures 6, 7).

Table (6): Effects of GTE and atorvastatin on lipid profile

Parameters / Groups	Total cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Group I	69.25 ± 2.81	30.33 ± 2.76	41.35 ± 2.89
Group II	148.31 ± 2.23	123.36 ± 2.58	20.4 ± 1.77
Percent change (%)	↑ > 100 %	↑ > 100 %	↓ 50.66 %
P_1	< 0.001	< 0.001	< 0.001
Group III	109.85 ± 4.67	89.41 ± 2.74	26.75 ± 2.33
Percent change (%)	↓ 25.93 %	↓ 27.52 %	↑ 31.12 %
P_2	< 0.01	< 0.05	< 0.05
Group IV	88.35 ± 2.91	61.4 ± 3.69	28.06 ± 2.3
Percent change (%)	↓ 40.42 %	↓ 31.32 %	↑ 37.54 %
P_3	< 0.001	< 0.001	< 0.05
P_4	< 0.05	< 0.001	> 0.05

Data represented as Mean ± SEM (n = 6)

Group I: Control group.

Group II: Hypercholesterolemic group.

Group III: GTE-treated hypercholesterolemic group.

Group IV: Atorvastatin-treated hypercholesterolemic group.

P1 : group II versus group I. **P2 :** group III versus group II.

P3 : group IV versus group II. **P4 :** group IV versus group III.

-Significant level at P < 0.05

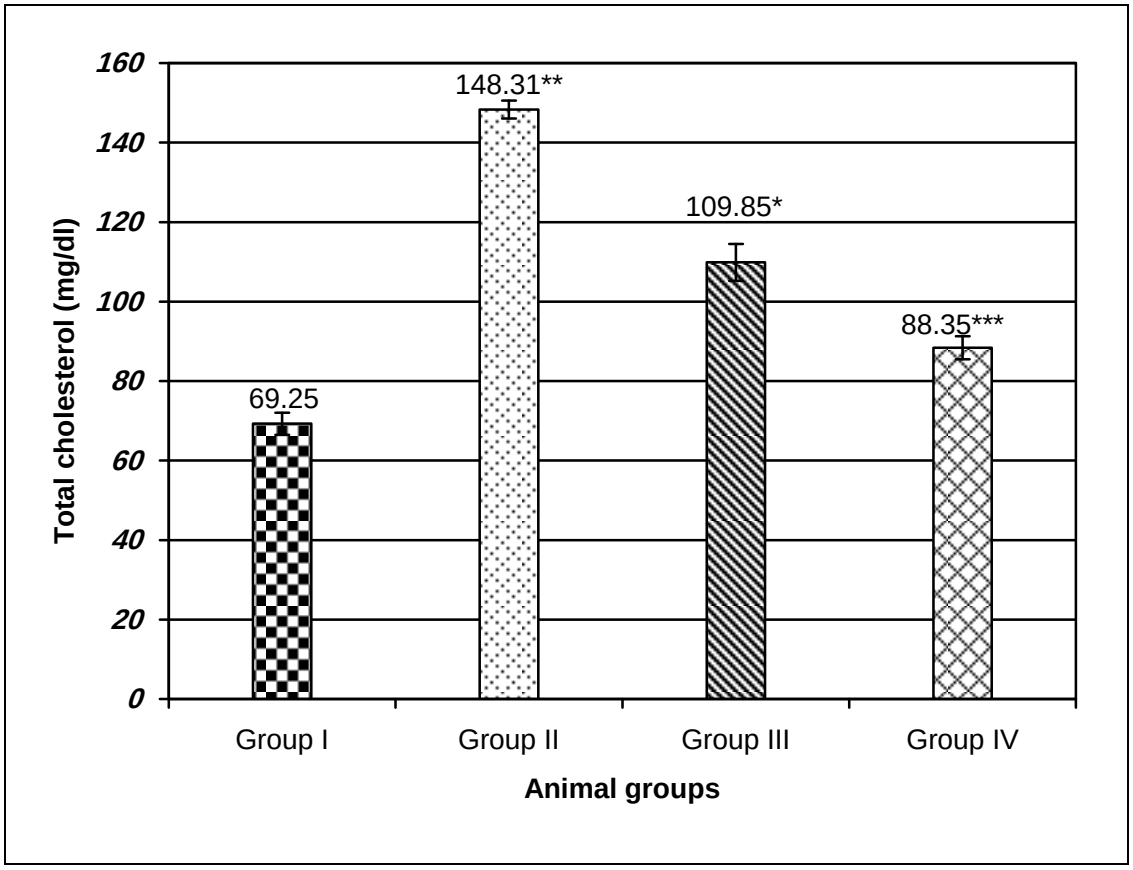

Figure (4): Histogram showing total cholesterol level in various groups.

* Significant (P < 0.01) versus group II.
* * Significant (P < 0.001) versus group I.
* * * Significant (P < 0.001) versus group II and (P < 0.05) versus group III.

Group I: Control.

Group II: Hypercholesterolemic group.

Group III: GTE-treated hypercholesterolemic group.

Group IV: Atorvastatin-treated hypercholesterolemic group.

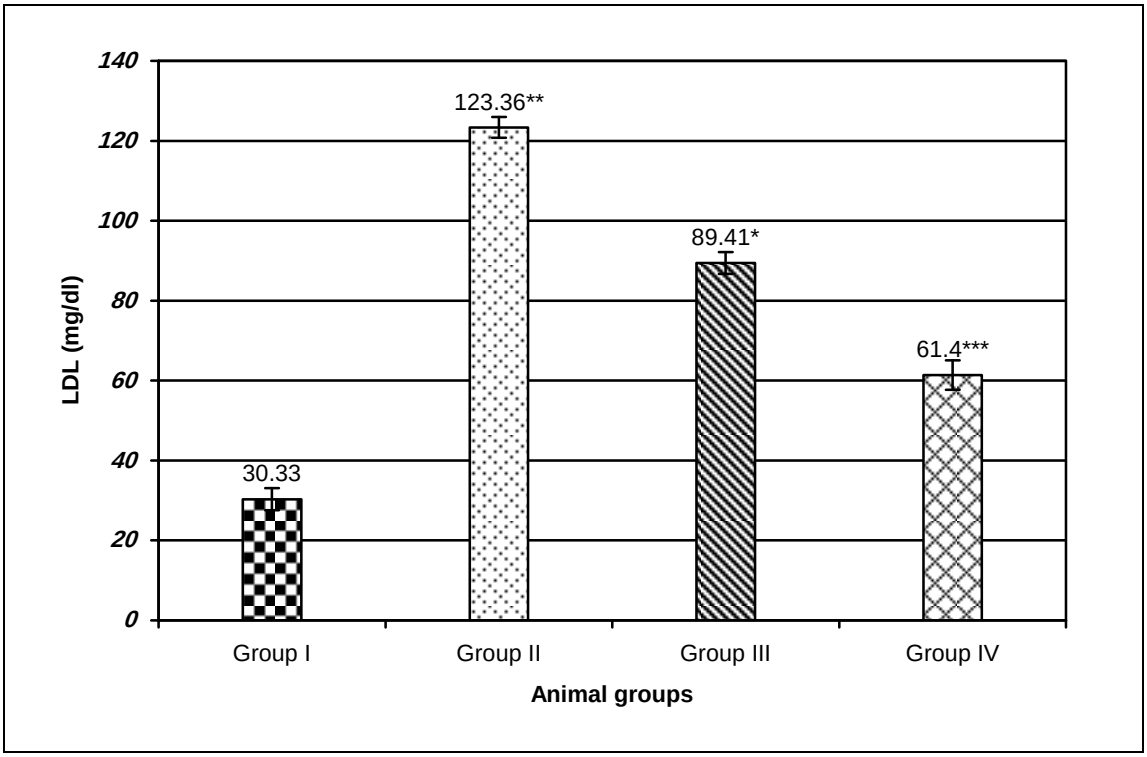

Figure (5): Histogram showing LDL level in various groups.

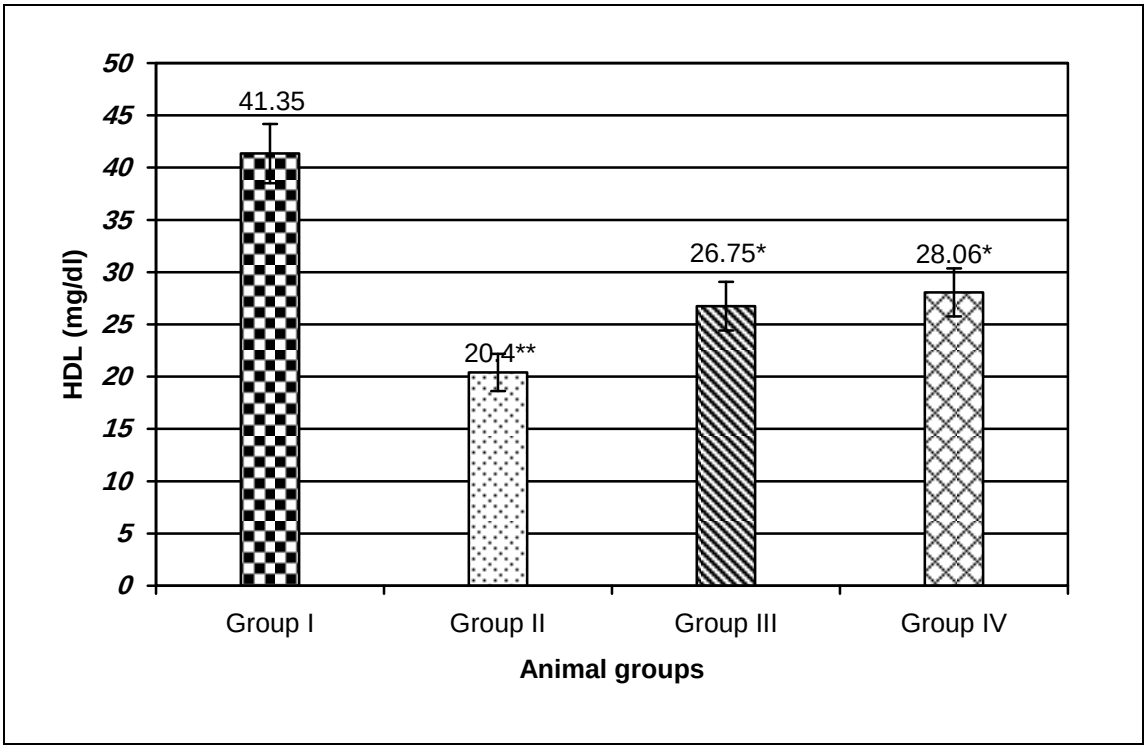

Figure (6): Histogram showing HDL level in various groups.

* Significant (P < 0.05) versus group II.
** Significant (P < 0.001) versus group I.
* * * Significant (P < 0.001) versus group II and group III.
Group I: Control.
Group II: Hypercholesterolemic group.
Group III: GTE-treated hypercholesterolemic group.
Group IV: Atorvastatin-treated hypercholesterolemic group.

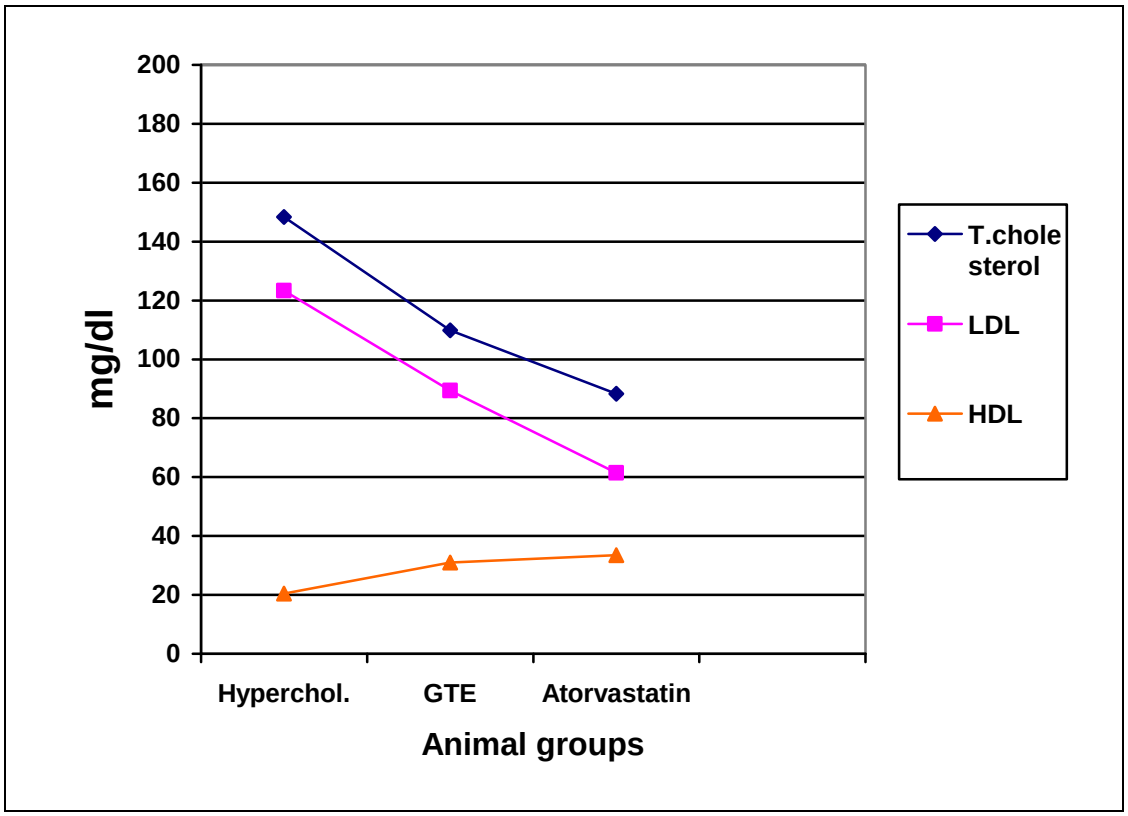

Figure (7): Histogram showing comparison between GTE (green tea extract) and atorvastatin in their effects on Total cholesterol, LDL, and HDL levels in hypercholesterolemic rats.

2. Effects of both GTE and atorvastatin treatment on body weight in hypercholesterolemic rats:

High fat diet supplementation (1% cholesterol and 10% coconut oil) for 7 weeks resulted in significant rise of body weight ($P < 0.05$) from 157.5 ± 4.9 gm in group (I) to 198.33 ± 4.01 gm in group (II). Treatment of group (III) with GTE reduced body weight significantly ($P < 0.05$) to 163.33 ± 6.14 gm compared to group (II). Atorvastatin treatment of group (IV) reduced body weight insignificantly ($P > 0.05$) to 192.5 ± 5.28 gm compared to group (II). Comparing the result of group (IV) to that of group (III), GTE was significantly ($P <0.05$) more effective than atorvastatin in reducing body weight (Table 7, Figure 8).

Table (7): Effects of GTE and atorvastatin on body weight

Groups	Initial body weight (gm)	Final body weight (gm)
Group I	116.66 ± 3.33	157.5 ± 4.9
Group II **Percent change (%)** **P_1**	115 ± 4.28	198.33 ± 4.01 ↑ 26 % < 0.05
Group III **Percent change (%)** **P_2** **P_3**	117.5 ± 4.03	163.33 ± 6.14 ↓ 17.64 % < 0.01 < 0.05
Group IV **Percent change (%)** **P_4**	115.16 ± 3.27	192.5 ± 5.28 ↓ 2.93 % > 0.05

Data represented as Mean ± SEM (n = 6)

Group I: Control group.
Group II: Hypercholesterolemic group.
Group III: GTE-treated hypercholesterolemic group.
Group IV: Atorvastatin-treated hypercholesterolemic group.
P1: group II versus group I. **P2:** group III versus group II.
P3: group III versus group IV. **P4:** group IV versus group II.
-Significant level at P < 0.05

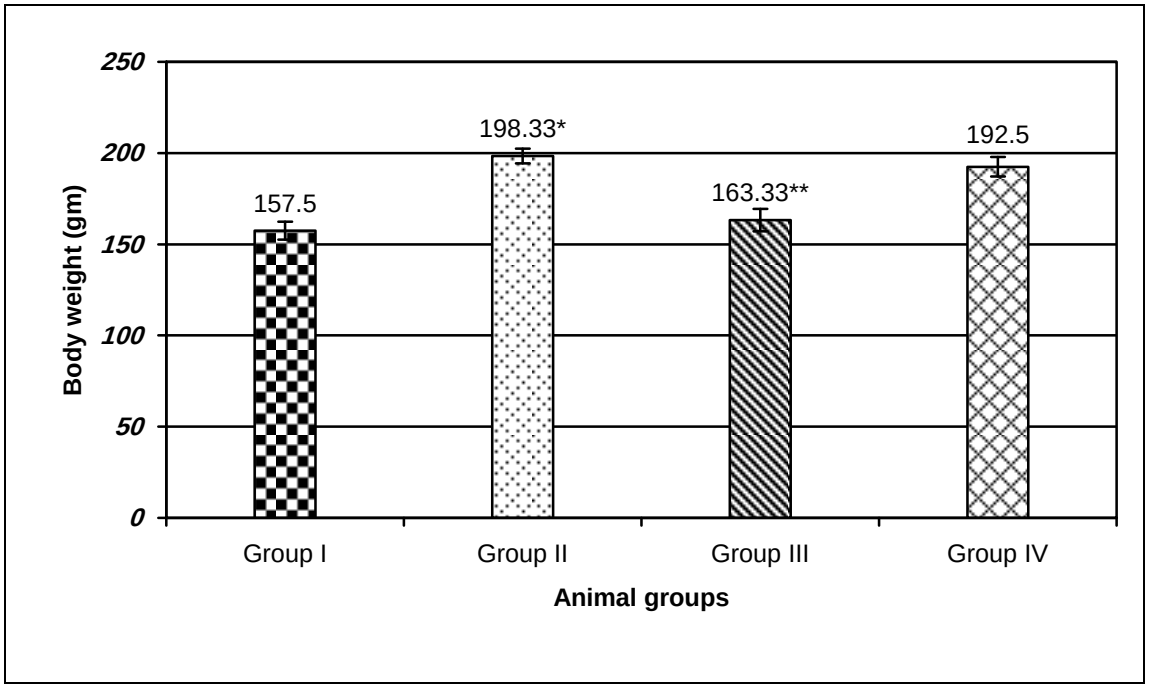

Figure (8): Histogram showing body weight in various groups.

* Significant (P < 0.05) versus group I.
* * Significant (P < 0.01) versus group II and (P < 0.05) versus group IV.
Group I: Control.
Group II: Hypercholesterolemic group.
Group III: GTE-treated hypercholesterolemic group.
Group IV: Atorvastatin-treated hypercholesterolemic group.

3. Effects of both GTE and atorvastatin treatment on oxidative activity in hypercholesterolemic rats:

High fat diet supplementation (1% cholesterol and 10% coconut oil) for 7 weeks resulted in significant rise of MDA (P < 0.001) from 3.2 ± 0.33 µmol/ml in group (I) to 7.11 ± 0.31 µmol/ml in group (II). Treatment of group (III) with GTE reduced MDA significantly (P < 0.01) to 4.63 ± 0.19 µmol/ml compared to group (II). Atorvastatin treatment of group (IV) reduced MDA significantly (P < 0.01) to 6.11 ± 0.4 µmol/ml compared to group II. Comparing the result of group (IV) to that of group (III), GTE was significantly (P <0.05) more effective than atorvastatin in reducing oxidative activity (Table 8, Figure 9).

Table (8): Effects of GTE and atorvastatin on MDA

Parameter Groups	MDA (µmol/ml)
Group I	3.2 ± 0.33
Group II **Percent change (%)** **P_1**	7.11 ± 0.31 ↑ > 100% < 0.001
Group III **Percent change (%)** **P_2** **P_3**	4.63 ± 0.19 ↓ 34.88 % < 0.01 < 0.05
Group IV **Percent change (%)** **P_4**	6.11 ± 0.4 ↓ 14.06 % < 0.01

Data represented as Mean ± SEM (n = 6)

P1: group II versus group I. **P2:** group III versus group II.

P3: group III versus group IV. **P4:** group IV versus group II.

-Significant level at P < 0.05

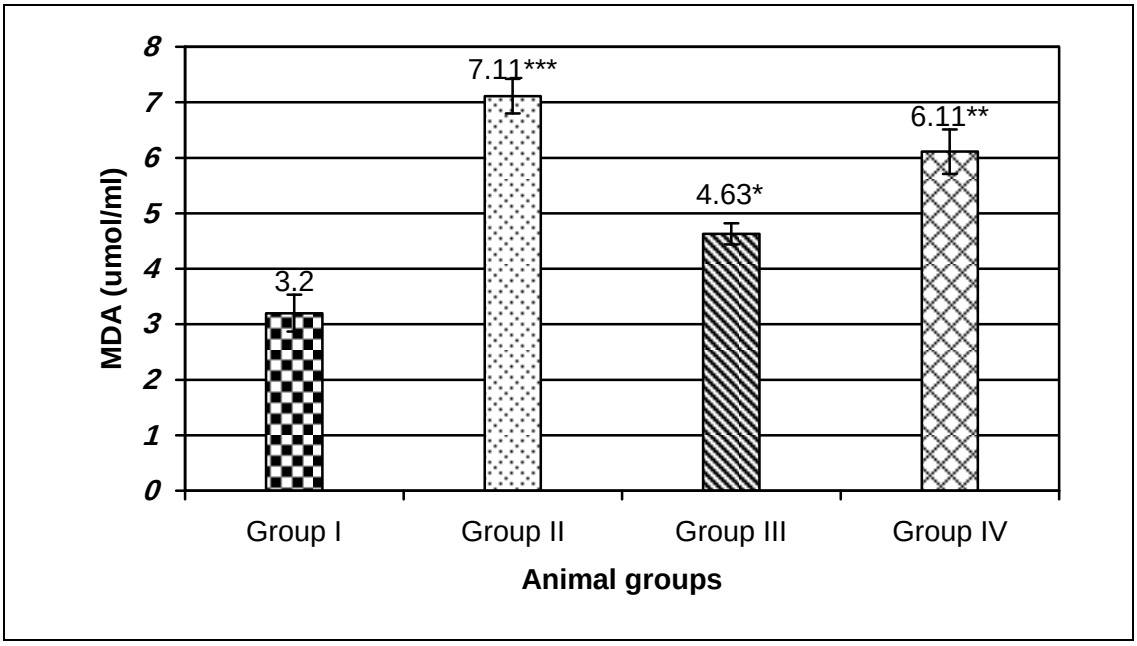

Figure (9): Histogram showing MDA level in various groups.

*Significant (P < 0.05) versus group IV and (P < 0.01) versus group II.

** Significant (P < 0.01) versus group II *** Significant (P < 0.001) versus group I.

Group I: Control group.

Group II: Hypercholesterolemic group.

Group III: GTE-treated hypercholesterolemic group.

Group IV: Atorvastatin-treatedhypercholesterolemic group.

4- Histopathological evaluation of the aorta:

Histological examination of a cut section of the aorta of control group (group I) showed that the wall of the aorta consists of a tunica intima, tunica media, and tunica adventitia. The tunica intima consists of an endothelial coat of flattened squamous cells resting on a complete basal lamina and is supported by a subendothelial loose connective tissue. The tunica media consists largely of elastic, concentric laminae and variable amounts of smooth muscle cells. The tunica adventitia contains bundles of collagen fibers and a few elastic fibers, both of which have a loose, helical arrangement (Figure 10).

In hypercholesterolemic group (group II); there were ulcerated endothelial cells of the intima with collection of foamy histocytes and fat globules and formation of fatty streaks with degeneration. The media and adventitia showed fibrosis and inflammatory cell infiltration (Figure 11).

In GTE-treated hypercholesterolemic group (group III), GTE treatment markedly decreased the size of fatty streaks, foamy and inflammatory cell infiltration. Regeneration of the endothelial cells of the intima was observed (Figure 12).

Atorvastatin-treated hypercholesterolemic group (group IV) showed decreased the size of fatty streaks and the number of foamy cells (Figure 13).

Figure (10): A photomicrograph of a cut section in the aorta of a control rat (group I) showing intact flat endothelial cell lining, elastic media and well fitted adventitia. (H x & E x 400).

Figure (11): A photomicrograph of a cut section in the aorta of a hypercholesterolemic rat (group II) showing ulcerated endothelial cells of the intima with collection of foamy histocytes and fat globules and formation of fatty streaks with degeneration. The media and adventitia showed fibrosis and inflammatory cell infiltration. (H x & E x 400).

Figure (12): A photomicrograph of a cut section in the aorta of a GTE treated rat (group III) showing marked decrease of the size of fatty streaks, foamy and inflammatory cell infiltration. Regeneration of the endothelial cells of the intima was observed. (H x & E x 400).

Figure (13): A photomicrograph of a cut section in the aorta of an atorvastatin treated rat (group IV) showing decreased the size of fatty streaks and the number of foamy cells. (H x & E x 400).

5. Effects of both GTE and enalapril treatment on arterial blood pressure in renovascular hypertensive rats:

▪Systolic arterial blood pressure (SBP):

Unilateral renal artery ligation for 7 weeks resulted in significant ($P < 0.001$) rise of systolic blood pressure from 119.16 ± 1.53 mmHg in control group (group I) to 197.5 ± 2.81 mmHg. Treatment of hypertensive rats of group (III) with GTE (each rat receiving 325mg daily) oral for 4 weeks reduced systolic blood pressure insignificantly ($P > 0.05$) to 194.5 ± 3.25 mmHg compared to group (II). Treatment of hypertensive rats in group (IV) with enalapril (30mg /kg/day) for 4 weeks reduced systolic blood pressure significantly ($P< 0.001$) to 138.33 ± 3.33 mmHg compared to group (II). Comparing the result of group (IV) to that of group (III), enalapril was significantly ($P <0.001$) more effective than GTE in reducing systolic blood pressure (Table 9, Figures 14, 16, 17).

▪ Mean blood pressure (MBP):

In control group (group I) the MBP was 90.27 ± 1.68 mmHg. Unilateral renal artery ligation for 7 weeks increased MBP significantly ($P < 0.001$) to 159.72 ± 5.86 mmHg in group (II). Treatment of hypertensive rats of group (III) with GTE reduced the mean blood pressure insignificantly ($P > 0.05$) to 155.94 ± 4.32 mmHg compared to group (II). Meanwhile, enalapril treatment of group IV reduced MBP significantly ($P < 0.001$) to 108.33 ± 2.5 mmHg compared to group (II). Comparing the result of group IV to that of group (III), enalapril was significantly ($P <0.001$) more effective than GTE in reducing MBP (Table 9, Figures 15, 16, 17).

Table (9): Effect of both GTE and enalapril treatment on arterial blood pressure of renovascular hypertensive anaesthetized rats.

Parameter / Groups	Systolic blood pressure (mmHg)	Mean blood pressure (mmHg)
Group I	119.16 ± 1.53	90.27 ± 1.68
Group II	197.5 ± 2.81	159.72 ± 5.86
Percent change (%)	↑ 65.74 %	↑ 77%
P_1	< 0.001	< 0.001
Group III	194.5 ± 3.25	155.94 ± 4.32
Percent change (%)	↓ 1.51 %	↓ 2.36 %
P_2	> 0.05	> 0.05
Group IV	138.33 ± 3.33	108.33 ± 2.5
Percent change (%)	↓ 29.9 %	↓ 32.17 %
P_3	< 0.001	< 0.001
P_4	< 0.001	< 0.001

Data represented as Mean ± SEM (n = 6)

Group I: Control group. **Group II:** Renovascular hypertensive group.

Group III: GTE-treated hypertensive group. **Group IV:** Enalapril-treated hypertensive group.

P1: group II versus group I. **P3:** group IV versus group II.

P2: group III versus group II. **P4:** group IV versus group III.

-Significant at P < 0.05

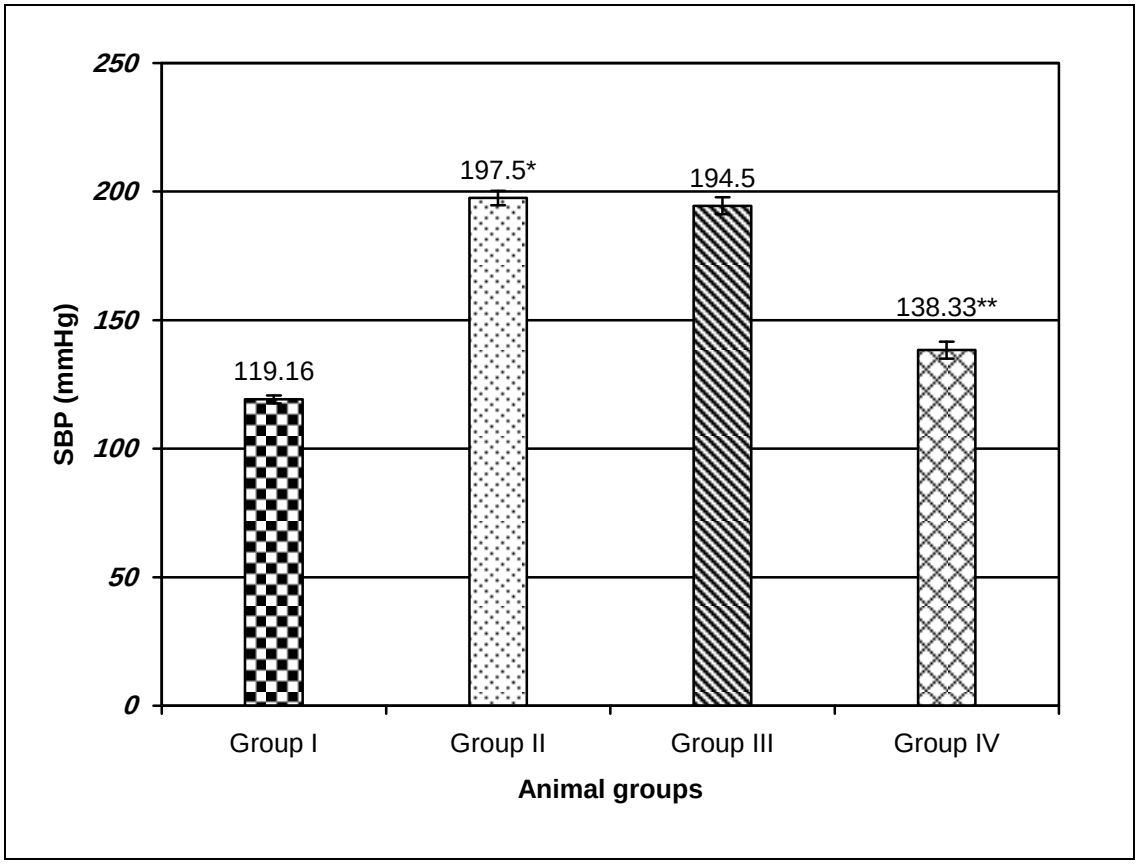

Figure (14): Histogram showing SBP in various groups.

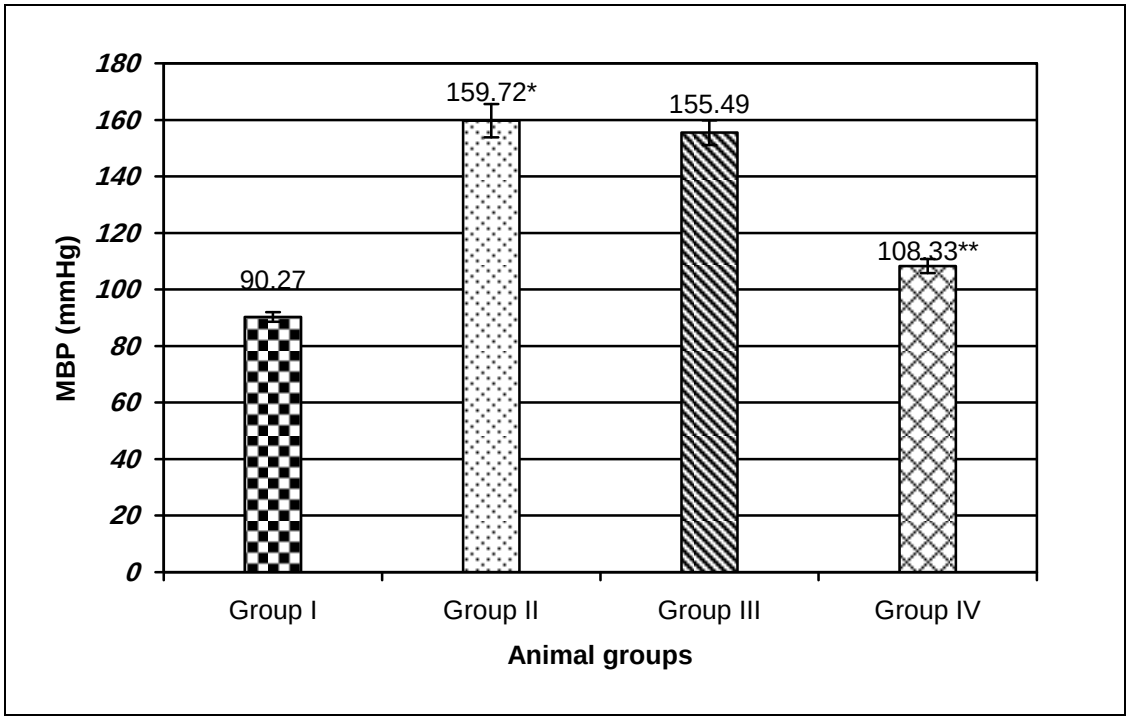

Figure (15): Histogram showing MBP in various groups.

* Significant (P < 0.001) versus group I.

* * Significant (P < 0.001) versus group II and group III.

Group I: Control group.
Group II: Renovascular hypertensive group.
Group III: GTE-treated hypertensive group.
Group IV: Enalapril-treated hypertensive group..

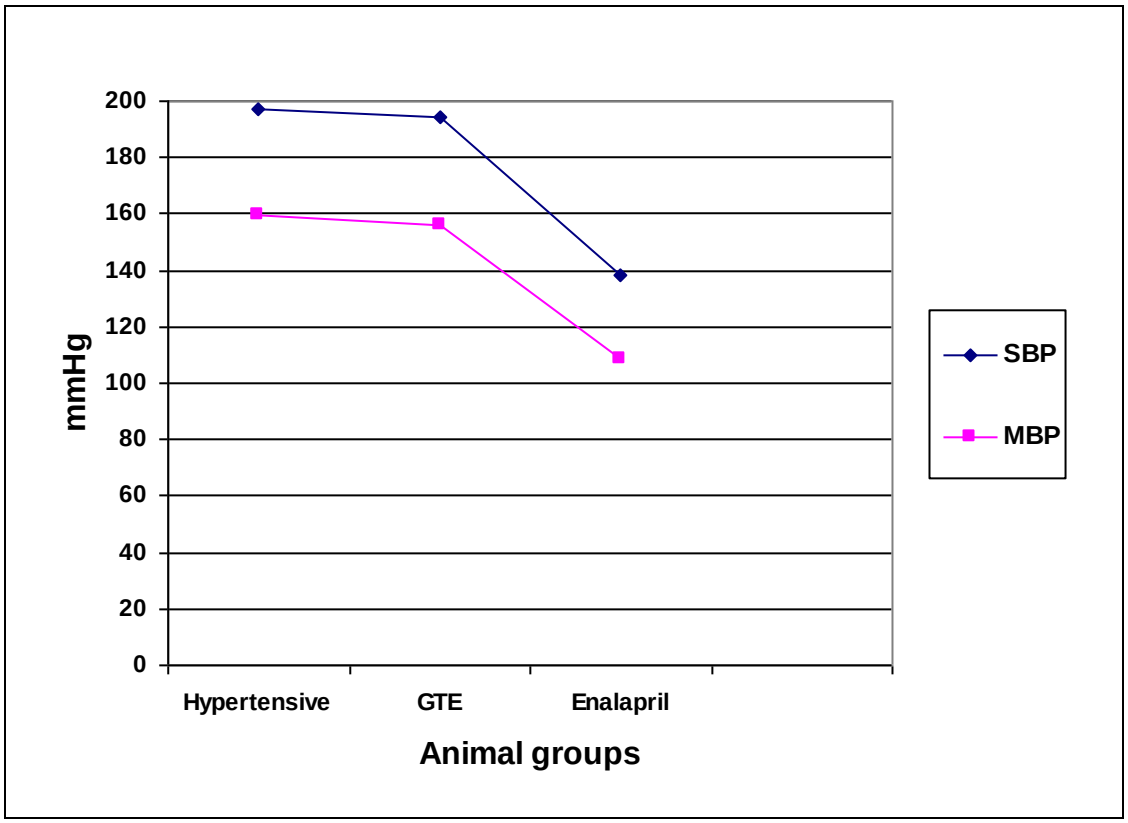

Figure (16): Histogram showing comparison between GTE and enalapril in their effects on SBP and MBP in hypertensive rats.

(a)

(b)

(c)

(d)

Figure (17): Diagrams showing arterial blood pressure in various groups.

(a) Control group (group I). **(b) Renovascular hypertensive group (group II).**

(c) GTE-treated hypertensive group (group III). **(d) Enalapril-treated hypertensive group (group IV).**

* Upper trace represents cuff pressure.

* Lower trace represents pulse blood flow.

*The SBP measured at the start of pulsation and referenced to the pressure curve.

*The MBP measured at the stability of pulsation and referenced to the pressure curve.

II. In vitro experiments:

1. Effects on isolated perfused rabbit's heart:

- **Effect of GTE on isolated perfused rabbit's heart:**

It was noticed that GTE in increasing doses (10 and 30 µg/bath) produced an increase in the force of spontaneous contraction of isolated perfused rabbit's heart in dose related manner. However GTE in doses (100, 300 and 1000 µg/bath) did not produce any change in the force of spontaneous contraction of the isolated perfused rabbit's heart. This increase of the force of spontaneous contractions of the isolated perfused rabbit's heart was significant ($P < 0.05$) with the dose of 10µg/bath with percentage of increase 41.17 %. Meanwhile, it was significant ($P < 0.01$) with the dose of 30µg/bath and with percentage of increase 68.23 % compared to basal value (Table 10, Figures 18, 19).

- **Site of action of GTE on isolated perfused rabbit's heart:**

It was observed that GTE added in dose of 10 µg/bath produced stimulatory effect on spontaneous rhythmic contractility of the isolated perfused rabbit's heart. This stimulatory effect of GTE was still present after blocking beta adrenergic receptors by using propranolol in a dose of 10 µg/bath (Figure 20). This indicates that positive inotropic effect produced by GTE is not mediated through beta adrenergic receptors.

Table (10): Effect of GTE on the amplitude of spontaneous contraction of isolated perfused rabbit's heart.

Dose of GTE µg/bath	Level of contraction before (cm)	Level of contraction after (cm)	Percentage changes (%)	P
10	4.25 ± 0.38	6.0 ± 0.35	↑ 41.17 %	< 0.05*
30		7.15 ± 0.18	↑ 68.23 %	< 0.01*

Data represented as Mean ± SEM of six experiments.

* Significant level at P < 0.05 compared to control value.

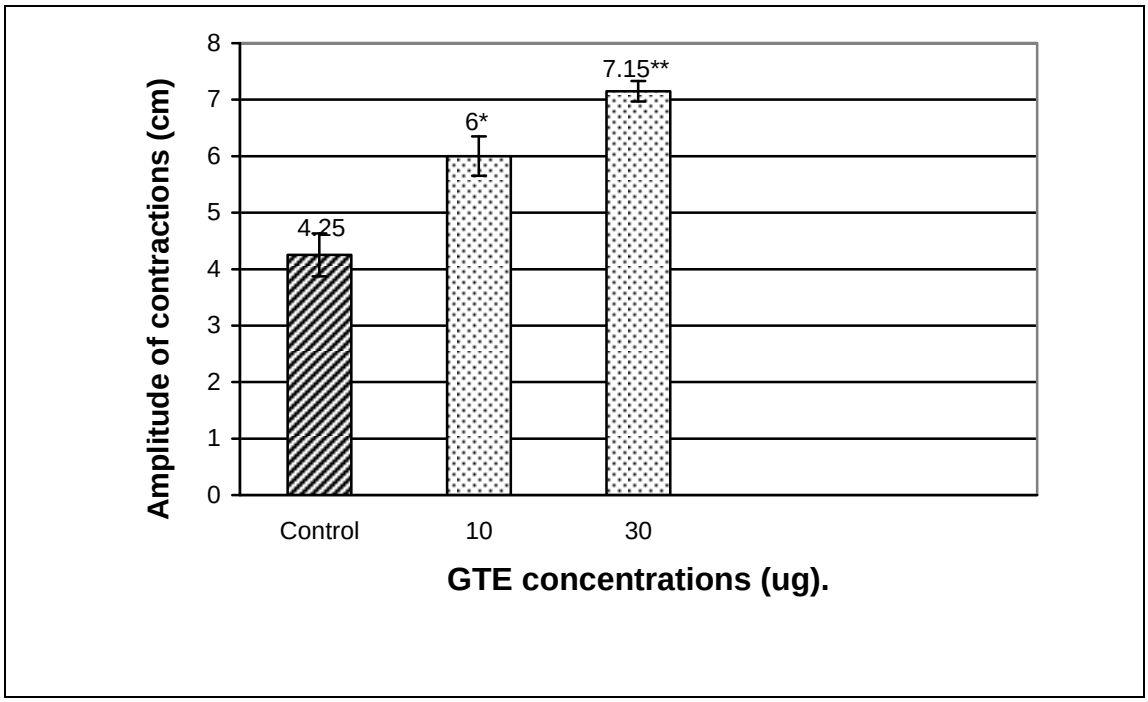

Figure (18): Histogram showing the effect of GTE on isolated rabbit's heart.

* Significant (P < 0.05) compared to control value.

** Significant (P < 0.01) compared to control value.

Figure (19): A record demonstrating the effect of gradually increasing doses of GTE on the isolated perfused rabbit's heart contractions.

Figure (20): A record demonstrating the site of action of GTE on the isolated perfused rabbit's heart.

2. Effects on isolated perfused rabbit's aortic spiral strip:

- **Effect of GTE on isolated perfused rabbit's aortic spiral strip:**

It was observed that addition of gradually increasing doses of GTE (10, 30, and 100 µg/ml bath) did not produce any response in the rabbit's aortic spiral strip. Meanwhile, the addition of GTE in higher doses (300 and 1000 µg/ml bath) induced contractile response of the rabbit's aortic spiral strip in dose dependent manner (Figure 22).

Interestingly, GTE was added in doses (300, 600, and 1200 µg/ml bath), the contractile response was significant ($P < 0.05$) with dose 300 and 600 µg/ml bath and significant ($P < 0.001$) with dose 1200 µg/ml bath compared to control value (Table 11, Figures 21, 23).

- **Site of action of GTE on isolated perfused rabbit's aortic spiral strip:**

It was observed that GTE added in submaximal dose of 600 µg/ml bath induced contractile response of the rabbit's aortic spiral strip. This contractile response of GTE was still present after blocking alpha adrenergic receptors by using prazocin in a dose of 1 µg/ml bath. This indicates that GTE induced contractile response independent of alpha receptors (Figure 24).

Table (11): Effect of GTE on isolated rabbit's aortic spiral strip.

GTE µg/ml bath	Control (cm)	Level of contraction after (cm)	Percentage changes (%)	P
300	0	0.26 ± 0.02	↑ 5.14 %	< 0.001*
600		2.26 ± 0.08	↑ 44.75 %	< 0.001*
1200		4.01 ± 0.1	↑ 79.4 %	< 0.001*

Data represented as Mean ± SEM of six experiments.

***Significant level at (P < 0.05) compared to control value.**

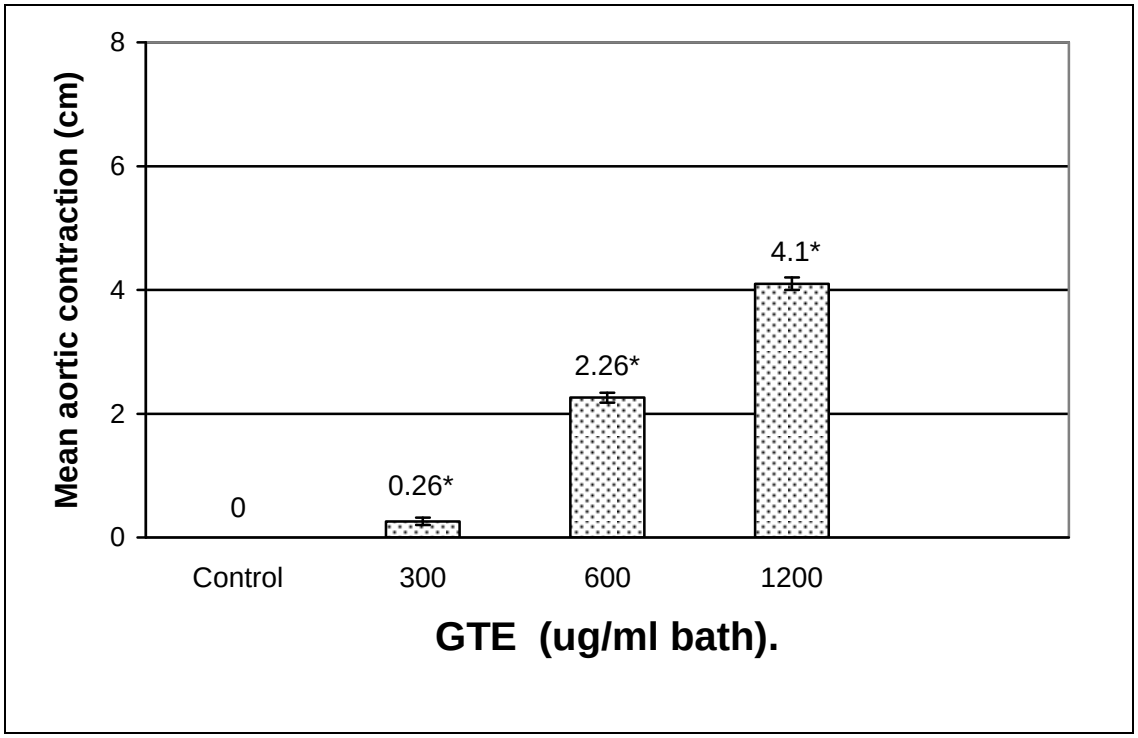

Figure (21): Histogram showing the effect of GTE on isolated rabbit's aortic spiral strip.

* Significant (P < 0.001) compared to control value

Figure (22): A record demonstrating the effect of GTE on isolated rabbit's aortic spiral strip.

Figure (23): A record demonstrating the dose dependent effect of GTE on isolated rabbit's aortic spiral strip.

Figure (24): A record demonstrating the site of action of GTE on isolated rabbit's aortic spiral strip.

3. Effects on isolated perfused rabbit's jejunum:

- **Effect of GTE on isolated perfused rabbit's jejunum:**

It was observed that addition of GTE in different dose levels (10, 30, and 100 μg/ml bath) produced dose related stimulation of rhythmic contraction of rabbit's jejunum. Addition of higher dose (300 μg/ml bath) also produced a stimulatory effect but with an amplitude less than the smaller doses used. (Table 12, Figures 25, 26).

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

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

Table (12): Effect of GTE on the amplitude of spontaneous rhythmic contraction of isolated perfused rabbit's jejunum.

Dose of GTE µg/ml bath	Level of contraction before (cm)	Level of contraction after (cm)	Percentage changes (%)	P
10	3.06 ± 0.3	4.31 ± 0.13	↑ 40.84 %	< 0.01*
30		5.05 ± 0.23	↑ 65.03 %	< 0.05*
100		5.31 ± 0.18	↑ 73.52 %	< 0.01*
300		4.1 ± 0.27	↑ 33.98 %	< 0.05*

Data represented as mean ± SEM of six experiments.
***Significant level at (P < 0.05) compared to control value.**

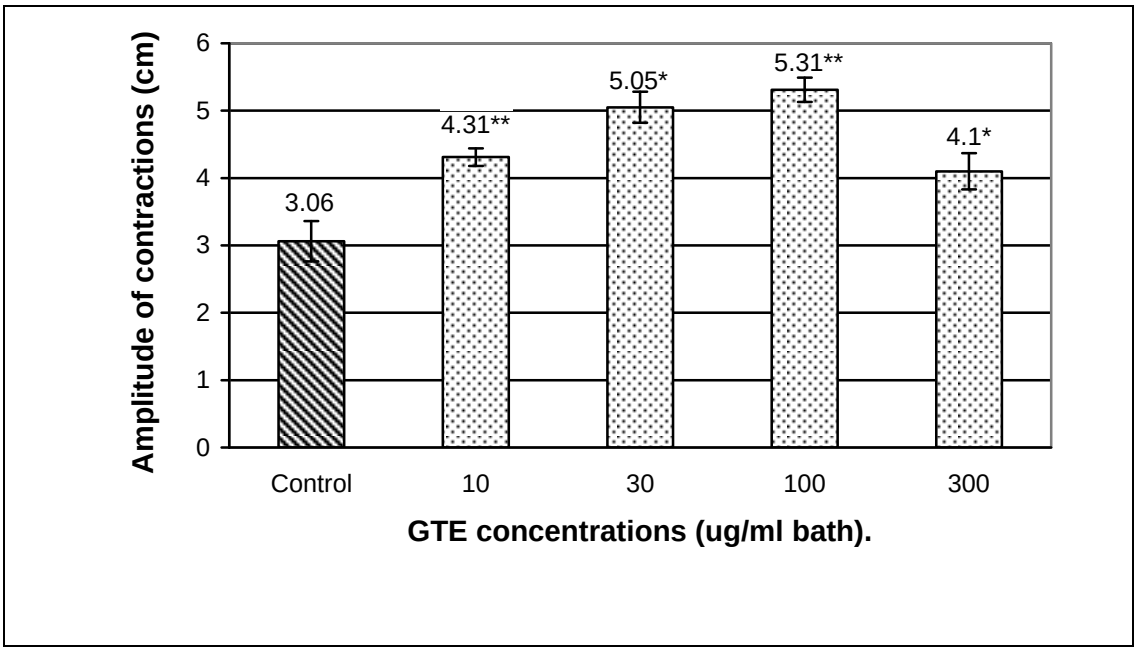

Figure (25): Histogram showing the stimulatory effect of GTE on the amplitude of spontaneous contraction of isolated perfused rabbit's jejunum.

* Significant (P < 0.05) compared to control value. ** Significant (P < 0.01) compared to control value.

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

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

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