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Original Research Article

Therapeutic Effects of Ethanolic Extract of Bay Leaves on Hormone-Induced Uterine Fibroids in Wistar Rats

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Abstract: ***Background****:* Histologically, fibroids are disordered smooth muscle cells buried in abundant quantities of extracellular matrix. The abundance of extracellular matrix in fibroids is responsible for the expansion of fibroids. Uterine fibroids are benign neoplasms. ***Materials and Method***: Adult female Wistar albino rats (200 ± 20g) were used for the present study. Animals were acclimatized for fourteen days in the animal house before being grouped into four groups of six (6) animals each. The eight experimental groups are as follows: ***Group A***: No fibroid induction, animals were provided with water and normal rat fed; ***Group B***: Uterine fibroid induced without treatment; ***Group C***:(treatment group): Mifepristone; ***Group D*** : (treatment group): Uterine fibroid induced+ Mifepristone; ***Group E***:(treatment group): Uterine fibroid induced+ 500 mg/kg of extract ; ***Group F***:(treatment group): Uterine fibroid induced+ 1500 mg/kg of extract; ***Group G***:(treatment group): 500 mg/kg of extract; ***Group H***:(treatment group): 1500 mg/kg of extract. Uterine fibroid was induced by administering Diethylstilbestrol (1.35 mg/kg/d), Progesterone injection of 1.0mg/180g intramuscularly three times a week, 0.9 mg/kg/d adrenal hydrochloride injection intramuscularly from week 5th to 6th week of the experiment (Zhao *et al.,* 2018 method). Uterine fibroid induction lasted for six (6) weeks. ***Results***: Results from the present study showed that estrogen and progesterone both increased in fibroid induced group when compared to the control, and both reduced in the treatment groups especially in the high dose group when compared to the fibroid induced group. Immunohistochemical studies showed weak positive stain for BCL-2 and strong positive stain for BAX in the ovaries. Weak positive stain for BCL-2 and strong positive stain for BAX in the uterus was also observed. ***Conclusion***: Bay leaves significantly reduced the levels of estrogen and progesterone caused by induced uterine fibroids. Bay leaves may likely reduce the size of uterine fibroids in rats due to its ability in the initiation of apoptosis in treatment groups. Bay leaves may be used as an anti fibroid agent in experimental rats.

Keywords: Apoptosis, BAX, Estrogen and Progesterone.

INTRODUCTION

Uterine fibroids are also more common uterine tumours, often known as leiomyomas or myomas. These are benign tumours of the female reproductive system's smooth muscle that involve various fibrous connective tissues (Victor *et al.,* 2025).

The origin of uterine fibroids appears to be genetic, manifesting as monoclonal tumors. Diverse features and mechanisms contribute to the growth and development of fibroids. Leiomyomas are experienced globally, the tumors appear to develop in those of African ancestry earlier than white women. While most leiomyomas are non-symptomatic, those that cause symptoms can adversely affect fertility, lifestyle, and physical function (Munro *et al*., 2025).

In recent times, the mechanism of action of several medicinal plants has been reported for various diseases, which enhance their acceptance among the population (Talukdar *et al*., 2018). In many rural locations around the world, plants have played important roles as medicine during pregnancy, birth, and postpartum care (Abdillahi and Van Staden, 2013).

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Plants have also been used for ages to treat infertility and other reproductive issues.The identification of novel components that can be successfully applied in a variety of biomedical applications, such as anticancer therapies, is aided by natural chemicals (Mann, 2002; Newman and Cragg, 2007; Cragg and Newman, 2013; Bailon-Moscoso *et al*., 2017). The idea that natural compounds are highly influential sources of new potential therapeutic agents is supported by the fact that over 60% of anticancer agents currently in use are derived from natural sources (Newman and Cragg, 2007; Cragg and Newman, 2013)

MATERIALS AND METHOD

Collection of Plant Materials and Extraction

Bay leaves were identified and authenticated in the department of Plant sciences and Biotechnology, Rivers State University. Bay leaves was pulverised and weighed. 4000g of the pulverised leaves were soaked in 5 litres of absolute ethanol for 72 hours with intermittent agitation and gyration. The solution was filtered twice; first using a chess cloth to remove large particles, and the filter paper which will remove finely pulverised particles. The filtrate was concentrated using water bath at a temperature of 350c, until dryness was achieved, leaving a paste.

Experimental Animals

Adult female Wistar albino rats (200 ± 20g) were used for the present study. The animals were purchased from the Department of Human Anatomy, Rivers State University and acclimatized for fourteen (14) days under standard laboratory conditions (good hygiene practices, 12-hourblight and dark cycle). The animals had access to clean drinking water and rat pellets.

Chemicals

Stilbesterol-5mg (Diethylstibesterol tablets BP 5 mg), manufactured by Kwality Pharmaceuticals LTD; Nam Stone Tablets (Mifepristone tablet 200mg), manufactured by NAMAN Pharma Drugs, Mumbai-2 (India); Progesterone B.P 20 mg/ml intramuscular injection, manufactured by Shanxi Shuguang Pharmaceuticals Co. LTD; Adrenaline injection B.P Img/Iml, R.No. 002289.

Tumor Induction: (Victor *et al*., 2025 method)

Uterine fibroid was induced by administering Diethylstilbestrol (1.35 mg/kg/d), Progesterone injection of 1.0mg/180g intramuscularly three times a week, 0.9 mg/kg/d adrenal hydrochloride injection intramuscularly from week 5th to 6th week of the experiment (Victor *et al*., 2025 method). Uterine fibroid induction lasted for six (6) weeks.

RESULTS

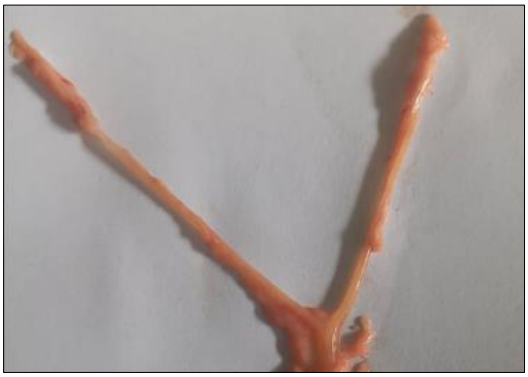


Fig. I: Animals received Distilled water

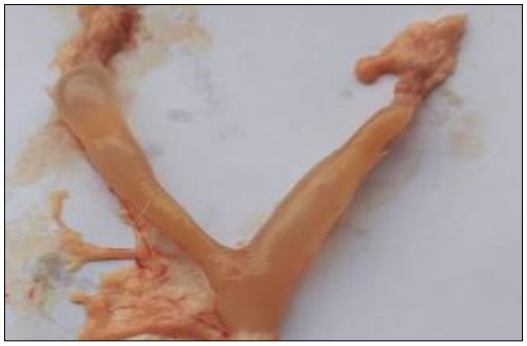


Fig. II: Animals induced with Uterine Fibroids

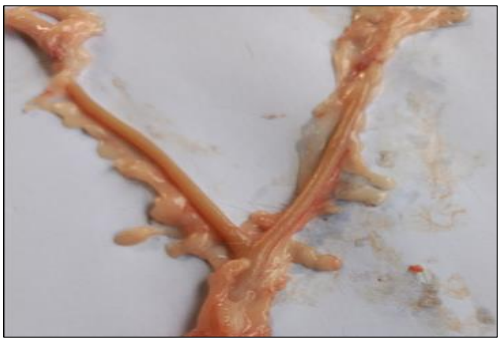


Fig. III: Animals treated with Mifepristone

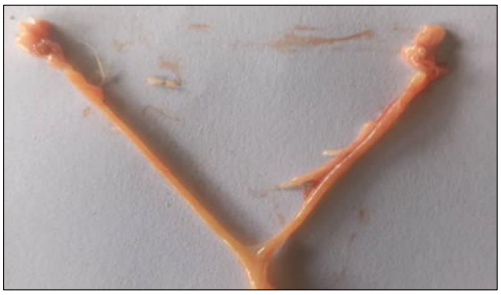


Fig. IV: Animals treated with Ethanolic Extract of Bayleaves (1500 mg/kg)

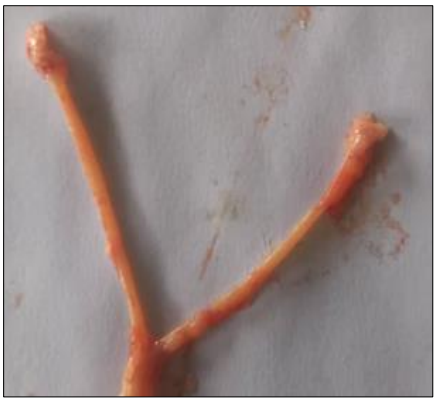


Fig. V: Animals treated with Ethanolic Extract of Bayleaves (500 mg/kg)

Effect of Ethanolic Extract of Bay Extract Treatment on Anti Oxidant Levels of Hormone-Induced Uterine Fibroids in Wistar Rats

In FIG VI, the control group had SOD concentration of 25.67 ±2.72. It increased significantly ($p<0.05$) in FI (44.44 ±2.82) compared with control and other treatment groups. FIG. VII shows that CAT concentration in FI (49.99 ±1.93) was significantly ($p<0.05$) higher compared with control and other experimental groups. It was in-turn significantly higher in other groups compared with control. In FIG. VIII, MDA concentrations in FI, Fibroid + Mifepristone and Fibroid + Bay (LD), Bay low and high doses group were significantly ($p<0.05$) high compared with control group.

Effect of Ethanolic Extract of Bay Extract Treatment on Female Reproductive Hormones of Hormone-Induced Uterine Fibroids in Wistar Rats

LH concentration was not significantly different among the different groups. LH in control group was 2.34 ±0.19.FIG. IX. FSH concentrations did not differ significantly among the different experimental groups, FIG. X. No significant difference was observed in prolactin concentration of the control (0.36 ±0.02) and FI (0.39 ±0.03) groups. It was however significantly (0.05) raised in Fibroid + Mifepristone compared with control, Bay low and high doses and with Mifepristone groups, FIG. XI. In FIG. XII, control group had progesterone concentration of 5.95 ±0.18. It increased significantly ($p<0.05$) in FI (9.66 ±0.58) and Mifepristone only (9.66 ±0.58) compared control, Fibroid + Mifepristone, Fibroid + Bay low and high doses groups. Estrogen levels also increased significantly ($p<0.05$) in FI (16.38 ±0.80) and Mifepristone alone (16.38 ±0.80) compared with control (11.14 ±0.63), Fibroid + Mifepristone (12.44 ±0.73) and Fibroid + Bay low dose (13.29 ±1.20) and high dose (12.42 ±1.51) and also with Bay low dose (12.63 ±0.34) groups, FIG. XIII.

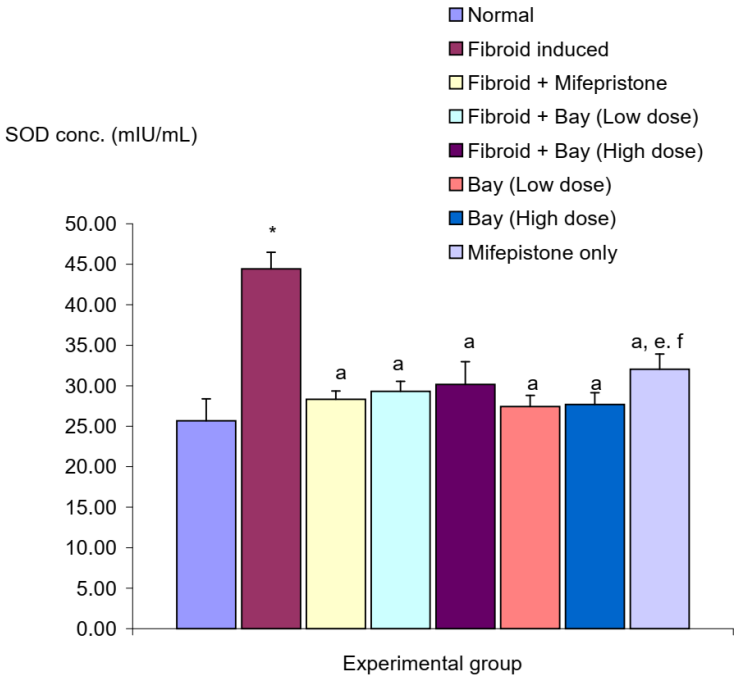


FIG. VI: Comparison of superoxide dismutase concentration in the different experimental groups of fibroid-induced rats.

Values are expressed as mean +SEM, n = 5.
* = significantly different from control at p<0.05
a = significantly different from fibroid-induced at p<0.05
e = significantly different from Bay (Low dose) at p<0.05
f = significantly different from Bay (High dose) at p<0.05

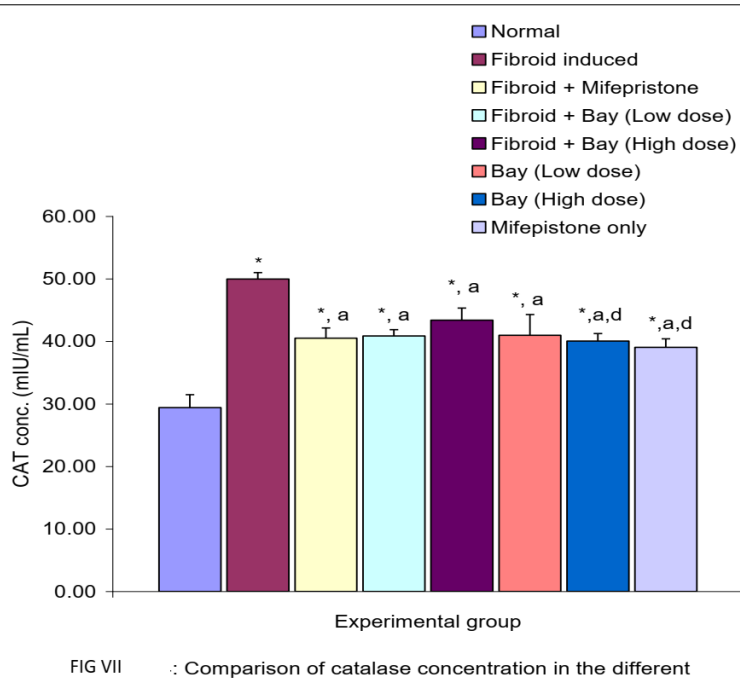


FIG VII .: Comparison of catalase concentration in the different experimental groups fibroid-induced rats.

Values are expressed as mean +SEM, n = 5.
* = significantly different from control at p<0.05
a = significantly different from fibroid-induced at p<0.05
d = significantly different from Fibroid + Bay (High dose) at p<0.05

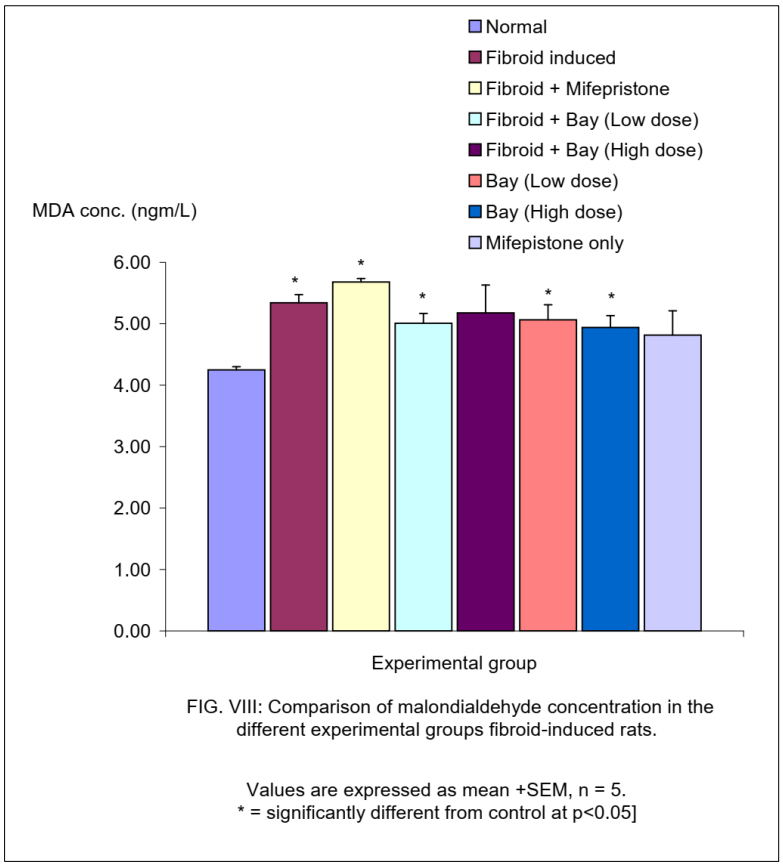


FIG. VIII: Comparison of malondialdehyde concentration in the different experimental groups fibroid-induced rats.

Values are expressed as mean +SEM, n = 5.
* = significantly different from control at p<0.05]

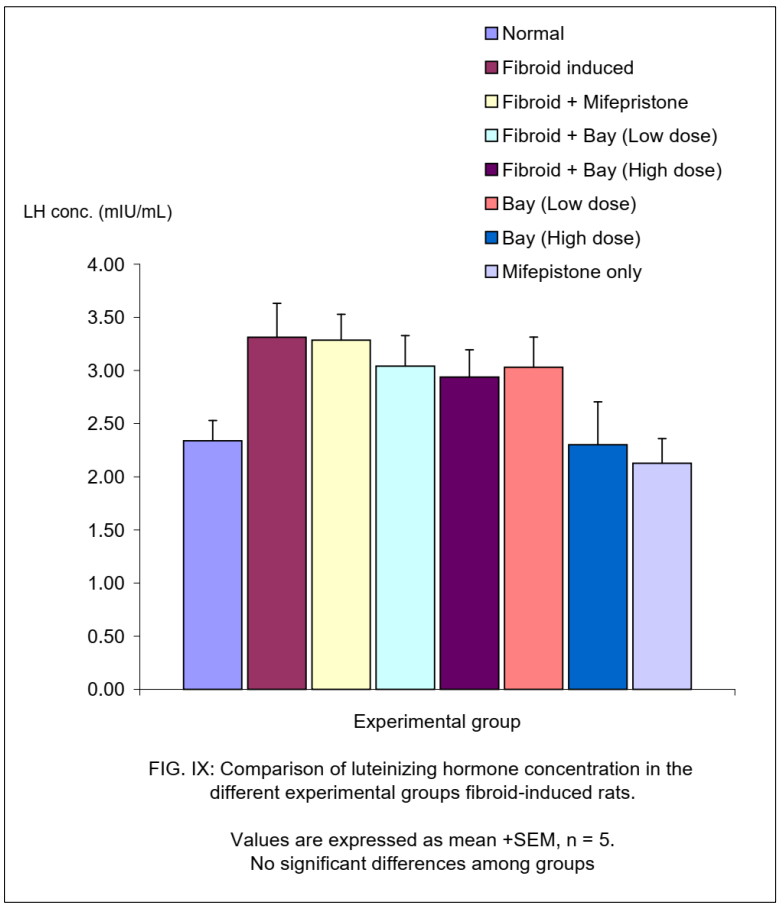


FIG. IX: Comparison of luteinizing hormone concentration in the different experimental groups fibroid-induced rats.

Values are expressed as mean +SEM, n = 5.
No significant differences among groups

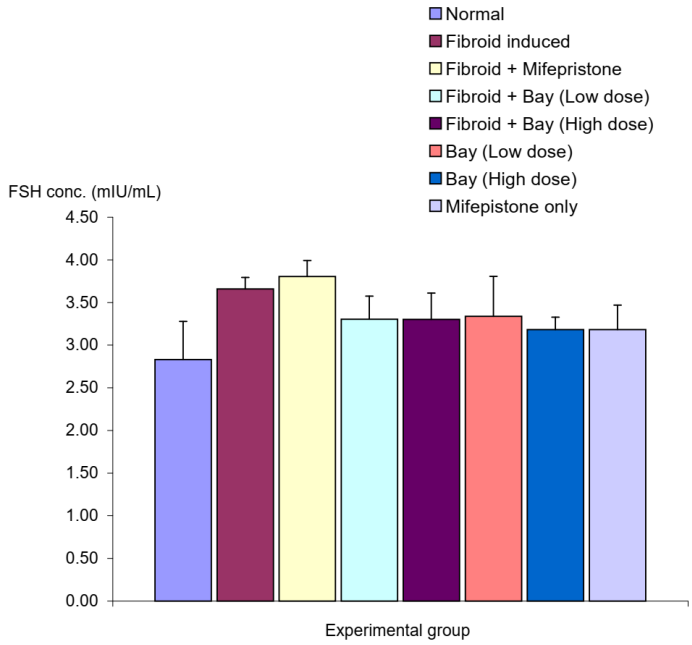


FIG. X: Comparison of follicle hormone concentration in the different experimental groups fibroid-induced rats.

Values are expressed as mean +SEM, n = 5.
No significant differences among groups

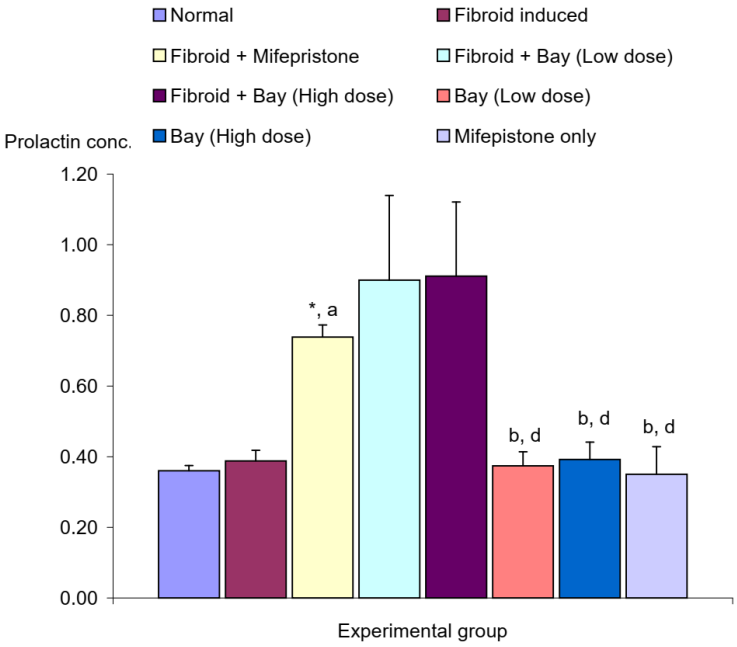


FIG. XI: Comparison of prolactin concentration in the different experimental groups fibroid-induced rats.

Values are expressed as mean +SEM, n = 5.
* = significantly different from control at p<0.05
a = significantly different from fibroid-induced at p<0.05
b = significantly different from fibroid + Mifeprostone at p<0.05
d = significantly different from Fibroid + Bay (High dose) at p<0.05

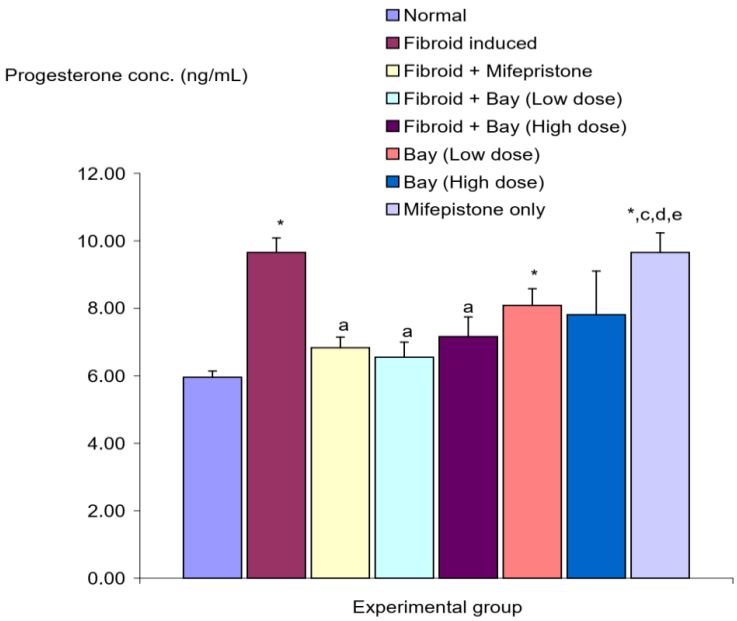


FIG. XII: Comparison of progesterone concentration in the different experimental groups fibroid-induced rats.

Values are expressed as mean +SEM, n = 5.
* = significantly different from control at p<0.05
a = significantly different from fibroid-induced at p<0.05
b = significantly different from fibroid + Mifeprostone at p<0.05
c = significantly different from Fibroid + Bay (Low dose) at p<0.05
d = significantly different from Fibroid + Bay (High dose) at p<0.05
e = significantly different from Bay (Low dose) at p<0.05

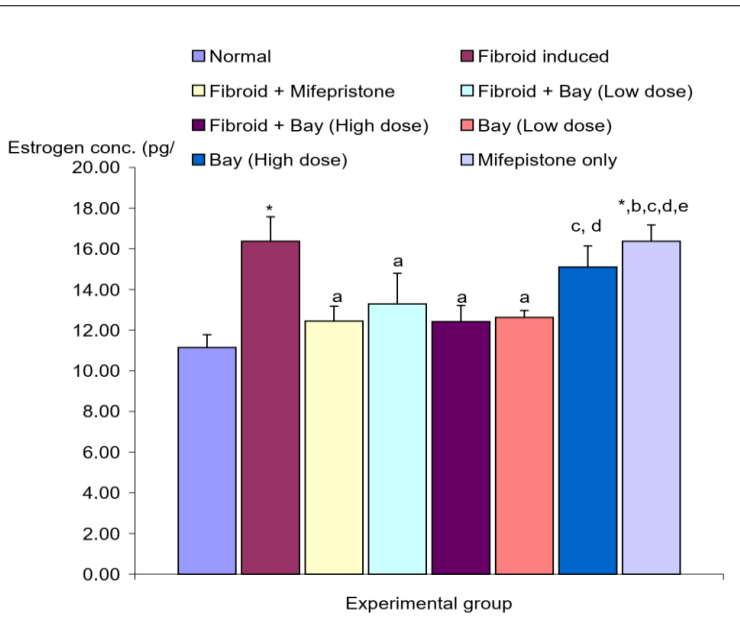


FIG. XIII: Comparison of estrogen concentration in the different experimental groups fibroid-induced rats.

Values are expressed as mean +SEM, n = 5.
* = significantly different from control at p<0.05
a = significantly different from fibroid-induced at p<0.05
b = significantly different from fibroid + Mifeprostone at p<0.05
c = significantly different from Fibroid + Bay (Low dose) at p<0.05
d = significantly different from Fibroid + Bay (High dose) at p<0.05
e = significantly different from Bay (Low dose) at p<0.05

Table 1: Comparison of SOD, CAT and MDA level in control, fibroid-induced, Mifepristone and Bay extract treated rats

	Superoxide dismutase (mIU/mL)	Catalase (mIU/mL)	Malon-dialdehyde (ng/mL)
Control	25.67±2.72	29.42±2.05	4.25±0.05
Fibroid-induced	44.44±2.82*	49.99±1.93*	5.34±0.45*
Fibroid + Mifepristone	28.34±1.02[a]	40.53±1.62*,[a]	5.68±0.06*
Fibroid + Bay (Low dose)	29.31±2.03[a]	40.88±1.01*,[a]	5.01±0.13*,[a]
Fibroid + Bay (High dose)	30.15±1.24[a]	43.41±1.01*,[a]	5.18±0.16*,[a]
Bay (Low dose)	27.43±1.36[a]	40.97±3.34*[a]	5.06±0.24*
Bay (High dose)	27.68±1.46[a]	40.07±1.18*[ad]	4.94±0.19*
Mifepristone	32.05±1.88*[aef]	39.07±1.35*[ad]	4.82±0.39

Values are expressed as mean ±SEM, n = 5.
* = significantly different from control at p<0.05
a = significantly different from fibroid-induced at p<0.05
b = significantly different from fibroid + Mifeprostone at p<0.05
d = significantly different from Fibroid + Bay (High dose) at p<0.05
e = significantly different from Bay (Low dose) at p<0.05
e = significantly different from Bay (High dose) at p<0.05

Table 2: Comparison of sex hormones level in control, fibroid-induced, Mifepristone and Bay extract treated rats

Variables	LH (mIU/mL)	FSH (mIU/mL)	Prolactin (ng/mL)	Progesterone (ng/mL)	Estrogen (pg/mL)
Control	2.34±0.19	2.83±0.45	0.36±0.02	5.95±0.18	11.14±0.63
Fibroid-induced	3.31±0.26	3.66±0.31	0.39±0.03	9.66±0.58*	16.38±0.80*
Fibroid + Mifepristone	3.29±0.24	3.80±0.19	±0.74±0.03*,[a]	6.83±0.32[a]	12.44±0.73[a]
Fibroid + Bay (Low dose)	3.04±0.32	3.30±0.14	0.90±0.24	7.16±0.43[a]	13.29±1.20[a]
Fibroid + Bay (High dose)	2.94±0.29	3.30±0.27	0.91±0.21	6.55±0.44[a]	12.42±1.51[a]
Bay (Low dose)	3.03±0.28	3.34±0.47	0.37±0.04[bd]	8.09±0.49*	12.63±0.34[a]
Bay (High dose)	2.30±0.40	3.18±0.15	±0.39±0.05[bd]	7.81±1.29	15.10±1.04[cd]
Mifepristone	2.13±0.23	3.18±0.29	±0.35±0.08[bd]	9.66±0.58*[cde]	16.38±0.80*[bcde]

Values are expressed as mean ±SEM, n = 5.
* = significantly different from control at p<0.05
a = significantly different from fibroid-induced at p<0.05
b = significantly different from fibroid + Mifeprostone at p<0.05
c = significantly different from Fibroid + Bay (Low dose) at p<0.05
d = significantly different from Fibroid + Bay (High dose) at p<0.05
e = significantly different from Bay (Low dose) at p<0.05

The mean MDA value was higher in fibroid induced group when compared to the control, MDA value reduced significantly in the treatment groups when compared to fibroid induced group (Table 1). Results from the present study showed that estrogen and progesterone both increased in fibroid induced group when compared to the control, and both reduced in the treatment groups especially in the high dose group when compared to the fibroid induced group (Table 2).

IMMUNOHISTOCHEMICAL STAINS

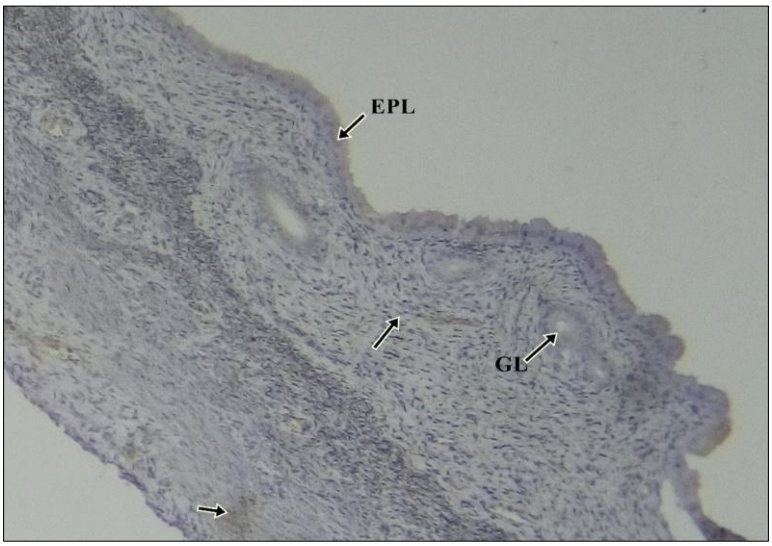


Plate I: (CONTROL): Section of the uterus stained for BCL2 and shows a weakly positive reaction. All the stroma cells and cells lining the glands are faintly stained brown as indicated by the unlabeled arrow x 100

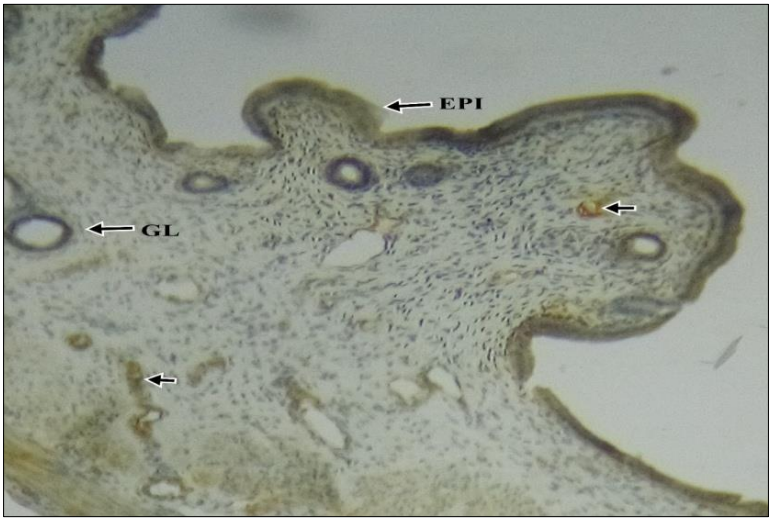


Plate II: (FIBROID INDUCED): Section of the uterus shows the endometrium consisting of the endometrial stromal cells and glands (GL) and superficial epithelial (EPI). The tissues show a positive reaction for BCL2

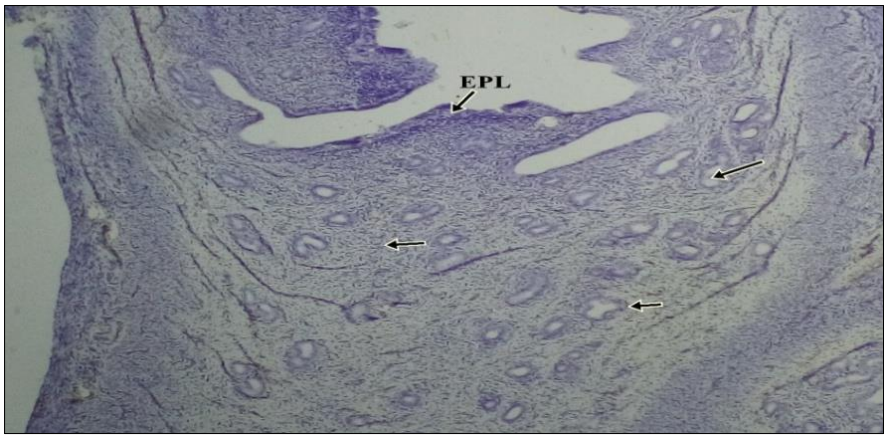


Plate III: (MIFEPRISTONE): Section of the uterus stained for BCL2 and shows a weakly positive reaction. All the stroma cells and cells lining the glands are faintly stained brown as indicated by the unlabeled arrow. x100

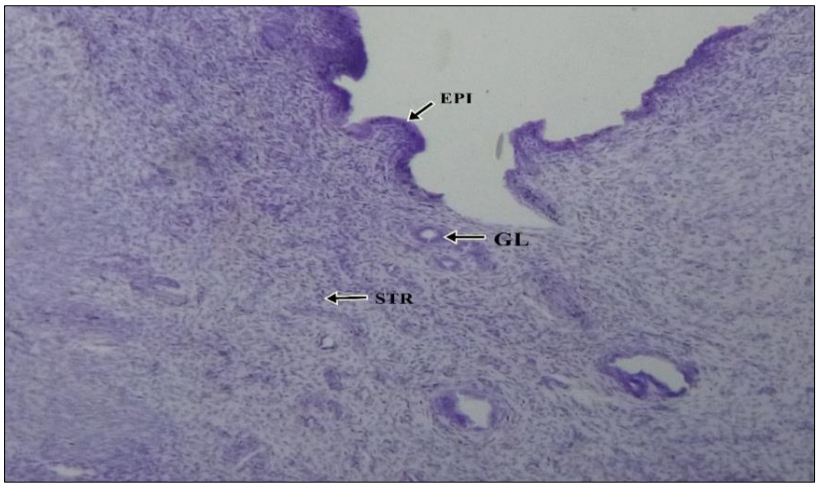


Plate IV: Section of the uterus from the low dose (500 mg/kg) treatment group. Section stained with BCL2 shows a negative reaction. X100

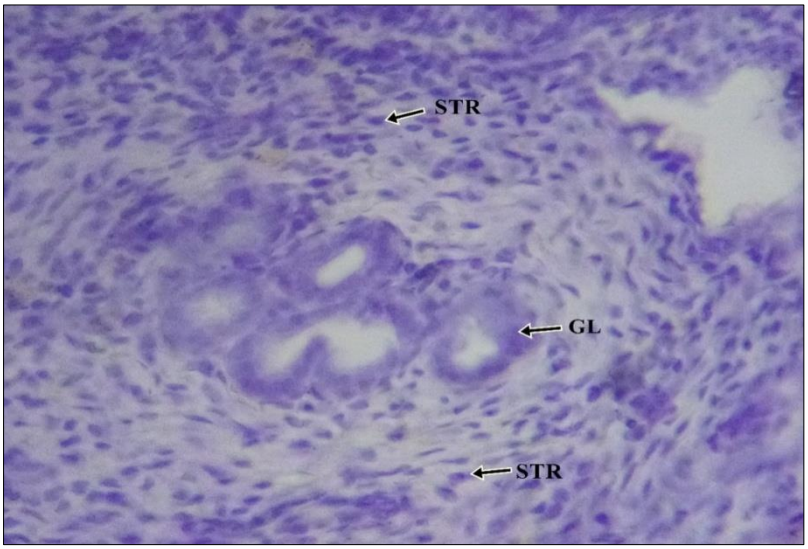
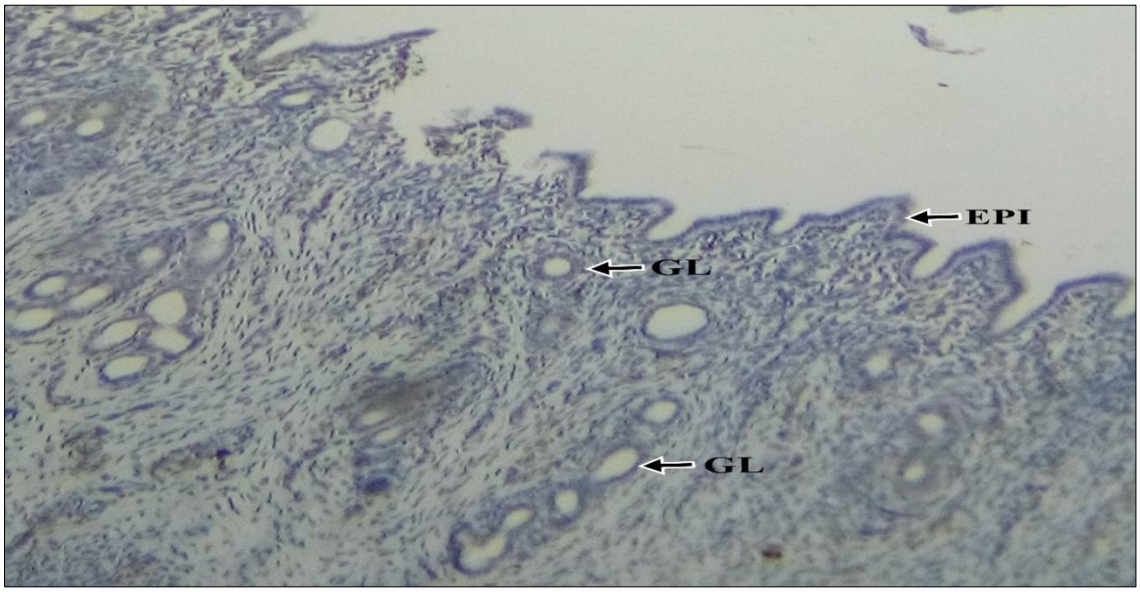


Plate V: Section of the uterus from the low dose (500 mg/kg) treatment group. Section stained with BCL2 shows a negative reaction. X400



N-1 X100 BAX

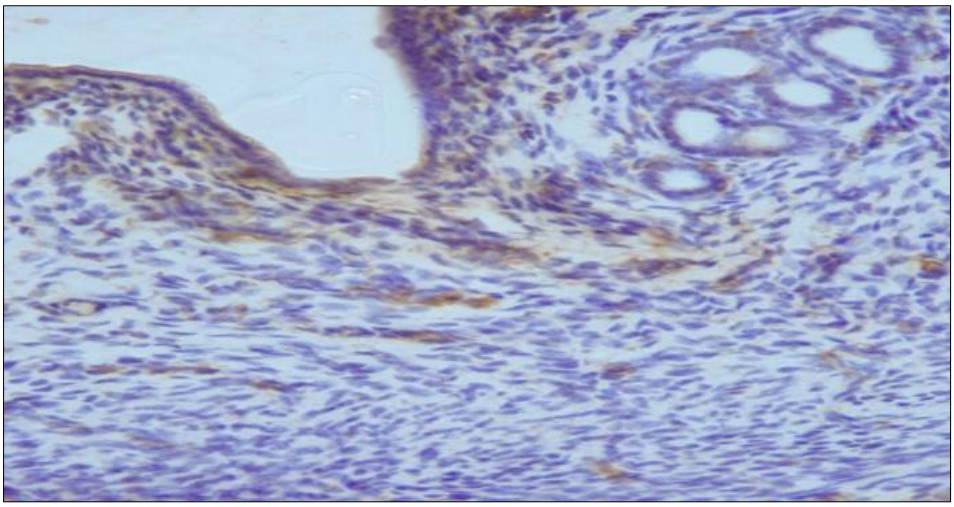


Plate VI: Section of the uterus from the low dose (500 mg/kg) treatment group. Section stained with BAX strong positive BK expression in the myometrium and endometrium cells X400

Immunohistochemical studies showed weak positive stain for BCL-2 and strong positive stain for BAX in the ovaries. Weak positive stain for BCL-2 and strong positive stain for BAX in the uterus was also observed.

DISCUSSION

Ovarian steroid hormones have been shown to be important biomarkers associated with the growth and development of uterine fibroid (Agbadua *et al*., 2020). Estrogen has been considered the major factor, there is growing evidence suggesting that progesterone and its receptors play a key role in the growth and development of uterine fibroid (Agbadua *et al*., 2020). Some authors have reported that estradiol and progesterone are important in the transformation of myometrial cells into leiomyoma cells, with progesterone required for the complete development and proliferation of leiomyoma cells (Reis *et al*., 2015). The key hormones in the pathogenesis of leiomyoma formation are estrogen and progesterone (Kim and Sefton, 2012).

A decrease in the serum levels of estrogen and progesterone observed in hormone fibroid-induced animals which were treated with ethanolic extract of bay leaves may explain the use of this treatment method by herbal practitioners. Progesterone and Estrogen levels were reduced significantly in Fibroid + Mifepristone, Fibroid + bay leaves and Fibroid + bay leaves high dose groups compared with fibroid induced groups (FI) (Figure 6 and 7) respectively. This implies that bay leaves extract reversed elevated estrogen and progesterone levels caused by diethylstilbestrol, adrenal hydrochloride, and progesterone injections (Agbadua *et al*., 2020). The possible mechanisms of action of bay leaves against uterine fibroid growth and development can be attributed to the inhibition of aromatase, thus preventing the synthesis of estrogen. Bay leaves may be protective because it decreases serum progesterone levels as observed in the study (Cermik, *et al*., 2002). This agrees with the findings of Oyebode *et al*., (2019) who studied the protective effects of the alpha stone decoction on monosodium glutamate-induced uterine fibroid. It also agrees with other studies by Obochi *et al*., (2009) and Olowofolahan *et al*., (2007), who investigated the effects of garlic extracts and extracts of Drymariacordota respectively, on MSG-induced uterine fibroid.

In fibroid-induced rats treated with bay leaves, it was observed that the size of the uterus and its weight were reduced. This suggests that bay leaves can be useful as an anti-fibroid agent. It is believed that plant extract capable of reducing the weight and size of the uterus can act as an anti-fibroid agent (Victor *et al*., 2025). This decrease in the size of the uterus of animals induced with fibroid and later administered with high and low doses of extracts bay leaves suggests that the plant extract may have played a pivotal role in the reduction of the size of the uterus and/or shrinking the growing tumor.

CONCLUSION

Bay leaves significantly reduced the levels of estrogen and progesterone caused by induced uterine fibroids. Bay leaves may likely reduce the size of uterine fibroids in rats due to its ability in the initiation of apoptosis in treatment groups. Bay leaves may be used as an anti fibroid agent in experimental rats.

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