Anti-inflammatory effect of natural heterocycle glucoside *vicine* obtained from *Vicia faba* L. and its aglucone (*divicine*) and their effect on some oxidative stress biomarkers in Albino rats

Mohammed Abdalla Hussein[1*]

[1*]*Department of Biochemistry, Faculty of Pharmacy, October 6th University, October 6th city, Egypt*

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ABSTRACT

Background: Inflammation is typically characterized by increased permeability of endothelial tissue resulting in oedema. Much of pyrimidine derivatives have been highly useful for treating acute and self-limited inflammatory conditions. This study was performed to explore the acute toxicity, anti-inflammatory activity and biochemical effect of natural heterocycle pyrimidine glucoside *vicine* obtained from *Vicia faba* L. and its aglucone (*divicine*) and their effect on some oxidative stress biomarkers in Albino rats. Also, the anti-inflammatory effect of the tested compounds were compared with diclofenac sodium (100 mg/kg bw). On the other hand, this article was extended to assess the impact of a very high dose of diclofenac sodium (100 mg/kg bw) on liver enzymes, which was used by many authors to explain the negative effects of diclofenac when used at this dose as a standard anti-inflammatory drug. **Methods:** The median lethal dose (LD_{50}) and histopathological effects of *vicine* and *divicine* was determined in order to assess their safety. Also, the inflammatory effect of the tested compounds was examined at doses (50–200 mg/kg bw) in rats, using fresh egg albumin-induced oedema and compared with standard anti-inflammatory drugs, diclofenac sodium (100 mg/kg bw). Finally, *vicine* and *divicine* treatment (50–200 mg/kg bw) was evaluated for 10 consecutive days prior in Albino rats in the serum levels of hepatic enzymes, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), gamma glutamyl transferase (γ-GT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), super oxide dismutase (SOD), reduced glutathione (GSH), glutathione peroxidase (GPx) and thiobarbituric acid reactive substances (TBARs). **Results:** *Vicine* and *divicine* were found to have an LD_{50} of 2100 and 1950 mg/kg bw orally, respectively and a significant ($p < 0.001$) dose-dependent anti-inflammatory activity with the highest percentage inhibition (59.56%) and (45.65%) for *vicine* and *divicine*, respectively, at 150 mg/kg against the fresh egg albumin-induced oedema in rats after 180 min. Also, the standard anti-inflammatory diclofenac sodium (100 mg/kg) showed 100% inhibition against the fresh egg albumin-induced oedema in rats after 180 minutes. In addition, administration of *vicine* and *divicine* orally to the rats at doses of 50–200 mg/kg bw for 10 days showed non-significant changes in liver enzymes and serum TBARs as compared with the control group. But administration of diclofenac sodium orally at a concentration of 100 mg/kg bw daily for 10 consecutive days to rats showed significant increase in liver enzymes and serum TBARs and significant decrease in blood GSH, SOD and GPx. Histopathology of the rat liver morphology of all the treated groups showed no disorder except for the photomicrograph of the cross section of liver from rats treated with diclofenac sodium 100 mg/kg for 10 consecutive days, shows pronounced dilatation of the hepatic portal vein. **Conclusion:** The observation allows conclusion that *vicine* and *divicine* at concentration of 50–150 mg/kg bw exhibited good anti-inflammatory effect and have no hepatotoxic activity.

Keywords: toxicity, anti-inflammatory, rats, *vicine* and *divicine,* fava beans (*Vicia faba*).

***Corresponding address:**
Mohammed A. Hussein, Ph.D. Biochemistry department, Faculty of Pharmacy, October 6th University, October 6th City, Egypt.
Tel.: +2 (012) 4832580; fax: +2 (02) 38353270.
E-mail : abdallamohammed_304@yahoo.com

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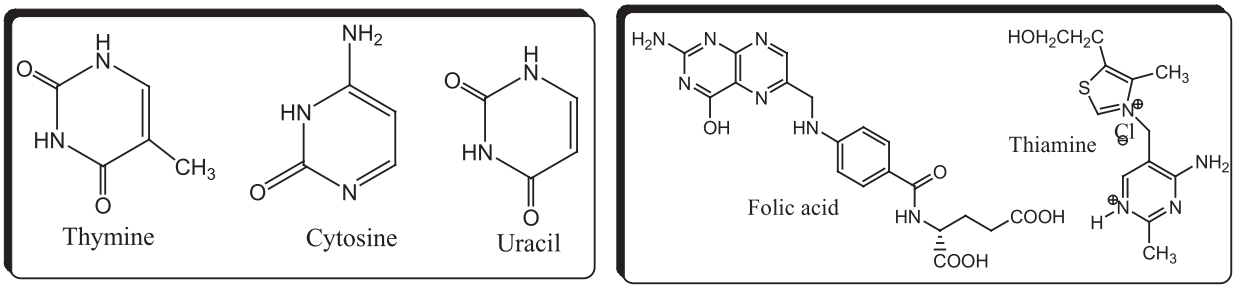
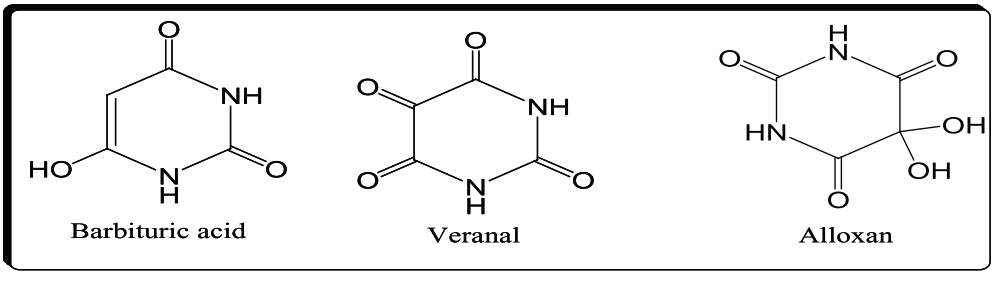
INTRODUCTION

Pyrimidine nucleus is one of the most important heterocycles exhibiting remarkable pharmacological activities. In medicinal chemistry, pyrimidine derivatives have been

well known for their therapeutic applications. The presence of a pyrimidine base in thymine, cytosine and uracil, which are the essential building blocks of nucleic acids, DNA and RNA is one of the possible reasons for their activities.[1]



Also, the pyrimidine ring is found in vitamins like riboflavin, thiamine and folic acid,[2] barbituric acid and its several derivatives (e.g. Veranal) which are used as hypnotics[3] and in alloxan, which is known for its diabetogenic action in a number of animals.[4]



Pyrimidine has a remarkable pharmacological efficiency and therefore intensive research has been focused on the anti-inflammatory activity of pyrimidine nucleus.[5,6] In addition, pyrimidine nucleus is also present in certain natural product sources such as *vicine* and *divicine*. Divicine is an aglycone derived from *vicine*, a glucosidic compound contained in fava beans.

Fava bean (*Vicia faba*; broad bean, horse bean) is an important member of the legume family with highly useful characteristics. It is widely grown and consumed, especially in China, North African countries and parts of Europe and North and South America, and is served in a large variety of forms, mostly based on the immature or mature seed. The highest concentration of *vicine* or *divicine* in *fava beans* was found in young seeds. The concentration decreased rapidly with maturity of seeds or the whole pod.[7] Also, *vicine* isolated from certain medicinal plants is used in folk medicine for various ailments,[8] the hypoglycemic properties could be verified as well as in animals as in humans.[9] In addition to this effect, it is used for treating stomach ache, colds and fevers, rheumatism, gout and to introduce abortion. Also, it has been described as a blood purifier, laxative and antihelmintic.[10]

Diclofenac, a non-steroidal anti-inflammatory drug (NSAID), is commonly employed in the treatment and/or management of rheumatoid arthritis and

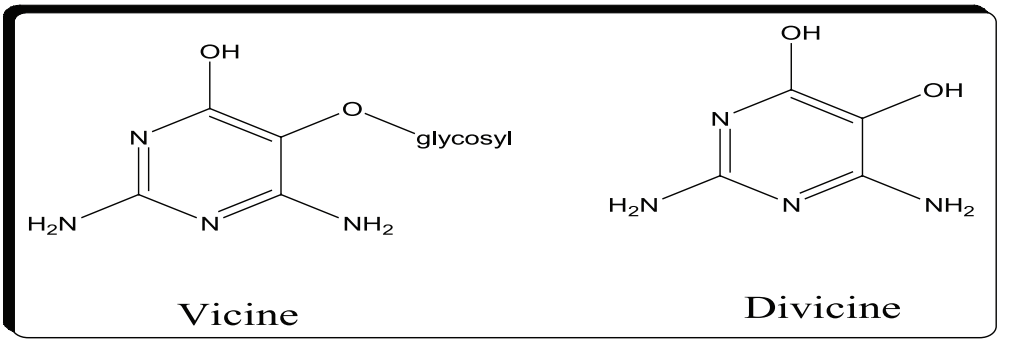


Figure 1. Structural formulas of *vicine* and *divicine* in *V. faba*.

osteo-arthritis[11,12] and for its anti-inflammatory and analgesic effects.[13] Diclofenac sodium reduces inflammation, swelling and arthritic pain by inhibiting prostaglandins synthesis and/or production.[14,15] The drug also affects polymorphonuclear leukocytes function *in vitro*, thereby reducing chemotaxis, superoxide toxic radical formation, oxygen-derived free radical generation and neutral protease production.[16] Diclofenac sodium has also been reported to suppress inflammation in experimental animal models.[17] As an extension of my interested research program in the extraction and therapeutic evaluation of medicinal plants,[18-21] I report herein, a facile route to calculate the median lethal doses (LD_{50}) and describe the anti-inflammatory effect of *vicine* and *divicine* isolated from fava beans (*Vicia faba*) which may pave the way for possible therapeutic application.

MATERIALS AND METHODS

Chemistry

Melting points were determined on Gallen-kamp melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on shimadzu MR 470 infrared spectrophotometer using the KBr pellets. Microanalytical data (C, H, N) was determined at the Microanalytical Centre, Cairo University, Egypt**.** Mass spectra were run using HP Model MS-5988.

Tested compounds

1. *Vicine* pure crystalline sample was extracted from mature seeds of fava beans (*Vicia faba*) according to the procedure described by Arbid and Marquardt.[22]
2. *Divicine* pure crystalline sample was obtained by acid hydrolysis from *vicine* according to the method described by Marquarott et al.[23]

METHODS

Animals

Male adult Albino rats weighing between 150–170 gm were obtained from the animal house Cairo University, Faculty of Veterinary Medicine. The animals were maintained on a 12 h light/dark cycle with *ad labium* access to food (commercial diet) during all periods of this study.

Determination of LD_{50}

Preliminary experiments were carried out for each tested compound on 6 main groups (10 rats/each dose/each group). *Vicine* and *divicine* were administrated orally in different doses to find out the range of doses which cause zero and 100% mortality of animals. A range dose was determined for each compound.

I used 120 rats divided into 12 main groups of 10 animals. In groups 1–6, of 10 animals each, LD_{50} of *vicine* was calculated by giving an oral dose of 700, 1400, 2100, 2800, 3500 and 4200 mg/kg bw, corresponding each dose to one group of 10 animals. In a similar way, in groups 7–12, LD_{50} of *divicine* was determined by oral administration of the following doses: 500, 1000, 1500, 2000, 2500 and 3000 mg/kg bw.

The LD_{50} was evaluated by Spearman and Karber method[24] on groups of rats, each of 10 animals. The test compounds were administrated orally at different doses. The number of animals which died within 24 h was recorded. The LD_{50} was then calculated by the application of the following formula:

$$LD_{50} = Dm - \frac{\Sigma(Z \cdot d)}{n}$$

D_m = the dose which killed all the rats in the group
Z = half the sum of the dead rats from 2 successive groups
d = the difference between 2 successive doses
n = number of animals in each group

Evaluation of anti-inflammatory property

The rats used were divided into five broad (A, B, C, D and E) experimental groups. All groups were injected in the right paw with albumin (0.5 ml/kg sp).

Group A (8 rats) control untreated group (neither treated with vicine nor divicine).
Group B (8 rats) received distilled water (3 ml/kg orally) only.
Group C 'test' (consisted of 4 sub groups, 8 rats in each subgroup) received *vicine* (50, 100, 150 and 200 mg/kg orally suspended in distilled water).
Group D 'test' (consisted of 4 subgroups, 8 rats in each subgroup) received *divicine* (50, 100, 150 and 200 mg/kg orally suspended in distilled water).
Group E 'test' (8 rats) received diclofenac sodium (100 mg/kg orally suspended in distilled water).[25,26]

The rat hind paw oedema was used as a model of acute inflammation. Rat hind paw oedema was induced by intraplantar injection of fresh egg albumin (0.5 ml/kg).[27] Oedema was always evident within 5–8 min following fresh egg albumin (0.5 ml/kg) injection. Linear diameter of the injected paw was measured (with a transparent

millimeter ruler) for 3 h at 30-minute intervals after the administration of the fresh egg albumin. Increases in the linear diameter of the right hind paws were taken as indicators of paw oedema. Oedema was assessed in terms of the difference in the 'zero time' (C_o) linear diameter of the injected right hind paw, and its linear diameter at 'time t' [(C_t) i.e., 30, 60, 90, 120 and 180 min] following fresh egg albumin administration. The increases in the right hind paw diameters induced by injections of fresh egg albumin were compared with those of the contra-lateral, non-injected left hind paw diameters.[28] *Vicine* and *divicine* were separately administered at doses of 50, 100, 150 and 200 mg/kg orally to each of the rats in the 'test' Groups C and D, respectively, 20 min before inducing inflammation with the injection of fresh egg albumin. Rats in the reference, comparative 'test' Group E received diclofenac sodium (100 mg/kg orally); while rats in the 'control' Group B received distilled water (3 ml/kg orally) only.

Percentage inhibition of the oedema was calculated from the formula:

$$[I_o - I_t/I_o] \times 100$$

[Where I_o is the average inflammation (hind paw oedema) of the 'control' Group A rats at a given time; and I_t is the average inflammation of the (Groups C and/or D) *vicine* and/or *divicine* or (Group E) diclofenac-treated rats at the same time].

BIOCHEMICAL STUDIES OF ANTI-INFLAMMATORY COMPOUNDS ON LIVER ENZYMES IN RATS

Experimental design

This experiment was carried out to examine the effect of anti-inflammatory compounds, *vicine* and *divicine* on liver enzymes. A solution of 50, 100, 150 and 200 mg/kg bw for each anti-inflammatory compound, *vicine* and *divicine* suspended in saline was prepared for intragastric intubation of rats. Groups of animals each consisting of 8 rats in each were treated daily for 10 days as follows:

Group A: Normal Group (untreated group).
Group B: Control (was given similar volume of distilled water po).
Group C_I: Was treated with *vicine* (50 mg/kg bw) suspended in distilled water orally in a single daily dose.[29]
Group C_{II}: Was treated with *vicine* (100 mg/kg bw) suspended in distilled water orally in a single daily dose.[29]
Group C_{III}: Was treated with *vicine* (150 mg/kg bw) suspended in distilled water orally in a single daily dose.[29]
Group C_{IV}: Was treated with *vicine* (200 mg/kg bw) suspended in distilled water orally in a single daily dose.[29]
Group D_I: Was treated with *divicine* (50 mg/kg bw) suspended in distilled water orally in a single daily dose.[29]
Group D_{II}: Was treated with *divicine* (100 mg/kg bw) suspended in distilled water orally in a single daily dose.[29]
Group D_{III}: Was treated with *divicine* (150 mg/kg bw) suspended in distilled water orally in a single daily dose.[29]
Group D_{IV}: Was treated with *divicine* (200 mg/kg bw) suspended in distilled water orally in a single daily dose.[29]
Group E: Was treated with diclofenac sodium (100 mg/kg bw) suspended in distilled water orally in a single daily dose.[29]

After 10 days of treatment, animals were killed by cervical dislocation, blood samples were withdrawn from the retro-orbital vein of each animal. The separated blood was used for the estimation of SGOT, SGPT, γ-GT, ALP, LDH, TBARS, GSH, GPx and SOD.

BIOCHEMICAL ASSAYS

Serum levels of glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvate transaminase (GPT) were determined according to Reitman and Frankel,[30] alkaline phosphatase (ALP) was accessed according to King,[31] Gamma Glutamyl transferase γ-GT was determined using the technique described by Fiala et al.,[32] lactate dehydrogenase was determined according to Buhl and Jackson[33] and TBARS in serum was accessed according to Uchiyama and Mihara.[34] Blood superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were determined using the technique described by Paglia and Valentine[35] and Marklund and Marklund,[36] respectively. Blood haemoglobin was accessed according to the method of Van Kampen and Zijlstra.[37]

HISTOPATHOLOGY

The liver tissues isolated from the test animals were fixed in formaline-saline for 48 hours. The fixed tissue were processed manually through graded ethanol, cleared in xylene, impregnated and embedded in paraffin wax. Thin sections were cut with a rotary microtome, stained by haematoxylin and eosin technique, examined microscopically for pathological changes according to the method of Bancroft and Steven.[38]

DATA ANALYSIS

Anti-inflammatory experimental data obtained from 'test' rats treated with *vicine*, *divicine* and diclofenac, as well as

those obtained from distilled water-treated 'control' rats, were pooled and expressed as means ± SD. The differences between *vicine*, *divicine* and diclofenac-treated 'test' means, and distilled water-treated 'control' means, were analyzed statistically by one way analysis of variance (ANOVA; 95% confidence interval), followed by Tukey-Kramer's multiple comparison test to assess the level of significance of the differences between the 'test' and 'control' group data means. Values of $p \leq 0.05$ were taken to imply statistical significance.

RESULTS

Table 1 shows the specific physicochemical properties of *vicine* and *divicine*. Melting point of *vicine* and *divicine* are 239–241 and 201–205, respectively. Microanalytical data in Table 1 shows that the calculate/found ratio of *vicine* and *divicine* (C, H, N).

Infra red spectrum of *vicine* exhibited bands at 3331.9, 3309.6 cm^{-1} (NH_2), 3201.6 cm^{-1} (OH), 2908, 2846 cm^{-1} (CH-aliphatic), 1680 cm^{-1} (C = N), 1473 cm^{-1} (C-O-C), 1218 cm^{-1} (C-N), 1064 cm^{-1} (C-O-C).

Mass spectrum of *vicine* revealed a molecular ion peak at m/z 304 (14.02%) with a base peak at 142 (100%), and other significant peaks were observed at 162 (15.71%), 90 (15.46%), 81 (15.65%).

Infra red spectrum of *divicine* exhibited bands at 3779.9, 3396 cm^{-1} (NH_2), 2930, 2846 cm^{-1} (CH-aliphatic), 1606 cm^{-1} (C=N), 1430 cm^{-1} (C-O-C), 1219 cm^{-1} (C-N).

Mass spectrum of *divicine* revealed a molecular ion peak and a base peak at m/z 143 (M^++H, 100%) and other significant peaks were observed at 138 (18%), 130 (17.46%), 90 (15%), 87 (19%).

DETERMINATION OF LD_{50} OF *VICINE* AND *DIVICINE* IN ADULT RATS

The results given in Table 2 show that oral administration of *Vicine* in doses of 700, 1400, 2100, 2800, 3500 and 4200 mg/kg bw resulted in mortalities of 0, 2, 6, 8, 9 and 10, respectively. The dose of *vicine* that killed half of the rats (LD_{50}) was 2100 mg/kg bw The results given in Table 3 show that oral administration of *divicine* in doses of 500, 1000, 1500, 2000, 2500 and 3000 mg/kg bw resulted in mortalities of 0, 1, 2, 5, 8 and 10, respectively. The dose of *divicine* that killed half of the rats (LD_{50}) was 1950 mg/kg bw.

DETERMINATION OF ANTI-INFLAMMATORY ACTIVITY OF *VICINE* AND *DIVICINE* IN ADULT RATS

The results presented in Table 4 shows that *vicine* (Group C) and *divicine* (Group D) (50–200 mg/kg orally) produced

Table 1 Physico-chemical properties and molecular formulae of *vicine* and *divicine*

Compd.	M.P. [°C]	Yield (%)	Mol. Formula (Mol. Wt.)	Elemental analyses Calcd./Found [%]		
				C	H	N
Vicine	239–241	–	$C_{10}H_{16}N_4O_7$ (304)	39.47	5.26	18.42
				39.25	5.30	18.30
Divicine	201–203	93	$C_4H_6N_4O_2$ (142)	33.80	4.23	39.43
				33.30	4.02	39.74

Table 2 Determination of LD_{50} of *vicine* given orally in adult rats

Group Number	Dose (mg/kg)	No. of animals/ group	No. of dead animals	(Z)	(d)	(Z.d)
1	700	10	0	1	700	700
2	1400	10	2	4	700	2800
3	2100	10	6	7	700	4900
4	2800	10	8	8.5	700	5950
5	3500	10	9	9.5	700	6650
6	4200	10	10	0	00	00

$$LD_{50} = Dm - \frac{\Sigma(Z \cdot d)}{n}$$

$$LD_{50} = \frac{4200 - 21000}{10} = 2100 \text{ mg/kg bw}$$

Table 3 Determination of LD_{50} of *divicine* given orally in adult rats

Group Number	Dose (mg/kg)	No. of animals/ group	No. of dead animals	(Z)	(d)	(Z . d)
1	500	10	0	0.5	500	250
2	1000	10	1	1.5	500	750
3	1500	10	2	3.5	500	1750
4	2000	10	5	6.5	500	3250
5	2500	10	8	9.0	500	4500
6	3000	10	10	0	00	00

$$LD_{50} = Dm - \frac{\Sigma(Z \cdot d)}{n}$$

$$LD_{50} = \frac{3000 - 10500}{10} = 1950 \text{ mg/kg bw}$$

Table 4 Effects of *vicine* and *divicine* (50–200 mg/kg, orally) and diclofenac sodium (DIC, 100 mg/kg, orally) on rat paw oedema induced by fresh egg albumin (0.5 ml/kg sp). Values quoted represent the mean ± SEM of 8 observations. Percent inhibitions of the egg albumin-induced inflammation by *vicine, divicine* and reference drug used are indicated as %

Experimental Group Dose (orally)		Time (in min) and paw diameter (in mm)				
		30	60	90	120	180
Control Group A (untreated)		12.45 ± 0.15	12.32 ± 0.42	12.26 ± 0.25	12.30 ± 0.31	11.50 ± 0.14
Control Group B (distilled water-treated) 3 ml/kg		12.50±0.23	12.40 ± 0.25	12.2 ± 0.36	12.35±0.49	11.45 ± 0.54
Group C Vicine treated	50 mg/kg	11.08 ± 0.14 (12.36 %)	10.74 ± 0.26 (12.82%)	9.35 ± 0.19* (23.7%)	9.09 ± 0.34* (26.09%)	9.95 ± 0.25* (13.47%)
	100 mg/kg	10.54 ± 0.21 (15.34%)	9.27 ± 0.13* (24.75%)	8.84 ± 0.25* (27.89%)	7.05 ± 0.22** (42.68%)	5.86 ± 0.32*** (49.04%)
	150 mg/kg	10.74 ± 0.52* (13.72%)	9.53 ± 0.34* (22.64%)	8.27 ± 0.16* (32.54%)	6.03 ± 0.07*** (50.97%)	4.65 ± 0.06*** (59.56%)
	200 mg/kg	11.10 ± 0.43 (10.83%)	9.76 ± 0.42* (20.77%)	8.40 ± 0.11* (31.48%)	6.64 ± 0.14*** (46.01%)	4.45 ± 0.04*** (61.30%)
Group D Divicine treated	50 mg/kg	11.53 ± 0.45 (7.38%)	11.28 ± 0.24 (8.44%)	10.73 ± 0.47 (12.47%)	10.30 ± 0.17 (16.26%)	9.46 ± 0.32* (17.73%)
	100 mg/kg	10.82 ± 0.37* (13.09%)	9.55 ± 0.38* (22.48%)	9.18 ±0.30* (25.12%)	8.26 ± 0.26* (32.84%)	8.08 ± 0.28** (29.73%)
	150 mg/kg	10.86 ± 0.17* (12.77%)	8.24 ± 0.61* (31.65%)	8.12 ±0.29* (33.76%)	7.10 ±0.07** (42.27%)	6.25 ± 0.2*** (45.65%)
	200 mg/kg	11.10 ± 0.25 (10.84%)	8.14± 0.15* (33.92%)	8.15 ±0.33** (33.52%)	7.23 ± 0.52** (41.21%)	6.28 ± 0.37*** (45.39%)
Group E (Diclofenac) (100 mg/kg)		11.89± 0.03 (4.49%)	4.43 ± 0.05*** (64.04%)	3.72 ± 0.05*** (69.65%)	2.44 ± 0.04*** (80.16%)	0.00 ± 0.00*** (100%)

Experimental groups were compared to control group A. Values are given as mean ± SD for groups of 8 animals each.
**Significantly different from control group at P < 0.05*
***Significantly different from control group at P < 0.01*
****Significantly different from control group at P < 0.001*

dose-related, significant ($p < 0.05 - 0.001$) anti-inflammatory activity with the highest percentage inhibition (59.56%) and (45.65%) for *vicine* and *divicine*, respectively, at 150 mg/kg against the fresh egg albumin-induced oedema in rats after 180 min. Also, the standard anti-inflammatory diclofenac sodium (100 mg/kg) showed 100% inhibition against the fresh egg albumin-induced oedema in rats after 180 minutes (Table 4). Sub plantar injections of fresh egg albumin (0.5 ml/kg) proved marked, time-related and progressive increases in the hind paw diameters of the 'control' untreated rats, maximal swelling and/or oedema occurred approximately 120 minutes following fresh egg albumin administration. Distilled water (3 ml/kg, orally) treatment alone had no any responses to the rat inflammatory oedema induced by fresh egg albumin administration.

BIOCHEMICAL STUDIES OF *VICINE* AND *DIVICINE*

Administration of *vicine* and *divicine* orally to the rats at doses of 50–150 mg/kg bw for 10 days showed non-significant

Table 5 Levels of glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvate transaminase (GPT), alkaline phosphatase (ALP), gamma glutamyl transferase (γ-GT), lactate dehydrogenase (LDH) and lipid peroxides (TBARS) in serum of normal and experimental groups of rats

Experimental Group Dose (orally)		SGOT U/l	SGPT U/l	ALP U/l	γ–GT U/l	LDH U/l	TBARs nmol/ml
Normal Group A (untreated)		12.45 ± 2.24	35.6 ± 3.8	42.4 ± 5.33	6.22 ± 2.09	247.36 ± 7.18	4.52 ± 1.68
Control Group B (Distilled water)		12.73 ± 3.25	34.65 ± 6.11	40.61 ± 4.08	6.20 ±1.66	244.55± 10.43	4.43 ± 2.1
Group C Vicine treated	50 mg/kg	11.43 ± 3.63	37.12 ± 5.07	40.04 ± 6.34	6.13 ± 1.47	246.35 ±11.56	4.51 ± 0.58
	100 mg/kg	11.87 ± 1.45	37.84 ± 4.35	45.80 ± 6.23	6.25 ± 1.62	249.54 ± 9.97	4.35 ± 1.25
	150 mg/kg	13.84 ± 3.11	39.76 ± 6.54	47.22 ± 7.32	6.49 ± 2.00	250.27 ± 15.68	4.54 ± 0.88
	200 mg/kg	15.92 ± 3.61*	37.48 ± 3.82	48.4 ± 4.76*	7.23 ± 2.60*	255.73* ± 18.36	4.12 ± 0.81
Group D Divicine treated	50 mg/kg	12.50 ± 2.46	36.54 ± 6.05	45.04 ± 6.34	6.27 ± 2.08	253.06 ±10.39	4.47 ± 0.82
	100 mg/kg	13.10 ± 3.12	40.42 ± 5.84	50.80 ± 6.23	6.13 ± 1.98	245.44 ± 14.60	4.69 ± 1.07
	150 mg/kg	13.56 ± 2.97	39.11 ± 4.33	52.22 ± 7.32	6.06 ± 2.67	252.58 ± 9.86	4.72 ± 1.34
	200 mg/kg	14.34 ± 4.11*	42.67 ± 5.62 *	55.4 ± 4.76*	6.10 ± 2.85	263.37 ± 16.94*	4.45 ± 0.96
Group E (Diclofenac) (100 mg/kg)		29.76 ± 4.29*	65.29 ± 6.72*	70.32 ± 5.11*	12.65 ± 2.51*	310.55 ± 13.73*	7.25 ± 1.47*

Vicine, divicine and diclofenac sodium were given orally as a single daily dose for 10 days. Control group was compared to normal group. Experimental groups were compared to control group. Values are given as mean ± SD for groups of 8 animals each.
** Significantly different from control group at $p < 0.05$*

changes in liver enzymes SGOT, SGPT, ALP, LDH, γ-GT and serum TBARs as compared with the control group (Table 5). But oral administration of *vicine* and *divicine* at 200 mg/kg bw showed a significant increase ($p < 0.05$) in liver SGOT, SGPT, ALP and LDH level. On the other hand, oral administration of diclofenac sodium (100 mg/kg wb) showed significant increase of serum SGOT, SGPT, ALP, LDH, γ-GT and TBARs as compared with the control group.

Table 6 shows the concentration of GSH, SOD and GPx in blood of normal and experimental groups of rats. The levels of GSH, SOD and GPx did not present significant changes in the groups treated with *vicine* (50–200) and *divicine* (50–150) mg/kg bw as compared with the control group. But oral administration of *divicine* at 200 mg/kg bw showed a significant decrease ($p< 0.05$) in liver GSH, SOD and GPx level, as well as administration of diclofenac sodium orally at dose of 100 mg/kg bw showed a significant decrease of blood SOD, GPx and GSH as compared with the control group.

LIVER TISSUE MORPHOLOGY

The varying doses of *vicine*, *divicine* (50, 100, 150 and 200 mg/kg/day for 10 days) administered orally to rats for 10 days did not induce pathologic changes in the morphology of the liver cells of the test animals. Also, oral administration of diclofenac sodium to rats for 10 days (100 mg/kg/d) shows pronounced dilatation of the hepatic portal vein (Figure 2) as compared with control (Figure 3).

DISCUSSION

Vicine [2,6-diamino-5-(β-D-glucopyranosyloxy)-4(1H)-pyrimidinone] and its aglycones *divicine* [2,6- diamino-1,6-dihydro-4,5-pyrimidinedione] are natural biologically active plant products (Figure 1). *Vicine* and/or *divicine* were detected in various species of family Fabaceae: *Vicia sativa* L.[39] and *Vicia faba* L.[40] Vicine was found also in *Pisum sativum* L.[41] and in *Lupinus albus* L.[42] Lupinus

Table 6 Level of reduced glutathione (GSH) and activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in blood of normal and experimental groups of rats

Experimental Group Dose (orally)		GSH (mg %)	SOD (U/g Hb)	GPx (U/g Hb)
Normal Group A (untreated)		59.48 ± 5.32	15.17± 2.64	163.95 ± 10.72
Control Group B (distilled water)		62.75 ± 5.42	15.35 ± 2.48	160.85 ± 7.66
Group C Vicine treated	50 mg/kg	58.37 ± 6.18	14.90 ± 3.05	165.26 ± 9.76
	100 mg/kg	59.26 ± 5.08	14.75 ± 2.74	160.14 ± 12.33
	150 mg/kg	55.72 ± 8.47	14.56 ± 4.27	155.48 ± 13.52
	200 mg/kg	56.43 ± 5.76	14.28 ± 2.11	159.75 ± 17.30
Group C Divicine treated	50 mg/kg	57.15 ± 3.85	14.76 ± 1.93	159.08 ± 9.46
	100 mg/kg	55.96 ± 6.15	14.10 ± 1.57	157.70 ± 19.13
	150 mg/kg	55.60 ± 4.90	13.73 ± 2.76	156.10 ± 8.97
	200 mg/kg	52.5 ± 5.39*	11.65 ± 2.05*	150.63 ± 11.63*
Group E (Diclofenac) (100 mg/kg)		45.32 ± 4.56*	9.58 ± 2.56*	130.19 ± 15.38*

Vicine, divicine and diclofenac sodium were given orally as a single daily dose for 10 days. Control group was compared to normal group. Experimental groups were compared to control group. Values are given as mean ± SD for groups of 8 animals each.
** Significantly different from control group at $p < 0.05$*

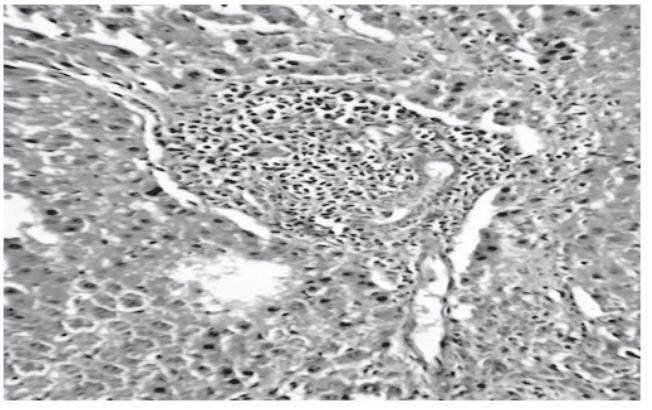


Figure 2. Photomicrograph of the cross section of liver from rat treated with diclofenac sodium 100 mg/kg/d for 10 days (H&E). Shows pronounced dilatation of the hepatic portal vein (x100).

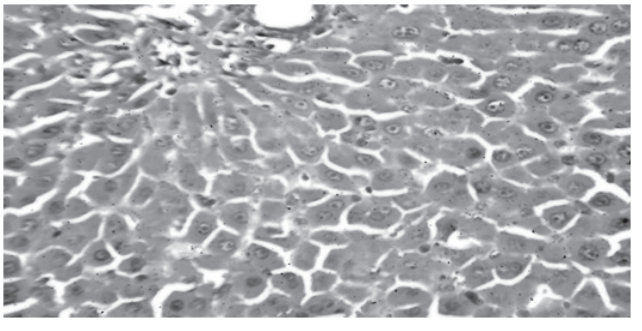


Figure 3. Photomicrograph of the cross section of liver of rat treated with normal distilled water daily for 10 days as control (H&E). Shows normal hepatic portal vein (x100).

species has been reported in a previous study as effective in the treatment of chronic eczema.[43] Some lupinus species exhibit anti-inflammatory capacity related mainly with the presence of phenolic compounds.[44] As part of a program aimed at evaluating the anti-inflammatory activity of *Vicia faba* L. phenolic compounds; *vicine* and *divicine* and study their effect on serum and blood liver enzymes levels in albino rats.

ISOLATION AND STRUCTURE ELUCIDATION OF *VICINE* AND *DIVICINE*

This study has investigated a possible method to isolate the active phenolic compound; *Vicine* from Fava beans (*Vicia faba*)[22] and by hydrolysing it, its aglucone *divicine* was obtained.[23] Structure elucidation of *vicine* and *divicine* were established based on both elemental analysis and spectral data. Infrared spectrum of *vicine* indicated the presence of (NH_2), (OH), (CH-aliphatic), (C = N), (C-O-C) and (C-O-C) groups. Mass spectrum of *vicine* revealed a molecular ion peak at m/z 304 (100%). The value of this peak in mass spectroscopy instrumental equals the elemental analysis data which equals the calculated molecular weight of *vicine*.

Infrared spectrum of *divicine* exhibited bands of (NH_2), (CH-aliphatic), (C = N), (C-O-C) and (C-O-C). Mass spectrum of *divicine* revealed a molecular ion peak and a base peak at m/z 143 (M^++H, 100%). The value of this peak in mass spectroscopy instrumental equals the elemental analysis data which equals the calculated molecular weight of *divicine*. Also, mass spectrum and elemental analysis of *divicine* indicated the breakdown of glycosidic bond in *vicine* by hydrolysis and lost one glucose moiety.

DETERMINATION OF THE MEDIAN LETHAL DOSES (LD_{50}) OF *VICINE* AND *DIVICINE*

The symptoms, histopathological changes and death of experimental rats shown in this study were obviously due to acute toxicity caused by the oral administration of vicine and divicine. Fava beans (*Vicia faba*), from which the *vicine* and *divicine* were isolated, has been known to be a strong hypoglycemic agent.[43] According to Deschroches et al.,[45] the LD_{50} data of *vicine* and its aglycone (*divicine*) 24 hours after intraperitoneal administration was 4000 mg/kg for *vicine* and 149 mg/kg for *divicine* related to the body weight of rats. In this study, the median lethal doses (LD_{50}) of *vicine* and its aglucone (*divicine*) 24 hours after oral administration were 2100 mg/kg for *vicine* and 1950 mg/kg for *divicine* (Tables 2 and 3). The median lethal doses (LD_{50}) of *vicine* and its aglucone (*divicine*) after oral administration have not been reported earlier to my knowledge, and this study is perhaps the first observation of its kind. The experimental animals could be classified into three categories: 1) animals that died early within the first few hours of ingestion; 2) animals that died later within the observation period (up to 48 hours); 3) animals that exhibited mild symptoms but were able to survive. Possible causes of death are heart failure that can occur by malfunction,[46] acute hypoglycaemia and hepato-damage. The immediate cause of death of animals that died early was probably heart failure. According to Kingsbury, at toxic levels, cardioactive glycosides produce cardiac irregularities and heart block.[47] Furthermore, in many lethal poisonings, heart failure can be brought about by malfunction of innervation or of the heart's conductive tissues, or it may be a result of a more direct effect on the heart musculature. As time after ingestion goes on, the glycosides have enough time to exert its action and death is likely to be due to acute hypoglycaemia rather than heart failure.

This probably explains the death of the animals that died later, since hypoglycaemia was confirmed in these animals by blood test.[48] Hepato-damage, seen in the animals that died later, is another possible cause of death, although apparently overshadowed by the acute hypoglycaemia.[43] The animals that survived escaped heart failure and acute hypoglycaemia and were also able to overcome hepato-damage by regeneration.

SCREENING OF ANTI-INFLAMMATORY ACTIVITY OF *VICINE* AND *DIVICINE*

The anti-inflammatory activities of the agent under study were investigated by using the fresh egg albumin-induced edema model described by Ekpendu et al. [33] The finding of anti-inflammatory activity exerted by *vicine* and *divicine* and the identification of active principles could support the use of these compounds for the treatment of inflammatory affections. The results obtained show that the effect exerted by *vicine* is higher than that exhibited by *divicine*. Previous studies concerning the mechanism of action of phenolic compounds as anti-inflammatory agent were shown that phenolics have anti-inflammatory activity in both proliferative and exudative phases of inflammation[49,50] while the mechanism of action of *vicine* and *divicine* as anti inflammatory agent may be due to the inhibition of some enzymes which participate in the synthesis of inflammatory substances derived from arachidonic acid.[50] Pyrimidines like *vicine* and *divicine* represent a broad class of compounds, which have received considerable attention due to their wide range of biological activities,[51] inhibit both the cyclooxygenase and 5-lipoxygenase pathways.[52] This inhibition reduces the release of arachidonic acid. Another anti-inflammatory property of phenolics is their suggested ability to inhibit neutrophil degranulation. This is a direct way to diminish the release of arachidonic acid by neutrophils and other immune cells.[53] The used dose of diclofenac sodium in this work was reported[25,26,54] and the results showed that diclofenac sodium enhanced the activity of liver enzymes. This finding suggests increased transmembrane transport of diclofenac sodium and metabolized it in the liver to 4-hydroxy diclofenac and other hydroxylated forms after glucoronidation and sulfation before being eliminated principally via urinary and biliary excretions,[55] where liver enzymes, especially, alkaline phosphatase, are involved in the absorption and transportation across canalicular membrane proteins.[56] On the other hand, alkaline phosphatase is a meta-enzyme and contains zinc as an integral part.[57] The nonspecific increase in the activity of alkaline phosphatase by diclofenac sodium exposure may be a result of the incorporation of the drug in the place of zinc atoms leading to an increase in the activity of this enzyme.[58] Hence, the present results might suggest a possible role of this enzyme in the catabolism of nucleic acids in the cells disintegrated by diclofenac sodium effect. The anti-inflammatory effect of *vicine* and its aglucone (*divicine*) after oral administration has not been reported earlier to my knowledge, and this study is perhaps the first observation of its kind.

EFFECT OF ANTI-INFLAMMATORY COMPOUNDS ON LIVER ENZYME LEVELS

Investigation of the effects of administration of *vicine* and *divicine* (50–150 mg/kg bw) isolated from fava beans (*Vicia faba*) on the serum levels of AST, ALT, γ-GT, and TBARS and levels of blood GSH, SOD and GPx in normal and experimental groups of rats revealed that acute treatments for 10 days resulted in a non significant rise in the serum and blood enzyme levels. The sharp significant increase was attenuated gradually after repeated administration of *vicine* and *divicine* (200 mg/kg bw) a period of 10 days. On the other hand, *vicine* (50–200 mg/kg) and *divicine* (50–150 mg/kg) treatment for 10 days caused a non significant reduction in the blood levels of GSH, SOD and GPx, respectively. But oral administration of *divicine* at 200 mg/kg bw, caused a significant reduction ($p < 0.05$) in the blood levels of GSH, SOD and GPx. Also, the sharp significant rise of serum levels of AST, ALT, γ-GT and TBARS and significant decreased in levels of blood GSH, SOD and GPx after repeated administration of diclofenac sodium (100 mg/kg orally) for a period of 10 days were reported. In addition, the photomicrograph of the cross section of liver from a rat treated with diclofenac sodium 100 mg/kg/d for 10 days shows pronounced dilatation of the hepatic portal vein. Graded non significant increase in serum and blood enzymes activity after treatment for 10 days might be an indication of negative feedback effect. This phenomenon was similar to the negative feedback mechanism involving the release of hormones.[59] Also, a sharp rise in the enzyme serum levels could probably be due to sudden physiological changes following acute *vicine* and *divicine* tissue interaction and not necessarily pathological. It has been reported that some substances are capable of inducing enzymes such as cytochrome P-450, monooxygenase, monoamine oxidase and other enzymes.[60, 61] These observations showed that repeated administration of *vicine* and *divicine* isolated from fava beans (*Vicia faba*) could stimulate the release of some enzymes into serum which could influence extrahephatic metabolism of

certain substances or drug biotransformation. Repeated administration of *vicine* and *divicine* for 10 days almost did not cause any significant change in serum and blood enzyme levels. These findings led to the suggestion that *vicine* and *divicine* isolated from fava beans (*Vicia faba*) is not like to be hepatotoxic. Finally, anti-inflammatory effect of natural heterocycle glucoside *vicine* obtained from *Vicia faba* L. and its aglucone (*divicine*) and their effect on serum and blood enzyme levels in Albino rats have not been reported earlier to my knowledge, and this study is perhaps the first observation of its kind.

In conclusion, the present study showed that *vicine* obtained from *Vicia faba* L. and its aglucone *divicine* have a considerable anti-inflammatory activity. Further studies are in progress to identify the possible mechanism of their effect.

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