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RESEARCH ARTICLE

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Study of analgesia caused by some commonly used herbs in the kingdom of Saudi Arabia

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ABSTRACT

The analgesic activity of the aqueous extracts of fifteen herbal drugscommonly used in folk medicine in treatment of various conditions were chosen for evaluating their analgesic activity and nothing was traced in the current literature about their analgesic effect. The selected plants arestar anise, lemon peel, halfa-bar, fennel, orange peel, becham, asafoetida, marjoram, clove, black pepper, cinnamon, benzoin, rhubarb, vanilla and Ammi-visnaga. Based on proper statistical analysis using the standard Edyys' hot plate method,the obtained results showed that some plants had analgesic effect and the most promising plants werebecham, marjoram,rhubarb and Ammi-visnagacompared to the negative control (normal saline) and positivecontrol (diclofenac sodium).

Keywords: commonly used herbal drugs; analgesic activity; hot-plate method.

INTRODUCTION

Pain is a common, highly unpleasant physical sensation caused by illness, injury or other stimuli. Analgesics or pain killers are drugs that used to achieve analgesia, it act in different ways either oncentral and/orperipheral nervous systems. Far from chemical medicaments, the search for better and safer ways of relieving pain throughout human historyled to the use of herbs as analgesic medicines. In this study, fifteen plants that are used in folk medicine in KSA for treatment of various conditions were extracted and investigated for their analgesic activity. Reviewing the current literature, nothing was traced about the analgesic activity of the selected plants. The investigated plants of this study include fruits of star anise, lemon peel, fennel, orange peel, black pepper, vanilla and *Ammi-visnaga*; aerial parts of half a-bar, barks of becham and cinnamon; leaves of marjoram; flowers of clove; rhizomes ofrhubarb; balsams of benzoin and resin combinations of asafoetida. Star anise**,** *Illiciumverum* Hook.(Illiciaceae),is an aromatic star-shaped fruit used as a spice and has been shown to be effective intreatment of inflammation, rheumatic pain, vomiting and insomnia. The plant is rich in lignans and sesquiterpenes which many studies have shown its neurotoxic and neurotrophic effects[1].Lemon peel is the outer part of lemon fruits of *Citrus limon*L. (Rutaceae). It

contains at least 2.5% of volatile oils, vitamin C, flavonoid hesperidin and other flavanone glycosides and mucilage. It is mainly used as flavouring agent and its volatile oil can be used for treatment of hypertension, dyspepsia, anaemia, acne and arthritis[2].Halfa bar**,** *Cymbopogonproximus*Stapf.(Graminae),is a wild perennial grass contains volatile oil and it is used in traditional medicine as a diuretic, antispasmodic and to help small stones removal from the urinary tracts[3]. The antimicrobial, antioxidant, antiemetic, hypotensive and anticonvulsant studies were done[3-5].Fennel, *Foeniculumvulgare* Mill.(Apiaceae), is an aromatic plant used as carminative, digestive, lactogogue, diuretic and in treating respiratory disorders.It containsphenols, phenolic glycosides and volatile oils[6].Orange peel, *Citrus aurantium*L. var. *sinensis* (Rutaceae), containsvolatile oil and flavonoids and it is used as flavouring agent[2]. Becham, *Commiphoraopobalsamum* L.Engl. belongs to the family Burseraceae. It has been used for treatment of chest, stomach and kidney complaints. It is also usedto relieve rheumatic pain, scurvy and jaundice. The plant containstriterpenes, flavonoids, syringic acid and volatile oils and has antihypertensive, hepatoprotective and ulcer protective activities[7-11]. Asafoetida, *Ferula assa-foetida* L. Qis obtained from the family Apiaceae. It consists ofa mixture of volatile oil, gum and resin exudates. It has been used as a spice and as aphrodisiac, a sedative and to produce diuresis[12]. Marjoram**,** *Origanummajorana*L.(family Lamiaceae) is a bushy widely used shrub. It contains a range of biologically active compounds, including flavonoids, phenolicterpenoids, phenolic glycosides, tannins, and sitosterol. Many studies showed that marjoram possess antiviral, bactericidal, antiseptic, antifungal, antioxidant, antitumor, hepatoprotective, pediculicides and ovicides activities [13-17].Clove,*Eugenia caryophyllata*Thunb.(Myrtaceae) is aromatic flower budsrich with volatile oil and used to relief toothache. Several studies demonstrated that clove has antifungal, antiviral, antimutagenic, anti-inflammatory, antiulcerogenic, antithrombotic and antiparasitic activities[18].Black pepper**,** *Piper nigrum*L., belongs to the family Piperaceae and it is one of the famous and most extensively used spices. Its activityis due to piperinealkaloid and many biological effects have been reported[19, 20]. Cinnamon, *Cinnamomumzeylanicum* Blume from the family Lauraceae, is an aromatic volatile oil containing plant having several applications in flavoring, perfumery, beverages and medicines[21, 22]. Biological studies showed anti diabetic, antioxidant, antifungal, antimutagenic, anticardiovascular, anti-inflammatory, antiulcer, antiviral, antihypertensive and cholesterol and lipid-lowering activities[21-26]. Benzoinis a balsamic resin obtained from the incised stem of *Styrax benzoin* Dry and (Styracaceae). It contains balsamic acids and their esters and triterpenoid. The tincture can be inhaled in steam for bronchitis and colds and internally, acts as an expectorant and antiseptic [2,27].Rhubarb**,***Rheum palmatum* L. (Polygonaceae), has been traditionally used as a laxative and an antidiarrheal agents due to the anthraquiononeand tannin contents[28, 29].Hot water extracts of rhubarb showed molluscicidal activity[30].Vanilla pod**,***Vanilla planifolia*Andr. (Orchidoceae) is the most popular flavoring agent in food industry. It is also used in cosmetic (perfumes) and in pharmaceutical industries due to vanillin content. The fruits of *Ammi-visnaga* L.Lam.(Apiaceae) isused in traditional medicine for treatment of urinary tract stones due to the presence of furanochromones[2, 31-34].

MATERIALS AND METHODS

Plant materials

The plants used in this study were purchased from a local market in Taif city which is located in the Western region of KSA. Each plant was separately air-dried in the shade and grounded.

Standard material for analgesic activity

Diclofenac sodium(Novartis, Basel, Switzerland) was obtained from local drug market.

Preparation of the crude plant extracts

Exact weight (50g) of each air-dried plant powder was separately extracted using hot water by infusion method until exhaustion. The total aqueous extract of each plant was concentrated under vacuum using rotary evaporator and lyophilized using a freeze-dryer. The dry weight obtained from each extract as follows: Star anise(4.5 g), Lemon peel (6.6 g), Half-bar (1.2 g), Fennel (3.3 g), Orange peel (6.2 g), Becham (2.6 g), Asafetida (1.9 g), Marjoram (1.9 g), Clove (9.9 g), Black pepper (1.4 g), Cinnamon (3.3 g), Benzoin (0.1 g), Rhubarb (1.2 g), Vanilla (1.1 g), *Ammi-visnaga* (2.6 g). All extracts were kept at – 20°C till used for analgesic study.

Animals

The experiment was performed using Wistar male mice, weighing 25–30 g, obtained from animal house of King Abdul-Aziz University, Jeddah, KSA. The mice were housed in standard environmental conditions and fed standard laboratory diet with free access to water and were kept for seven days with 12 h light/ dark cycle prior conducting the analgesic study. The present work followed the Ethics guidelines of EEC999 of Europe 1982.

Table 1:The analgesic activity of negative, positive controls and plant extracts

#	ID	AVG	STD	CV
-ve control	Normal Saline	8.38	2.38	-
+ve control	Diclofenac Sod.	15.27	4.00	-
1	Star anise	9.05	2.16	23.83
2	Lemon peel	9.78	1.09	11.14
3	Half-bar	10.48	1.32	12.56
4	Fennel	10.71	2.39	22.27
5	Orange peel	11.85	2.93	24.71
6	Becham	14.89	3.08	20.72
7	Asafoetida	10.31	0.57	5.55
8	Marjoram	15.27	2.63	17.21
9	Clove	11.21	0.79	7.09
10	Black pepper	13.12	3.83	29.2
11	Cinnamon	12.8	1.67	13.02
12	Benzoin	13.95	3.21	23.04
13	Rhubarb	14.39	3.8	26.4
14	Vanilla	12.08	1.84	15.26
15	*Ammi-visnaga*	15.3	2.21	14.48

ID: identity *AVG: averageSTD: standard deviation* *CV: coefficient of variation*

Table 2:Means for survival time for plants (1-12) obtained using the nonparametric estimator of the function of survival using the Kaplan Meier test

			95% Confidence Interval	
Factor	Estimate	Std. Error	Lower Bound	Upper Bound
1- Star anise	10.311	1.488	7.394	13.227
Diclofenac Sod.	15.272	1.787	11.770	18.774
Overall	12.566	1.340	9.940	15.192
2- Lemon peel	9.252	.664	7.950	10.553
Diclofenac Sod.	15.272	1.787	11.770	18.774
Overall	11.988	1.265	9.509	14.467
3- Half-bar	10.484	.537	9.431	11.537
Diclofenac Sod.	15.272	1.787	11.770	18.774
Overall	12.660	1.108	10.489	14.832
4- Fennel	10.712	1.066	8.622	12.802
Diclofenac Sod.	15.272	1.787	11.770	18.774
Overall	12.992	1.241	10.560	15.424
5- Orange peel	11.852	1.196	9.507	14.197
Diclofenac Sod.	15.272	1.787	11.770	18.774
Overall	13.406	1.123	11.206	15.607
6- Becham	14.890	1.259	12.423	17.357
Diclofenac Sod.	15.272	1.787	11.770	18.774
Overall	15.064	1.008	13.088	17.039
7- Asafoetida	11.243	.951	9.380	13.107
Diclofenac Sod.	15.272	1.787	11.770	18.774
Overall	13.075	1.109	10.901	15.248
8- Marjoram	14.405	1.296	11.865	16.945
Diclofenac Sod.	15.272	1.787	11.770	18.774
Overall	14.799	1.028	12.784	16.814
9- Clove	11.085	.318	10.462	11.708
Diclofenac Sod.	15.272	1.787	11.770	18.774
Overall	12.988	1.021	10.987	14.989
10- Black pepper	12.229	1.659	8.978	15.480
Diclofenac Sod.	15.272	1.787	11.770	18.774
Overall	13.612	1.249	11.164	16.061
11- Cinnamon	12.322	.777	10.799	13.844
Diclofenac Sod.	15.272	1.787	11.770	18.774
Overall	13.663	.980	11.742	15.584
12- Benzoin	13.152	1.420	10.369	15.934
Diclofenac Sod.	15.272	1.787	11.770	18.774
Overall	14.115	1.114	11.932	16.299

Table 3: Means for survival time for plants (13-15) obtained using the nonparametric estimator of the function of survival using the Kaplan Meier test

			95% Confidence Interval	
Factor	Estimate	Std. Error	Lower Bound	Upper Bound
13- Rhubarb	13.173	1.819	9.608	16.737
Diclofenac Sod.	15.272	1.787	11.770	18.774
Overall	14.127	1.262	11.654	16.600
14-Vanilla	15.300	1.280	12.791	17.809
Diclofenac Sod.	15.272	1.787	11.770	18.774
Overall	15.282	1.147	13.034	17.531
15- *Ammi-visnaga*	12.080	.921	10.275	13.885
Diclofenac Sod.	15.272	1.787	11.770	18.774
Overall	13.853	1.159	11.582	16.124

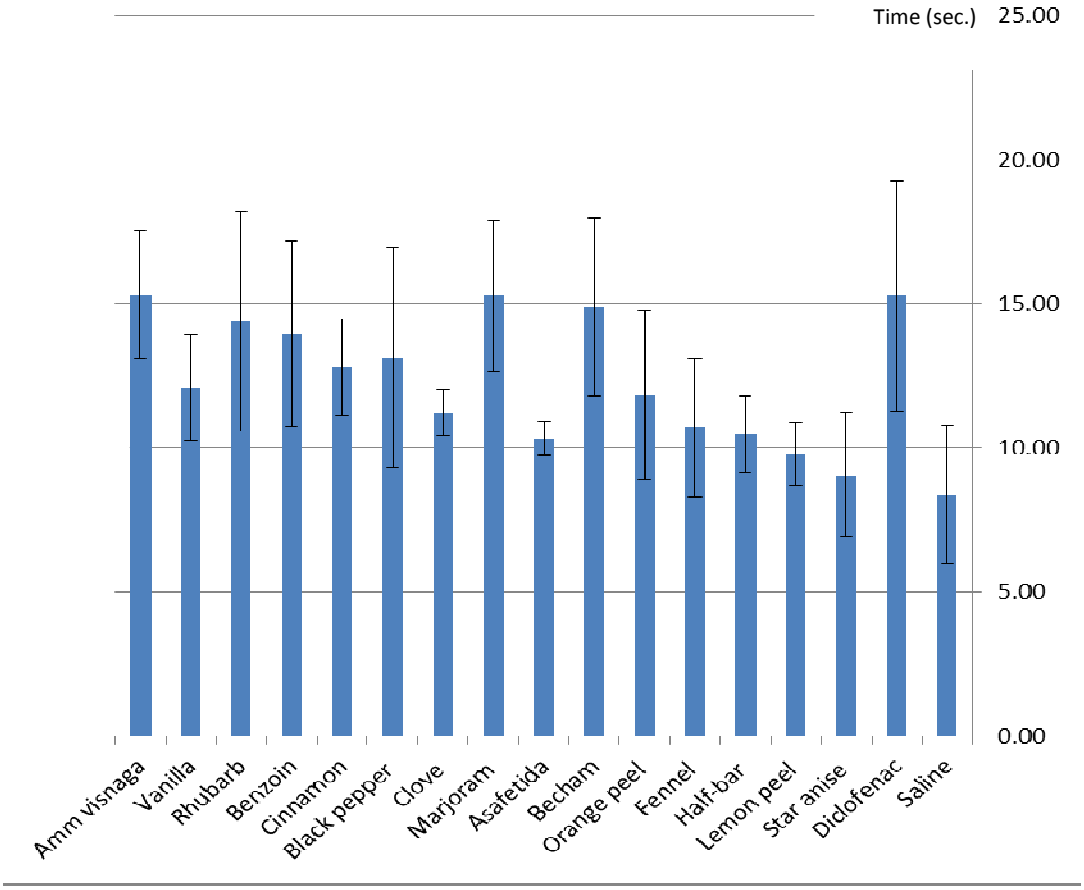

Fig. 1: The analgesic activity of negative control, positive control and different plant extracts

Analgesic study

The analgesic study was performed by the standard"hot-plate" method which is specifically used for the screening of centrally acting analgesics,according to Eddy and Leimback[35]with slight modifications. Analgesics which act centrallycause prolongation of the latency times of response i.e. jumping and licking of the paws. In this study,the mice were divided into 17groups each of 6 mice. Group 1 (negative control) received normal saline, group 2 (positive control) received diclofenac sodium (1 mg/kg) via intraperitoneal injection and groups 3-17 received plant extracts(300 mg/kg).The mice in each group were left at room temperature for one hour period prior to pain induction using Eddys'hot plate. The mice were placed on Eddys' hot plate at`a temperature of 55 ± 0.50°C in order to note the animal's reaction to heat pain (licking of the forepaws or jumping). A cut off time of 15 sec, was set to prevent any damage of the paw.

Statistical analysis

The statistical significances were examined using analysis of variance (ANOVA). All statistical analysis was conducted via SPSS program version 14.0.1. The significance of the differences was determined at a 95% confidence interval, and values of $p< 0.05$ were considered to be statistically significant. Survival analysis was performed using Kaplan-Meier of SPSS 14.0.1.

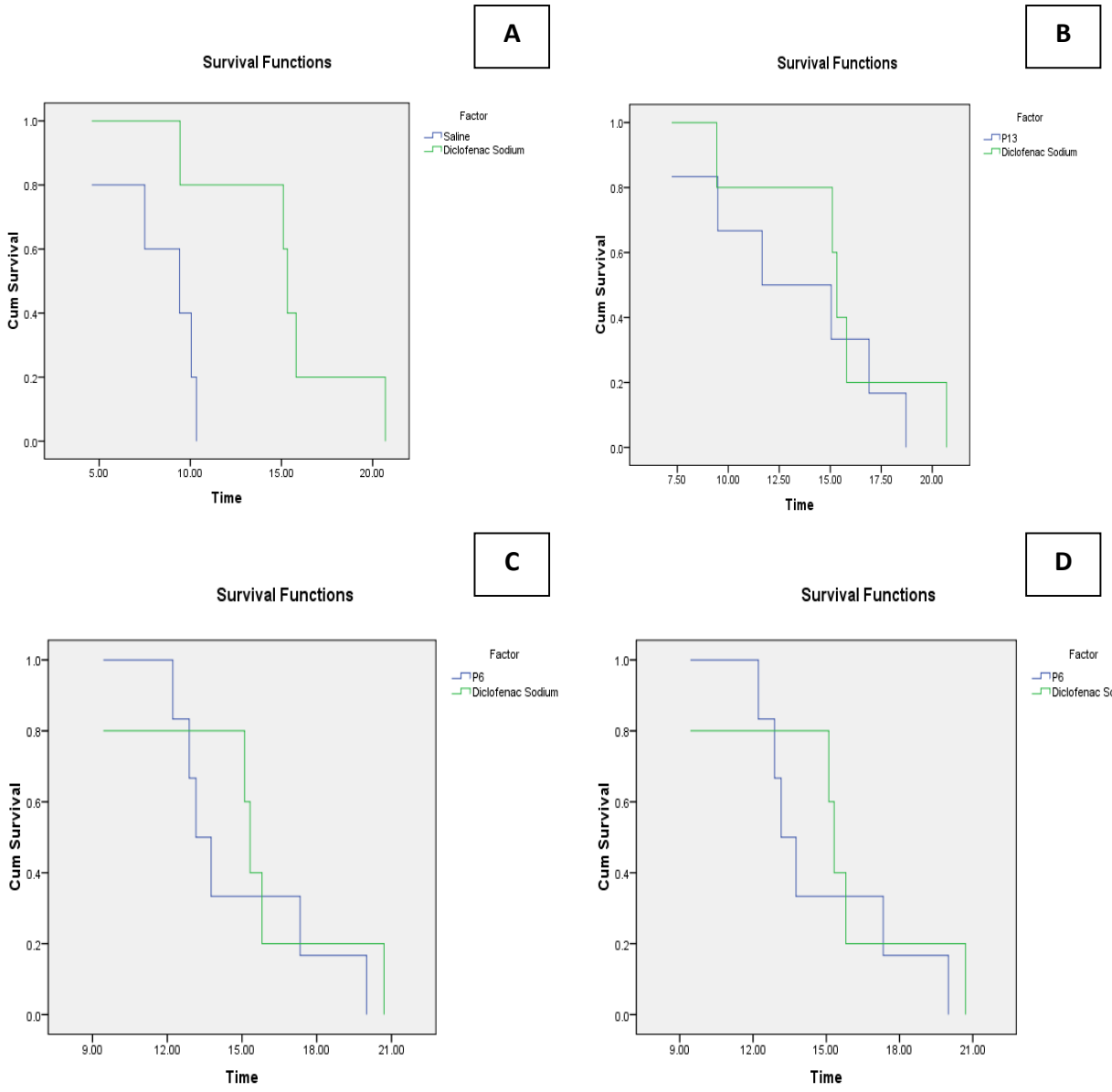

Fig. 2: Survival analysis using the Kaplan Meier test of SPSS.

A: animals treated with normal saline and those treated with diclofenac sodium

B: animals treated with diclofenac sodium and those received extract of Rhubarb (13)

C: animals received diclofenac shows cumulative survival against time (sec) of animals treated with diclofenac sodium and those mice received extract of Marjoram (8)

D: represents SA of animals received diclofenac and those received extract of Becham (6).

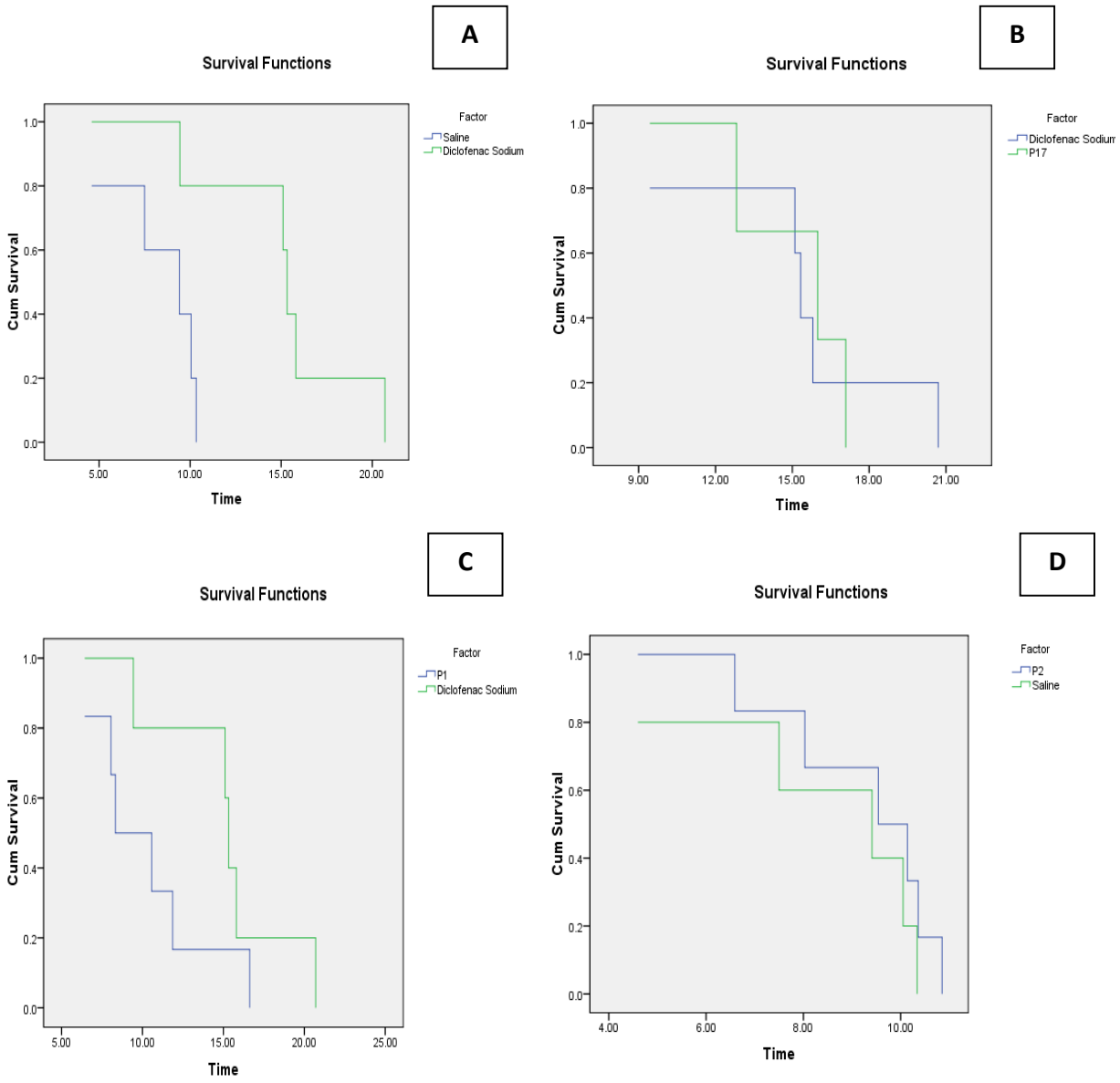

Fig. 3: Survival analysis using the Kaplan Meier test of SPSS.
A: animals treated with normal saline and those treated with diclofenac sodium,
B: animals treated with diclofenac sodium and those received extract of Ammi-visnaga (15)
C: animals received normal saline andthose treated with extract of Star anise (1)
D: animals received normal saline and animals treated with extract of lemon peel (2).

RESULTS AND DISCUSSION

The analgesic activity results are listed in Table 1 and illustrated in Fig. 1. The results showed that some plants as becham (6), marjoram (8), black pepper (10), cinnamon (11), benzoin (12),rhubarb(13) and *Ammi-visnaga* (15)had analegsic effect. The most promising plants are becham (6), marjoram (8),rhubarb (13)and *Ammi-visnaga* (15) compared to the negative control received normal saline, however, these plants showed analgesic activities but statistically insignificant (p> 0.05) compared to the positive control that received diclofenac sodium. The remaining plants didn't show singificant analgesic activity (p> 0.05) compared tothe positive control.The activity of rhubarb and becham may be attributed to the tannin and terpenoid compunds respectively while in *Ammi-visnaga*itis attributed to furanochromone derivatives as khellin and visnagin which reported to have antiinflammatory and analgesic activities [36-38]. The activity of marjoram may be attributed to its tannin andflavonoid content.

Survival analysis was performed using Kaplan-Meier of SPSS, because the outcome factor is the time until the happening of an event of interest (licking forepaws). For example, if the event is licking of forepaws by a mouse in response to induced heat pain, then the survival time can be the time in seconds until that mouse develops a response (licking forepaws). Another advantage of using survival analysis is censoring, which is a significant issue in this kind of analysis. When an animal died before the end of the experiment and did not experience the event (licking forepaws). This animal's survival time can be censored. Furthermore, the Kaplan-Meier method can generate the characteristic "stair step" survival curves.Mean for survival analysis for the positive control and the fifteen plants are shown in Table 2 and 3. Since the most promising plants in terms of analgesic activities are becham (6), marjoram (8), rhubarb (13) and *Ammi-visnaga* (15), the discussion using the survival analysis (SA) will directed toward the above mentioned plants. The significance of the differences was calculated at a 95% confidence interval. SA showed confidence interval (CI) of becham (6) 12.423-14.197, while the CI of the positive control (diclofenac sodium) was 11.77-18.774 (Table 2). Upon careful testing of these two CI, it was clear that there was an overlapping among them. This overlapping was further confirmed via visual inspection of the survival curve (Figure 2C). The X- axis of the survival curve (Figures 2 & 3) showed the time (sec) to event (response), while the Y- axis showed the probability of survival. Any point on the curve indicates the possibility that any mouse of a given group will respond to the heat stimuli and remains in the study past that time. Overlapping was very clear at the upper limit of CI, this means that analgesic activity of bechamwassimilar to the positive control but statistically insignificant (p>0.05). CI of marjoram (8) was 11.865-16.945, while the corresponding CI of diclofenac sodium was 11.77-18.774 (Table 2). Results of SA clearly indicated overlapping between two CIs especially at the upper limit. This overlapping was confirmed upon testing the survival curve (Figure 2D), which pointed out that this plant displayed certain analgesic activity close to the diclofenac but still statistically insignificant (p>0.05). The same trend was noted for the rhubarb (13) and *Ammi-visnaga* (15) (Table 3, Figure 2B and Figure 3B).

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