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Anti-diarrheal, antipyretic and phytochemical investigation of methanolic extract of *Ficus lyrata* leaves

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Abstract: The leaves of *Ficus lyrata* warb (Moraceae) is used to treat GI disorder such as diarrhea in folklore use. The aim of present research is to explore the antidiarrheal and antipyretic activity of methanolic extract of leaves of *F. lyrata*. The diarrhea was induced by using castor oil. The anti-pyretic effects were also explored on yeast induced pyrexia. The physicochemical analysis of powder such as (total ash, water soluble ash, acid insoluble ash, moisture content, foaming index and swelling index) along with organoleptic evaluation of leaf of *F. lyrata* in order to access the quality Purity of the substance was also done. The phytochemical analysis of methanolic extract of *F. lyrata* leaves confirmed the presence of polyphenols, tannins, terpenoids, cardiac glycosides, flavonoids and reducing sugar. The antidiarrheal activity of MEFL at 100 and 200 mg/kg body weight showed 62% and 76% reduction in defecation respectively. The decrease in intestinal fluid accumulation at same dose revealed 67.2% and 75% reduction in fluid accumulation in intestine of Wister albino rats. The methanolic extract of *F. lyrata* showed reduction in distance travelled by charcoal meal such as 54% and 72% at dose 100 and 200 mg/kg respectively. The antipyretic effect at a dose of 100 and 200 mg/kg showed significant reduction in temperature. The anti-diarrheal activity of the *F. lyrata* might be due to the presence of flavonoids.

Keywords: Ethnopharmacology, conventional use of plants, antidiarrheal, antipyretic, *Ficus lyrata.*

INTRODUCTION

Ficus lyrata (F. lyrata) also known as fiddle leaf belongs to the genus Ficus which has 800 species (Farag *et al.,* 2014). Another name of Moraceae family is Mulberry family and fig family. About 40 genera and 1100 species are present in this family (Rahman and Khanom, 2013). Rationally leaves and fruits of ficus were used both in fresh and dry form. The roots, bark and stems contain flavonoids, terpenoids, alkaloids, phenolic and tannins, while the fruits and leaf extract of *F. lyrata* has large quantity of phenolic compounds, tannins, flavonoids and triterpenoids (Zhang *et al*., 2019). Several studies has shown the presence of phenolic acid conjugates (O-caffeoyl quinic acid, and gentisic acid) and flavonoids (apigenin and leutolin C glycosides) in significant concentration (Wahyudha *et al*., 2020). *F. lyrata* is traditionally used to treat many disorders such as gastrointestinal problems, anthelmintic, diabetes, antitumor activity, asthma, astringent, bleeding, bone fracture, antiseptic, cough, sexual disorders, ear-ache and toothache, migraine, eye troubles, scabies, gonorrhea, paralysis and anti-diarrhea (Haider and Zhong, 2014). The ethnobotanical study of leaves showed that it has anti-diarrheal activity. The control in inhibition of defecation may be due to the presence of flavonoids, polyphenols and triterpenoids through the inhibition of peristalsis movement. Since *F. lyrata* also contain beta-sitosterol, numerous pentacyclic compounds such as (ursolic acid), ellagic acid, quercetin and other flavonoids compounds which shows control in diarrhea by inhibiting peristalsis activity and hydro-electrolyte secretion (Awad *et al.,* 2019). It is possible that anti-secretory, anti-inflammatory and antioxidant property are responsible for antidiarrheal activity. The antipyretic effect at a dose of 100 and 200mg/kg showed significant reduction in temperature. The methanolic extract of *F. lyrata* has some influence on prostaglandin biosynthesis because prostaglandin is believed to be regulated the body temperature. In this study, the attempt is made to explore the mechanism involved in the anti-diarrheal activity and to establish the anti-pyretic potential of the *F. lyrata*.

MATERIALS AND METHODS

Collection of plant material

F. lyrata is a perennial plant and was collected in October 2020 from Bahawalpur, Pakistan. The plant was identified and authenticated by Taxonomist, Prof. Dr. Zaheer

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Extraction of plant material

Extraction of the plant was carried out by following the method previously used (Buabeid *et al.,* 2022) and Percentage yield was calculated. The obtained extract was then transferred in glass vials and stored in refrigerator at 4ºC for further use.

Pharmacognostic features

Macroscopic evaluation

Macroscopic and Microscopic evaluation was done by using the previously adopted method by (Awad *et al*., 2019).

Phytochemical screening

The procedure was adopted for qualitative phytochemical analysis. Different phytochemical tests were performed on methanolic extract for the presence of different chemical constituents (Chanda, 2014).

Physicochemical analysis

Physicochemical analysis includes moisture content, fluorescence analysis, total ash value, acid insoluble ash, water soluble ash, foaming index and swelling index was determined by using previously used methods and formula, respectively (Jeevitha *et al*., 2021).

Determination of extractive values

Water soluble extractive and alcohol soluble extractive value was determined by following previously used methods (Adham, 2015).

Experimental animal

Adult healthy male wistar albino rats of 150-200g were used for experimental study. The animals were placed in clean restrainers under specified laboratory condition 25 $^{\circ}$C for about 7 days to acclimatize the environment. Animals were given fresh water and food regularly. The animals were housed in animal house Department of Pharmacy, The University of Lahore). Prior to the study all the protocols and procedures were approved by research ethics committee (IREC 2021-26) of Faculty of Pharmacy; The University of Lahore (Mason and Matthews, 2012).

Acute toxicity study

In order to check the lethal dose of methanol extract, toxicity study was performed on animals. For this purpose, animals were grouped in to three (six animals each). The rats in these groups were given oral dose of methanolic extract of *F. lyrata* 250, 500, 1000mg/kg body weight. The animals were observed for 15 days to note any toxic effect and safest dose was selected for experimental purpose (Awad *et al*., 2019).

Antidiarrheal activity

Rats were divided into four groups. Each group contains six animals. Diarrhea was induced in these rats by giving 1ml castor oil. Control group 1 was given 2ml/kg intraperitoneal (i.p) normal saline, group 2 received 3 mg/kg atropine i.p and group 3 and 4 was given 100, 200 mg/kg i.p MEFL. All these groups were treated one hour before castor oil administration per oral (p.o). Number of stools were calculated after every 1 hour interval till 4 hours by placing plain paper under each group. The mean number of diarrheal stools in treated group was compared with control group (Antonisamy *et al*., 2015). Following formula is used to calculate the percentage inhibition of diarrhea in rats.

$$\text{Inhibition}(\%) = \frac{\text{Average number of feaces in control group} - \text{Average number of feaces in test group}}{\text{Average number of feaces in control group}} \times 100$$

Castor oil induced enteropooling

The rats divided in 4 groups were fasted overnight. Before administering the castor oil, group 1 was given 2ml/kg normal saline i.p, group 2 was administered with standard atropine 3mg/kg i.p experimental group 3 and 4 received 100 and 200 mg/kg methanol extract i.p respectively. After two hours' rats were sacrificed. Small intestine of all the rats was removed and tied with thread from both ends. Its length was measured and intestinal fluid was poured in to graduated cylinder to check the volume of intestinal contents. The weight of empty and full intestine was also calculated (Chitme *et al*., 2004).

$$\text{Mean inhibition}(\%) = \frac{\text{MVICC - MVICT}}{\text{MVICC}} \times 100$$

MVICC= Mean volume of intestinal content in control group
MVICT= Mean volume in intestinal content in test group

Small intestinal transit

The overnight fasted rats were divided in to 5 groups. Group 1 was administered 2ml normal saline orally. Group 2 was given 2ml castor oil orally with 2ml/kg normal saline i.p. Group 3 received atropine 3mg/kg i.p. Group 4 and 5 was administered 100 and 200 mg/kg, respectively plant extract i.p 1 hour before administration of castor oil. After 1 hour of castor oil administration, 1 ml of (10% charcoal suspension in 5% gum acacia was administered orally. After 1 hour rats were sacrificed. The distance travelled by charcoal meal from pylorus to caecum was measured. Percentage of inhibition of distance moved by charcoal was calculated by following formula (Antonisamy *et al*., 2015).

$$\text{Inhibition}(\%) = \frac{\text{A - B}}{\text{A}} \times 100$$

Where,
A= Distance travelled by charcoal in control group
B= Distance travelled by charcoal in test group
Peristalsis index is calculated by following formula:
Mean distance travel by charcoal in test group

Table 1: Fluorescence analysis of dried powder of *F. lyrata.*

Treatment	Day light	UV Light	
		254 nm	366nm
Powder + H_2O	Dark golden	Dark brown	Brown
Powder + MeOH	Dark green	Dark brown	Reddish brown
Powder + Chloroform	Yellowish green	Green	Golden
Powder + $FeCl_3$	Amber	Dark green	Brownish black
Powder + I_2	Light golden	Brown	Light green
Powder + 10% NaOH	Reddish brown	Dark brown	Reddish green
Powder + KOH	Bronish green	Green	Reddish orange
Powder + H_2SO_4	Black	Reddish brown	Black
Powder + dil. NH_3	Dark green	Brown	Reddish brown

Table 2: Effect of extract on castor oil induced enteropooling in rats.

Treatment	Dose	Mean weight of intestinal content (g)	% Inhibition
Control Group	Normal saline 2ml/kg i.p	1.933 ± 0.233	----
Standard Group	Atropine 3mg/Kg i.p	0.916 ± 0.14**	52.61%
F. lyrata extract	100 mg/kg i.p	0.63 ± 0.15***	67.2%
F. lyrata extract	200 mg/kg i.p	0.48 ± 0.12***	75%

Table 3: Castor oil induced intestinal transit.

Treatment	Dose	Distance traveled by Charcoal (cm)	% Inhibition of distance traveled by Charcoal	Peristalsis Index %
Control group	C.O 2ml p.o+ N.S 2 ml/kg i.p	23.7 ± 1.6	----	51.43
Standard group	C.O 2ml p.o + atropine 3mg/kg i.p	16 ± 1.84	33.33	37.90
F. lyrata 100 mg/kg	C.O 2ml p.o +Extract 100 mg/kg	10.83 ± 1.1*	54.1	28.80
F. lyrata 200 mg/kg	C.O 2ml p.o+ Extract 200 mg/kg	4.83 ± 0.7**	79	13.90

Effect of castor oil on intestinal transit. Results are shown in mean and ± SEM of experiment on rats. Where n = 6. Analysis was done by One Way ANNOVA followed by (Daunnet Test). P value adjusted as *P=.033, **P= .002, ***P<.001.

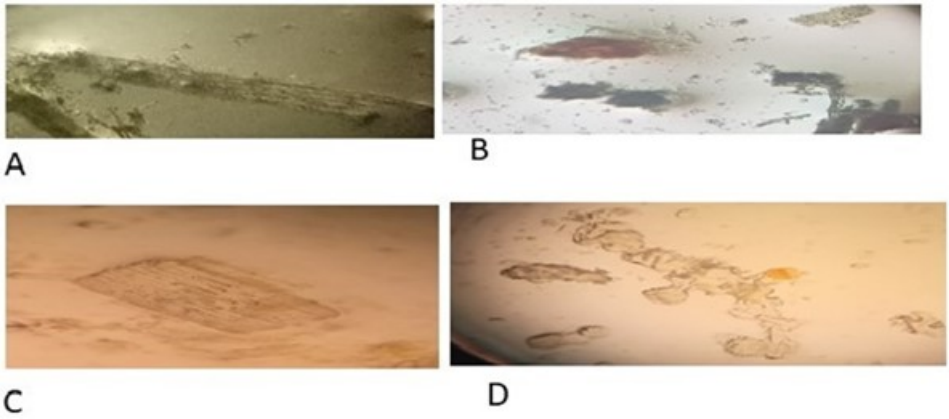

Fig. 1: Showing the microscopic images (A) Fibers and sclereids, (B) Starch granules (C): Fibro vascular tissue (D): Calcium oxalate crystals.

$$\text{Peristalsis index}\,(\%) = \frac{\text{Mean distance traveled by charcoal in control group}}{\text{Mean distance travel by charcoal in test group}} \times 100$$

Antipyretic activity

The rats were kept in separate cages for one day before experiment. Brewer's yeast was used to induce pyrexia in rats. Before experiment rectal temperature of these animals was noted and animals having normal body temperature were selected for further experiment. By subcutaneous injection of 20% Brewer's yeast suspension at the dose of 10ml/kg was used to induce pyrexia in rats. Using digital thermometer, rectal temperature was measured after 12 hours of yeast injection. The rats showed increase in body temperature 0.7ºC were further

used for experiment. The rats were divided in to 4 groups each having 6 animals. Group 1 was control group and given distilled water and yeast suspension, group 2 served as standard and was given 150 mg/kg paracetamol along with yeast suspension p.o Group 3 and 4 were experimental groups and given MEFL 100 and 200 mg/kg of body weight p.o (Sajeesh *et al.,* 2011).

STATISTICAL ANALYSIS

The data was stated as mean ± SEM, evaluated by one way ANOVA followed by Dunnett's test. $P<0.05$ was considered as statistically significant by using the software GraphPad prism version 8.0.

RESULTS

Macroscopic evaluation and microscopic evaluation

F. lyrata Leaves are bright fleshy or green in color. Leaves are lure shape and odorless. The size of leaves is 33-44 cm length, 22-30cm wide. The taste of leaves is bitter and rough in texture. The powder microscopy of *F. lyrata* showed that plant contain fibro vascular tissue, calcium oxalate crystals, fiber and sclereids and starch granules as shown in fig. 1.

Phytochemical analysis

Phytochemical analysis revealed the presence of phenolic compounds, flavonoids, glycosides, tannins and terpenoids in leaves of *F. lyrata*. Alkaloids and saponins are absent in leaves. The moisture content of *F. lyrata* was found to be 5.03% which is less than 10%.

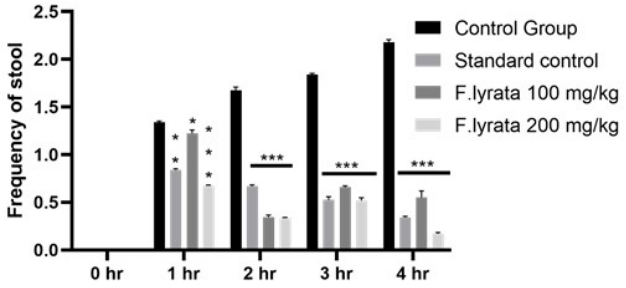

Fig. 2: Effects of extract on castor oil induced diarrhea on rats. Results are shown in Mean and ± SEM from the experiment. Analysis was performed by (Two Way ANNOVA) followed by Dunnet Test. Where P values are expressed as P=.033(*), .002 (**), <.001 (***) (n=6).

Fluorescence analysis

The color change of crude powder with different solvent revealed the property of solvent with powder constituents and results obtained are mentioned in table 1.

Physicochemical analysis

Total ash value

The total ash value of *F. lyrata* was found to be 22.66% which shows the existence of inorganic salt, acid insoluble ash is 10.76% while water soluble ash is 03%.

Foaming index

The height of foam in all of the ten test tubes shows more than 1 cm it means that foaming index is more than 1000 which was calculated. The foaming index calculated was 107.40.

Acute toxicity test

In toxicity study no mortality was observed at a dose of 250, 500 and 1000mg/kg of body weight. Any other observed effect of drug also was not shown in all three groups of animals for 15 days. Thus, safest dose for further experimental purpose was selected for present study.

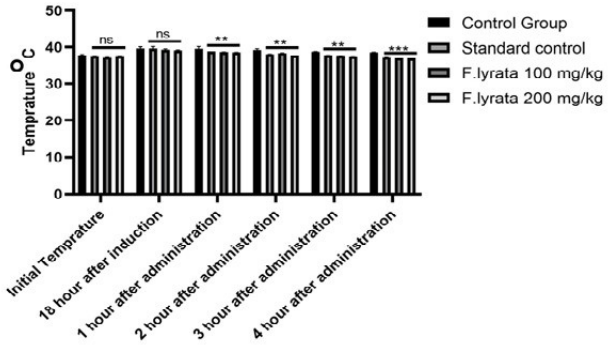

Fig. 3: Antipyretic effect of F. lyrata leaves in rats. Analysis was performed by (One Way ANNOVA) followed by Dunnet Test. Where P values are expressed as P=.033(*), .002 (**), <.001 (***) (n=6).

Anti-diarrheal activity

Test doses of 100 and 200 mg/kg having anti-diarrheal potential of MEFL are shown in fig. 2. inhibit defecation in rats by 62% and 76%. While standard drug atropine showed 65% inhibition at a dose of 3mg/kg body weight when compared with control group respectively.

Enteropooling effect

Castor oil induced intestinal transit time

The distance travelled by charcoal meal from pylorus to caecum was calculated and % inhibition of distance travelled by control group and treated group along with standard group are mentioned in table 3.

Antipyretic effect of F. lyrata leaves

Different test doses of 100 and 200 mg/kg having anti-pyretic potential of methanolic extract of *F. lyrata* are shown in fig. 3.

DISCUSSION

Fluorescence is a phenomenon of illumination. In case of powder of plant many chemical components in plant material shows illumination. These are alkaloids and berberine. If the chemicals do not show fluorescent by nature, other reagents can typically be used to transform them into fluorescent derivatives or breakdown products

(Jeevitha *et al*., 2021). Statistical analysis showed that by comparing standard and MEFL with control group there was significant reduction in inhibition of defecation in rats. It was also shown from experimental data that at dose dependent manner MEFL showed greater inhibition as compared to standard drug atropine. The control in defecation may be due to three mechanism of antidiarrheal activity such as increase the reabsorption of NaCl and water from intestine, inhibits the secretion of prostaglandins and intestinal motility may be decreased by the 100 or 200 mg/kg extract. As anticholinergics block the parasympathetic moments in GI tract and thus diarrhea was controlled (Chitme *et al*., 2004). From statistical analysis it was concluded that MEFL showed satisfactory results at a dose of 100 and 200 mg/kg body weight and the percentage inhibition of fluid accumulation was 67.2% and 75% respectively. While standard drug atropine at dose of 3mg/kg body weight showed inhibition of 52% which was less than *F.lyrata* extract. It showed that MEFL was more significant in controlling diarrhea than atropine. The fluid accumulation due to castor oil was due to the inhibition of reabsorption of NaCl and water caused by ricinoleic acid secreted by castor oil thus lead to the inflammation of intestinal mucosa. The atropine thus is anti-muscarinic agent and inhibit the fluid accumulation in intestinal lumen. The plant extract exhibit antidiarrheal activity by stimulation of fluid absorption in intestine and may also act as anti-prostaglandin agent as shown in table 2 (Ezeja *et al*., 2012). The present study showed the peristalsis index inhibition by dose dependent manner when compared to control group. Results were analyzed by One Way ANOVA where $p<0.001$. The MEFL show more significant results than atropine in dose dependent manner such as 54.1% and 79% at a dose of 100, 200 mg/kg respectively. The atropine is anticholinergic drug and hence decrease peristalsis movements and thus decrease peristalsis movement by 33.3% (Antonisamy *et al*., 2015). *F. lyrata* methanol extract contain significant amount of flavonoids and thus its shows hypothermic effect in case of yeast induced pyrexia. At dose of 100 mg/kg *F. lyrata* extract show comparable effect with standard drug paracetamol. *F. lyrata* extract show significant effect of antipyretic activity compared to control group at a dose of 200mg/kg as shown. Yeast induced pyrexia may be due to the synthesis of prostaglandins in hypothalamus of brain. It thus causes the elevation of body temperature. Most of the NSAID's used as antipyretic shows their mechanism of action by inhibiting the prostaglandin synthase. It will stop the synthesis of prostaglandin in brain and body temperature decreases from high to low. The medicinal plants used for antipyretic activity may show this effect probably due to the presence of metabolites such as saponins, flavonoids, alkaloids. These constituents thus play the role of NSAIDs in lowering body temperature (Sajeesh *et al*., 2011).

CONCLUSION

The recent study involves the pharmacological and pharmacognostic study of *F. lyrata* plant. Phytochemical analysis of methanolic extract of *F. lyrata* leaves confirmed the presence of polyphenols, tannins, terpenoids, cardiac glycosides, flavonoids and reducing sugar. Antidiarrheal activity of MEFL at 100 and 200 mg/kg body weight showed 62% and 76% reduction in defecation respectively. The decrease in intestinal fluid accumulation at same dose revealed 67.2% and 75% reduction in fluid accumulation in intestine of wistar albino rats. Methanolic extract of *F. lyrata* showed reduction in distance travelled by charcoal meal such as 54% and 72% at dose 100 and 200 mg/kg respectively. Antipyretic effect at a dose of 100 and 200 mg/kg showed significant reduction in temperature. Thus, further studies can be done on fruit or stem of *F. lyrata* for further separation, identification pharmacological uses of constituents. Further studies are required to establish the underlying mechanism.

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REFERENCES

Adham AN (2015). Comparative extraction methods, phytochemical constituents, fluorescence analysis and HPLC validation of rosmarinic acid content in *Mentha piperita, Mentha longifolia* and *Osimum basilicum*. *J. Pharmacogn. Phytochem,* **3**(6): 130-139.

Antonisamy P, Duraipandiyan V, Ignacimuthu S and Kim JH (2015). Anti-diarrhoeal activity of friedelin isolated from *Azima tetracantha* Lam. in wistar rats. *SIJBS,* **1**(1): 34-37.

Awad NE, Abdelqawy MA, Hamed MA and Aboshehata MM (2019). Quantitation and characterization of phenolic compounds in butanol fraction of *Ficus lyrata* leaves and in vivo assessment of hepatoprotective and hypocholesterolemic activities in rats. *J. Herbs Spices Med. Plants,* **25**(4): 330-346.

Buabeid M, Arafa ES, Rani T, Ahmad F, Ahmed H, Hassan W and Murtaza G (2022). Effects of *Solanum lycopersicum* L. (tomato) against isoniazid and rifampicin induced hepatotoxicity in wistar albino rats. *Braz. J. Biol.,* **84**: 234-245

Chanda S (2014). Importance of pharmacognostic study of medicinal plants: An overview. *J. Pharmacogn. Phytochem.,* **2**(5): 69-73.

Chitme HR, Chandra R and Kaushik S (2004). Studies on anti-diarrhoeal activity of *Calotropis gigantea* R. Br. in experimental animals. *JPPS*, **7**(1): 70-5.

Ezeja IM, Ezeigbo II, Madubuike KG, Udeh NE, Ukweni, IA, Akomas SC and Ifenkwe DC (2012). Antidiarrheal

activity of *Pterocarpus erinaceus* methanol leaf extract in experimentally-induced diarrhea. *Asian Pac. J. Trop. Med.,* **5**(2): 147-150.

Farag MA, Abdelfattah MS, Badr SE and Wessjohann LA (2014). Profiling the chemical content of *Ficus lyrata* extracts via UPLC-PDA-qTOF-MS and chemometrics. *J. Nat. Prod.,* **28**(19): 1549-1556.

Haider M and Zhong L (2014). Ethno-medicinal uses of plants from district Bahawalpur, Pakistan. *Curr. Res. J. Biol.,* **6**(5): 183-190.

Jeevitha M, Ravi PV, Subramaniyam V, Pichumani M & Sripathi SK (2021). Exploring the phyto-and physicochemical evaluation, fluorescence characteristics and antioxidant activities of Acacia ferruginea Dc: an endangered medicinal plant. *Future J. Pharm. Sci.,* **7**(228): 1-17.

Mason TJ and Matthews M (2012). Aquatic environment, housing, and management in the eighth edition of the guide for the care and use of laboratory animals: Additional considerations and recommendations. *JAALAS,* **51**(3): 329-332.

Rahman A and Khanom A (2013). Taxonomic and ethno-medicinal study of species from Moraceae (Mulberry) family in Bangladesh flora. *J. Plant Sci.,* **1**(3): 53-57.

Sajeesh T, Arunachalam K and Parimelazhagan T (2011). Antioxidant and antipyretic studies on *Pothos scandens* L. *Asian Pac. J. Trop. Med.*, **4**(11): 889-899.

Wahyudha D, Mardawati E, Djali M and Balia R (2020). The characterization of *Ficus lyrata* Warb fruit extract and the effect on toxicity, physicochemical and microbiology properties of chicken carcass. *Int. J. Adv. Sci. Eng. Inf. Technol.,* **10**(1): 362-367.

Zhang Q, Onstein RE, Little SA and Sauquet H (2019). Estimating divergence times and ancestral breeding systems in Ficus and Moraceae. *Ann. Bot.,* **123**(1): 191-204.